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Phomopsis husk rot of macadamia in Australia and South Africa caused by novel *Diaporthe* species

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Phomopsis husk rot (PHR) in macadamia is a disease of economic importance in major commercial production areas in Australia and South Africa. Effective control of PHR is hindered by limited knowledge about its aetiology and epidemiology. The diversity and pathogenicity of more than 50 isolates of *Diaporthe* associated with PHR in macadamia orchards in Australia and South Africa was assessed. Multilocus phylogenetic analyses of DNA sequences of the ITS, *tef1α*, and *tub2* gene loci revealed four novel clades that are described as *Diaporthe australiana* sp. nov., *D. drenthii* sp. nov., *D. macadamiae* sp. nov., and *D. searlei* sp. nov. Pathogenicity tests with representative isolates found that all four species caused PHR of varying severity between and within species, as well as between the two macadamia cultivars HAES 344 and HAES 816. The Australian species, *D. australiana*, was the most aggressive species compared with the three South African species. This study improves our understanding of the aetiology of PHR in macadamia and paves the way for more effective disease management.

Keywords

disease severity, fruit pericarp, *Phomopsis*, Proteaceae, tree nut

1. Introduction

Macadamia (*Macadamia integrifolia* and *M. tetraphylla*) is indigenous to the rainforests of south-eastern Australia, and highly valued as a cultivated tree nut. The macadamia kernel is relatively soft, protected by a hard testa and an outer pericarp (husk). Commercial macadamia plantations are found in several tropical and subtropical countries in Africa, Asia, and North and South America (Trueman, 2013; Howlett *et al*., 2015). Australia and South Africa are the leading producers and suppliers of macadamia nuts, with both accounting for over 56% of global production (INC, 2019). As the macadamia industry continues to expand, yield loss due to diseases that affect tree and fruit health has become a critical problem to production. Two diseases that affect the fruit pericarp, husk spot caused by *Pseudocercospora macadamiae* (Akinsanmi and Drenth, 2012) and husk rot caused by *Diaporthe* species (Akinsanmi and Drenth, 2017) cause significant yield losses.

Husk rot symptoms in macadamia are caused by *Colletotrichum*, *Diaporthe*, *Lasiodiplodia*, and *Stilbella* (Akinsanmi and Drenth, 2017). The disease name phomopsis husk rot (PHR) was given to symptoms in macadamia associated with *Diaporthe* spp. (Akinsanmi and Drenth, 2017). *Diaporthe* species can cause blights, cankers, decays, dieback, wilts, leaf spots, fruit and root rots across a diverse range of plant species (Du *et al*., 2016), including serious diseases in cultivated blueberry (Polashock and Kramer, 2006), citrus (Guarnaccia and Crous, 2017), grapevine (van Niekerk *et al*., 2005), kiwifruit (Díaz *et al*., 2017), and sunflower (Thompson *et al*., 2011). Further, many *Diaporthe* species may persist transiently as saprobes and endophytes (Udayanga *et al*., 2012; Lawrence *et al*., 2015) with the potential to become latent pathogens when triggered by environmental stressors (Carroll, 1988; Santos *et al*., 2016). The mechanisms governing the switch from harmless endophyte to parasite are unclear, although host signals and environmental factors may underlie these transitions (Müller and Krauss, 2005; Schulz and Boyle, 2005).

PHR is a major threat to macadamia production in Australia and South Africa, causing premature fruit drop in several cultivars (Campbell, 2015; Schoeman *et al*., 2016; Akinsanmi and Drenth, 2017). The symptoms of PHR are characterized by the rapid and sporadic formation of soft, spongy, black lesions about 5–10 mm in diameter that contrast against the green pericarp of healthy fruit (Akinsanmi and Drenth, 2017). These lesions may coalesce and degrade the entire pericarp, leading to premature fruit drop (Akinsanmi and Drenth, 2017).

The description of new species in *Diaporthe* is largely based on multilocus phylogenies (Udayanga *et al*., 2012; Santos *et al*., 2017; Long *et al*., 2019). The five very informative loci that have been identified for the genus are the internal transcribed spacer (ITS), β-tubulin (*tub2*), translation elongation factor 1-α (*tef1α*), calmodulin (*cmd*), and histone H3 (*his3*) (Santos *et al*., 2017). Of the five loci, *tef1α* is the most informative gene locus for delineating species within the genus. Therefore, species description should include *tef1α* with at least a combination of ITS and either *his3* or *tub2* molecular data. The combined ITS and *tub2* molecular data indicated the existence of at least two *Diaporthe* species associated with PHR in Australia (Akinsanmi and Drenth, 2017).

There are now over 50 macadamia varieties grown in commercial plantations worldwide (Hardner, 2016), with increasing reports of PHR. It is unclear whether there is variation in aggressiveness between isolates and species of *Diaporthe* associated with PHR. There is limited

information on the aetiology, pathogen biology, and epidemiology of PHR in macadamia (Akinsanmi and Drenth, 2017). For example, little is known about the process of infection; the influence of weather and host genotype on infection; disease development; and whether the same species cause PHR epidemics in both Australia and South Africa. The aims of this study were first to assess the ability of isolates of *Diaporthe* collected from macadamia plantations in Australia and South Africa to cause PHR, and secondly to determine whether the same *Diaporthe* species caused PHR epidemics in Australia and South Africa.

2. Materials and methods

2.1 Fungal isolates

Macadamia fruits with PHR symptoms were collected from commercial macadamia orchards in Australia and South Africa. Approximately 3–5 mm sections were excised from the margin of the diseased pericarp. The tissue pieces were surface-sterilized in 2.5% wt/vol sodium hypochlorite (NaOCl) solution for 3 min, rinsed in three changes of sterile distilled water, dried with sterile blotting paper, and plated on ½-strength potato-dextrose agar (PDA; Difco Laboratories Inc.) amended with 30 mg/L streptomycin sulphate. The plates were incubated at 20 ± 2 °C for 7 days. Monoconidial cultures of *Diaporthe* isolates (15 from Australia and 14 from South Africa) that represent a range of geographical regions were obtained following the hyphal tip method as described by Harteveld *et al*. (2013).

2.2 DNA extraction and PCR amplification

Genomic DNA of the isolates was extracted from about 40 mg of mycelium from monoconidial isolates using the Promega Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. Total DNA was quantified using the BioDrop Duo spectrophotometer (Biodrop) with the adjusted working stock concentration of 25 ng/µl. PCR was used to amplify the ITS region, with the primers ITS5 and ITS4 (White *et al*., 1990). A partial region of the *tub2* gene was amplified using primers T1 and Bt2b (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997), and a partial region of the *tef1α* gene was amplified with primers EF1-728F and EF1-986R (Carbone and Kohn, 1999). PCR was conducted with the Phusion Master Mix (Thermos Fisher Scientific) in a Supercycler thermal cycler (Kyratec). PCR amplification used a 24 µl reaction

mixture that contained 10 µl of 10 mM Phusion MasterMix, 1 µl of each 10 mM forward and reverse primer, 11 µl of nuclease-free water, and 1 µl template DNA. Thermocycler settings were an initial denaturation for 60 s at 98 °C; followed by 35 cycles at 98 °C for 10 s, 72 °C for 30 s, 72 °C for 45 s; with a final extension step at 72 °C for 5 min. PCR amplicons were purified using the QIAquick PCR purification kit (QIAGEN), and sequenced in both directions by Macrogen Incorporated (Seoul, South Korea).

2.3 Phylogenetic analysis of PHR isolates

All generated sequences were assembled in Geneious v. 11 (Biomatters Ltd). The sequences of each locus were aligned separately and manually adjusted. Alignment gaps were treated as missing character states and all characters were unordered and of equal weight. The sequences generated in this study and used in the phylogenetic study were deposited in GenBank (Table 1). The sequences were compared against the NCBI's GenBank nucleotide database using BLAST to determine the closest phylogenetic relatives. These sequences were aligned with selected sequences of ex-type or authentic representatives of *Diaporthe* species (Table 2) using the MAFFT alignment algorithm (Katoh and Toh, 2008) in Geneious. To establish the identity of the isolates at the species level, phylogenetic analyses were conducted first for each locus (data not shown), and then for the three loci combined (*tub2* + ITS + *tef1α*). *Diaporthe ocoteae* strain CBS 141330 was included as the outgroup (Table 2). Maximum likelihood (ML) analysis was run using RAxML v. 7.2.8 (Stamatakis and Alachiotis, 2010) in Geneious, and started from a random tree topology using the general time-reversible (GTR) model. The nucleotide substitution model used was GTR with a gamma-distributed rate variation. The Bayesian analysis was performed with MrBayes v. 3.2.1 (Ronquist and Huelsenbeck, 2003) in Geneious. To remove the need for a priori model testing, the Markov chain Monte Carlo (MCMC) analysis was set to sample across the entire GTR model space with a gamma-distributed rate variation across the sites. Five million random trees were generated using the MCMC procedure with four chains. The sample frequency was set at 1,000 and the temperature of the heated chain was 0.1. Burn-in was set at 25%, after which the likelihood values were stationary.

2.4 Morphological characterization of PHR isolates

Isolates were grown on PDA, oatmeal agar (OMA), and 2% wt/vol tap water agar supplemented with sterile wheat straws (WSA), incubated at 23 ± 2 °C under a 12 hr photoperiod to induce

sporulation. Colony characters and pigment production were determined after 14 days' growth on PDA and OMA. Colony colours were determined according to the charts of Rayner (1970). Cultures were examined periodically for the development of ascomata and conidiomata. Colony diameters were measured after 7 and 10 days. Morphological characteristics were determined by mounting fungal structures in clear lactic acid. Images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast illumination. Conidial widths were measured at the widest part of each conidium. Means and standard deviations were calculated from at least 20 measurements. Novel species were registered in MycoBank (Crous *et al*., 2004).

2.5 Pathogenicity of PHR isolates

Conidial suspensions of 15 Australian and 14 South African isolates of *Diaporthe* associated with PHR were prepared (Harteveld *et al*., 2013) and adjusted to 10^6 conidia/ml using a haemocytometer. Mature symptomless fruits of macadamia cultivars HAES 344 and HAES 816 were collected from the tree canopies of healthy trees. The fruits were surface sterilized in 12.5% wt/vol NaOCl solution for 5 min, rinsed in three changes of sterile water, and air dried in a laminar flow cabinet. A pathogenicity assay for PHR, as described by Akinsanmi and Drenth (2017), was used to inoculate each fruit pericarp by puncturing with a sterile needle at five points approximately 5 mm apart with a total of 20 µl conidial suspension. Three fruits were used as replicates for each treatment and fruits inoculated with sterile water served as negative controls. Each inoculated fruit was placed in a Petri dish and kept upright with sterile cotton wool. The Petri dishes were placed in plastic boxes lined with damp sterile paper towels to maintain high relative humidity (>90%) (Akinsanmi and Drenth, 2017). The boxes were incubated at room temperature at 20 ± 2 °C for 14 days and the trial was repeated. The percentage of surface area of each fruit with PHR lesions, relative to the total area, was recorded at14 days post-inoculation (Akinsanmi and Drenth, 2017).

2.6 Data analysis

The disease severity data were examined for homogeneity of variance, differences between repeats and their interactions using the generalized linear model procedure in IBM SPSS Statistics v. 25. Disease severity data of the repeated trials were combined because there was no significant variation between repeats. Variations in the lesion area between cultivars, the *Diaporthe* species,

isolates within each species, and their interactions were analysed using GLM procedure. Significant means were separated and compared using Tukey's HSD test in SPSS at $p < .05$.

3. Results

3.1 Phylogenetic analysis

Approximately 600 bp of the ITS, 695 bp of the *tef1α*, and 700 bp of the *tub2* gene regions were sequenced from the Australian and South African *Diaporthe* isolates (Table 1). After removing ambiguously aligned regions, the ITS, *tef1α*, and *tub2* alignments were trimmed to 548, 419, and 702 bp, respectively. The ITS phylogeny was able to resolve 53 out of 58 *Diaporthe* species, including three of the new taxa (data not shown). The *tef1α* phylogeny inferred three of the new species, and the *tub2* phylogeny resolved all four of the new species (data not shown). As the topologies of the single locus phylogenies for the tree data sets did not show any conflicts, they were analysed in a concatenated alignment. The concatenated alignment consisted of 82 sequences, including the outgroup *D. ocoteae* strain CBS 141330, of which 418 characters were parsimony-informative, 371 were variable, and 880 were constant. The phylogenetic tree inferred from the concatenated alignment resolved the 18 *Diaporthe* isolates from PHR used in this study into four well-supported and unique clades (Figure 1), which are described as novel species.

3.2 Taxonomy of novel *Diaporthe* species from PHR in macadamia

Diaporthe australiana R.G. Shivas, Akinsanmi and Y.P. Tan, sp. nov. — MycoBank MB 833827; Figure 2.

Etymology. In reference to the genus, *Macadamia*, which is indigenous to Australia.

Conidiomata on OMA pycnidial, aggregated in scattered groups up to 2.5 mm diameter, globose or irregular, up to 1 mm diameter, dark brown to black, without necks, whitish to pale yellow conidial drops exuded from central ostioles. *Conidiophores* with an irregularly polygonal basal cell from which 1 or 2 conidiogenous cells arise, hyaline, smooth, densely aggregated, 15–25 µm. *Conidiogenous cells* phialidic, hyaline, cylindrical, 10–20 × 1–2.5 µm, straight or flexuous. *Paraphyses* intermingled among conidiophores, hyaline, smooth, 1–3-septate, up to 70 µm long,

tapered, apex 1–2 µm wide. *Alpha conidia* hyaline, aseptate, fusiform, guttulate and acute at both ends, 5–8.5 × 1.5–2 µm. *Beta conidia* not observed.

Culture characteristics — Colonies on OMA and PDA after 7 days at 25 °C cover the entire surface with mycelium. Colony on OMA with abundant conidiomata; mycelium adpressed and ropey, white. Reverse off-white to pale brown.

Materials examined. AUSTRALIA, New South Wales, Lindendale, from husk rot of *Macadamia* sp., Feb. 2017, *O.A. Akinsanmi* (BRIP 66145 – holotype; includes culture ex-type); Alstonville, *ibid*, Jan. 2017, *ibid* (BRIP 66149); Lindendale, *ibid*, Feb. 2017, *ibid* (BRIP 66142); *ibid* (BRIP 66146); *ibid* (BRIP 66147); Queensland, Amamoor, *ibid*, 6 Jan. 2009, *ibid* (BRIP 64233); Gympie, *ibid*, 6 Jan. 2009, *ibid* (BRIP 64235); *ibid* (BRIP 64236); *ibid* (BRIP 64237); *ibid*, 25 Jan. 2009, (BRIP 64238); Kin Kin, *ibid*, 6 Jan. 2009, *ibid* (BRIP 64243).

Notes —*Diaporthe australiana* was placed in a clade that included *D. hongkongensis* and *D. lithocarpi* (nom. inval. Art. 41.1 Shenzhen Code) (Figure 1). *Diaporthe australiana* was distinguished from *D. hongkongensis* (97% in ITS, 97% in *tef1α*, and 94% in *tub2*), and from *D. lithocarpi* (96% in ITS, 94% in *tef1α*, and 92% in *tub2*). *Diaporthe australiana* has longer conidiogenous cells (10–20 versus 5–12 µm), more tapered paraphyses (1–2 versus 2–8 µm wide), and narrower alpha conidia (1.5–2 versus 2–3 µm) (Gomes *et al*., 2013) than *D. hongkongensis*. *Diaporthe australiana* is only known to occur in Australia.

Diaporthe drenthii Y.P. Tan, Akinsanmi and R.G. Shivas, sp. nov. — MycoBank MB 833828; Figure 3.

Etymology. Named after Professor Andre Drenth, an internationally renowned plant pathologist, in recognition of his significant contributions to the study of tropical crop diseases.

Conidiomata on OMA pycnidial, aggregated in scattered groups up to 1 mm diameter, globose or irregular, dark brown to black. *Conidiophores* hyaline, smooth, densely aggregated, 15–25 µm. *Conidiogenous cells* phialidic, hyaline, cylindrical, 10–20 × 1–2.5 µm, straight or flexuous. *Alpha conidia* hyaline, aseptate, fusiform, acute at both ends, 5.5–8.5 × 1.5–2 µm. *Beta conidia* sparse, curved 25–35 × 1 µm.

Culture characteristics — Colony on OMA after 7 days at 25 °C cover the entire surface with mycelium, felty, ochreous to umber. Colony on PDA 7 days at 25 °C reaching 7–8 cm diameter, dense and felty, margin adpressed, reverse centre citrine to olivaceous buff to pale luteous at margin.

Materials examined. SOUTH AFRICA, KwaZulu-Natal, Ramsgate, from husk rot of *Macadamia* sp., 28 Jan. 2016, *M.H. Schoeman* (BRIP 66524 — holotype, culture ex-type); *ibid* (BRIP 66519); *ibid* (BRIP 66521); *ibid* (BRIP 66523).

Notes — *Diaporthe drenthii* was placed in a clade that included *D. arecae*, *D. cercidis*, *D. pterocarpicola*, and *Diaporthe searlei* (described below) (Figure 1). *Diaporthe drenthii* was distinguished by *tub2* sequence similarity from *D. arecae* (95%), *D. cercidis* (95%), *D. pterocarpicola* (96%), and *Diaporthe searlei* (96%). *Diaporthe drenthii* is only known to occur in South Africa.

Diaporthe macadamiae Y.P. Tan, Akinsanmi and R.G. Shivas, sp. nov. — MycoBank MB 833829; Figure 4.

Etymology. Named after the host plant, *Macadamia*.

Conidiomata on OMA pycnidial, aggregated in scattered groups up to 3 mm diameter, globose or irregular, up to 1 mm diameter, dark brown to black, with short necks c.250 µm long, immersed in agar. *Conidiophores* densely aggregated, 15–25 µm. *Conidiogenous cells* phialidic, hyaline, cylindrical, 10–20 × 1–2.5 µm, straight or flexuous. *Alpha conidia* hyaline, aseptate, cylindrical, rounded at both ends, 5.5–9.5 × 1.5–2.5 µm, with conspicuous globose guttules. *Beta conidia* sparse, curved, 15–25 × 1 µm.

Culture characteristics — Colonies on OMA and PDA after 7 days at 25 °C cover the entire surface with mycelium. Colony on OMA white on surface, dense and felty. Reverse on OMA and PDA centre olivaceous to grey olivaceous.

Materials examined. SOUTH AFRICA, Mpumalanga, Nelspruit, from husk rot of *Macadamia* sp. cv. Nelmak, 23 Feb. 2016, M.H. Schoeman (BRIP 66526 — holotype, culture ex-type); *ibid* (BRIP 66525).

Notes — *Diaporthe macadamiae* is sister to a clade that contains 30 species, including the three novel species described in this study (Figure 1). *Diaporthe macadamiae* is distinguished by each of the three loci ITS, *tef1α*, and *tub2*. *Diaporthe macadamiae* is only known to occur in South Africa.

Diaporthe searlei R.G. Shivas, Akinsanmi and Y.P. Tan, sp. nov. — MycoBank MB 833830; Figure 5.

Etymology. Named after Dr. Chris Searle, an Australian horticulturalist, who has made many significant contributions to the Australian macadamia industry.

Conidiomata on OMA pycnidial, aggregated in scattered groups up to 3 mm diameter, globose or irregular, up to 1 mm diameter, dark brown to black, without necks. *Conidiophores* hyaline, smooth, densely aggregated, 15–45 µm. *Conidiogenous cells* phialidic, hyaline, cylindrical, 10–35 × 1–2.5 µm, straight or flexuous. *Alpha conidia* hyaline, aseptate, fusiform, acute at both ends, 5–9 × 1.5–2 µm.

Culture characteristics — Colonies on OMA and PDA after 7 days at 25 °C almost cover the entire surface with mycelium. Colony on PDA margin coralloid, adpressed, scattered stromata, grey olivaceous, reverse patches of pale luteous to ochreous. Colony on OMA with abundant conidiomata, adpressed, ropey. Reverse pale luteous to amber.

Material examined. SOUTH AFRICA, Mpumalanga, Nelspruit, from husk rot of *Macadamia* sp. cv. Nelmak, 23 Feb. 2016, *M.H. Schoeman* (BRIP 66528 — holotype, culture ex-type).

Notes — *Diaporthe searlei* was placed in a clade that included *D. arecae*, *D. cercidis*, *D. pterocarpicola*, and *Diaporthe drenthii* (Figure 1). *Diaporthe searlei* was distinguished by *tub2* sequence similarity from *D. arecae* (96%), *D. cercidis* (97%), *D. pterocarpicola* (98%), and *Diaporthe drenthii* (96%). *Diaporthe searlei* is only known to occur in South Africa.

3.3 Pathogenicity of Australian and South African *Diaporthe* isolates

Each of the four newly described species, *D. australiana*, *D. drenthii*, *D. macadamiae*, and *D. searlei*, caused PHR on macadamia cultivars HAES 344 and HAES 816. There were significant variations in disease severity between the two cultivars ($p < .0001$), among the four species ($p =$

.034), and among the isolates within species (p = .017). However, there was no significant species × cultivar interaction (p = .082) (Figure 6). The mean disease severity in HAES 816 was significantly higher than in HAES 344 for each of the *Diaporthe* species (Figure 6). The overall mean PHR severity (48%) of the cultivars inoculated with the Australian species, *D. australiana*, was significantly (p < .001) higher than each of the three South African species, *D. drenthii* (24%), *D. macadamiae* (24%), and *D. searlei* (8%).

4. Discussion

This study has identified and described four novel species of *Diaporthe* that cause PHR in macadamia. Phylogenetic analyses of the concatenated ITS, *tef1α*, and *tub2* gene loci supported the establishment of *D. australiana*, *D. drenthii*, *D. macadamiae*, and *D. searlei* as novel species. These findings support an earlier study that showed multiple *Diaporthe* species were responsible for PHR (Akinsanmi and Drenth, 2017). The four species were phylogenetically distinct and caused PHR in two macadamia cultivars. However, there were significant variations in their aggressiveness on the two cultivars.

Many studies on a range of economically important crops such as grapevine (Guarnaccia *et al*., 2018), sunflower (Thompson *et al*., 2011), soybean (Santos *et al*., 2011), and citrus (Guarnaccia and Crous, 2017), have shown that different *Diaporthe* species can cause the same disease symptoms. PHR of macadamia caused by *D. australiana*, *D. drenthii*, *D. macadamiae*, and *D. searlei*, is another example. The identification of multiple *Diaporthe* spp. capable of causing PHR poses questions as to their distribution across macadamia cultivars, orchards, regions, and continents. Further studies are needed to determine the factors that affect the distribution of these and possibly further undiscovered *Diaporthe* species in macadamia orchards.

Many new *Diaporthe* species have been described in recent years, based on multi-locus phylogenetic studies (Gao *et al*., 2017; Guarnaccia *et al*., 2018; Long *et al*., 2019). Unfortunately, it is rare that much new or useful biological information accompanies these newly described taxa. In this study, we have demonstrated that *D. australiana*, *D. drenthii*, *D. macadamiae*, and *D. searlei* can cause PHR in macadamia. However, very little else is known about these fungi, including for example, whether these species are associated with other plant diseases.

Surveys of macadamia orchards in Australia from 2000 to 2007 found that PHR incidence differed between orchards and cultivars (Akinsanmi and Drenth, 2017). In addition, *Diaporthe* species have been associated with phomopsis graft dieback and canker (Drenth *et al*., 2009) and as endophytes in leaves, woody parts, fruit, and roots in macadamia. Many pathogenic *Diaporthe* spp. are known to exist as both endophytes and latent pathogens (Gomes *et al*., 2013). However, there is no information on whether *D. australiana*, *D. drenthii*, *D. macadamiae*, and *D. searlei* have either saprobic, endophytic, or opportunistic/latent pathogenic capacities (Carroll, 1988; Schulz and Boyle, 2005). For instance, *D. phaseolorum* infects young soybean tissues but only causes disease symptoms in mature plants (Sun *et al*., 2012). The potential exists for endophytic *Diaporthe* spp. to cause PHR in a similar latent manner. Future research prospects need to elucidate the diversity and potential interplay of endophytic and pathogenic *Diaporthe* species in macadamia production systems.

Pathogenicity assays demonstrated that *D. australiana*, *D. drenthii*, *D. macadamiae*, and *D. searlei* can cause PHR in macadamia. We found significant variation in the aggressiveness of different species, and even within isolates from the same species on the two macadamia cultivars tested. Further studies are required to examine the virulence and varietal susceptibilities on major commercial cultivars and new macadamia germplasm in breeding programs. The fact that lesion areas caused by most of the isolates were similar at 14 days post-inoculation support the need for early control intervention at the onset of PHR symptoms. This study has improved our understanding of *Diaporthe* species associated with PHR in Australia and South Africa, which begs further ecological and epidemiological studies. For example, it is assumed that the infection process for PHR in the field is initiated by rain-splashed conidia that land onto wounded immature fruit (Akinsanmi and Drenth, 2017). The role of endophytic *Diaporthe* spp. in the pathosystem has not been studied, and this information is required for effective PHR control.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1 Phylogenetic tree inferred from a maximum-likelihood analysis of three combined genes (ITS, *tef1α*, and *tub2*). RAxML bootstrap values (bs) greater than 70% and Bayesian posterior probabilities (pp) greater than 0.8 are given at the nodes (bs/pp). The outgroup is *Diaporthe ocoteae* ex-type strain CBS 141330. Novel species are in bold.

Figure 2 *Diaporthe australiana* ex-type BRIP 66145. (a) Colony on oatmeal agar, (b) conidiophores and conidia, (c) paraphyses and conidia, (d) α-conidia. Scale bars: 1 cm (a), 10 µm (b–d).

Figure 3 *Diaporthe drenthii* ex-type BRIP 66524. (a) Colony on oatmeal agar, (b) conidiophores and conidia, (c) α and β conidia. Scale bars: 1 cm (a), 10 µm (b,c).

Figure 4 *Diaporthe macadamiae* ex-type BRIP 66526. (a) Colony on oatmeal agar, (b) conidiophores and conidia, (c) α-conidia. Scale bars: 1 cm (a), 10 μm (b–d).

Figure 5 *Diaporthe searlei* ex-type BRIP 66528. (a) Colony on oatmeal agar, (b) conidiophores and conidia, (c) α-conidia. Scale bars: 1 cm (a), 10 μm (b–d).

Figure 6 Mean phomopsis husk rot severity (square root-transformed percentage lesion area on fruit surface) caused by isolates of four *Diaporthe* species on two macadamia cultivars. Lines on the bars indicate standard error.

Table 1**:** PHR isolates obtained from macadamia in Australia and South Africa used in the phylogenetic study.

Species	**Strain** [1]	**Locality** [2]	**GenBank accessions** [3]		
			ITS	***tef1α***	***tub2***
Diaporthe australiana sp. nov.	BRIP 64233	Australia, QLD, Amamoor	KU985011		KU985024
	BRIP 64234	Australia, QLD, Kin Kin	KU985012		KU985025
	BRIP 64235	Australia, QLD, Gympie	KU985013		KU985026
	BRIP 64236	Australia, QLD, Gympie	KU985014		KU985027
	BRIP 64237	Australia, QLD, Gympie	KU985015		KU985028
	BRIP 64238	Australia, QLD, Gympie	KU985009		KU985022
	BRIP 66142	Australia, NSW, Lindendale	MN708221	MN696521	MN696529
	BRIP 66145 [T]	Australia, NSW, Lindendale	MN708222	MN696522	MN696530
	BRIP 66146	Australia, NSW, Lindendale	MN708223		MN696531
	BRIP 66147	Australia, NSW, Lindendale	MN708224	MN696522	MN696532
	BRIP 66149	Australia, NSW, Alstonville	MN708225	MN696524	MN696533
Diaporthe drenthii sp. nov.	BRIP 66519	South Africa, KwaZulu-Natal, Ramsgate	MN708226		MN696534
	BRIP 66521	South Africa, KwaZulu-Natal, Ramsgate	MN708227		MN696535
	BRIP 66523	South Africa, KwaZulu-Natal, Ramsgate	MN708228	MN696525	MN696536
	BRIP 66524 [T]	South Africa, KwaZulu-Natal, Ramsgate	MN708229	MN696526	MN696537
Diaporthe macadamiae sp. nov.	BRIP 66525	South Africa, Mpumalanga, Nelspruit		MN696527	MN696538
	BRIP 66526 [T]	South Africa, Mpumalanga, Nelspruit		MN696528	MN696539
Diaporthe searlei sp. nov.	BRIP 66528 [T]	South Africa, Mpumalanga, Nelspruit	MN708231		MN696540

[1] BRIP, Queensland Plant Pathology Herbarium, Brisbane, Queensland, Australia; CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands.
[2] NSW, New South Wales; QLD, Queensland.
[3] ITS, internal transcribed spacer region; *tef1α,* translation elongation factor 1-α; *tub*, β-tubulin.
[T] ex-type strain.

Table 2: *Diaporthe* species used in the phylogenetic analysis.

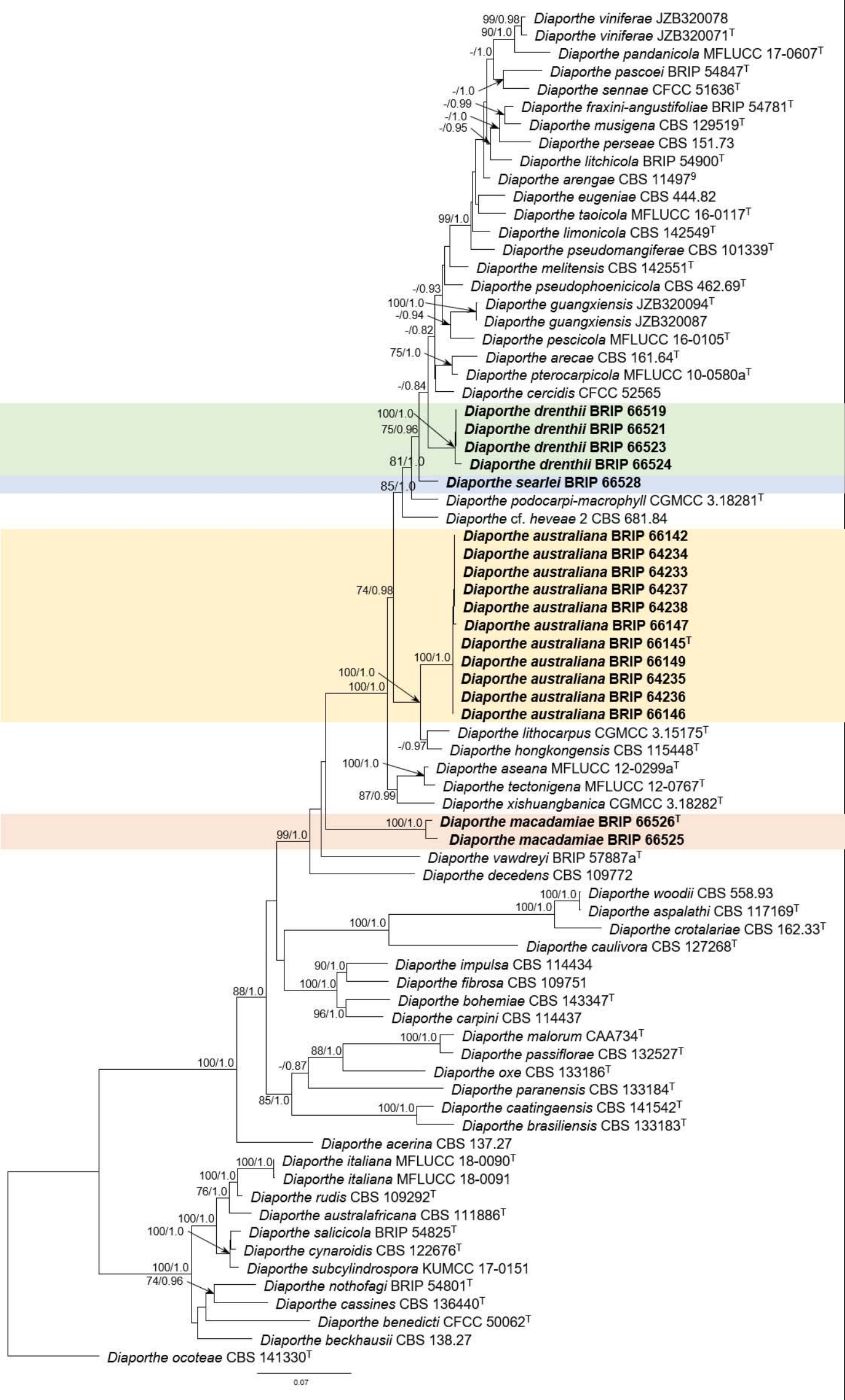
Species	**Strain** [1] [2]	**Host/substrate**	**Locality**	**GenBank accessions** [3]		
				ITS	***tef1α***	***tub2***
Diaporthe acerina	CBS 137.27	*Acer saccharum*	unknown	KC343006	KC343732	KC343974
Diaporthe arecae	CBS 161.64 [T]	*Areca catechu*	India	KC343032	KC343758	KC344000
Diaporthe arengae	CBS 114979 [T]	*Arenga engleri*	Hong Kong	KC343034	KC343760	KC344002
Diaporthe aseana	MFLUCC 12–0299a [T]	unknown dead leaf	Thailand	KT459414	KT459448	KT459432
Diaporthe aspalathi	CBS 117169 [T]	*Aspalathus linearis*	South Africa	KC343036	KC343762	KC344004
Diaporthe australafricana	CBS 111886 [T]	*Vitis Vinifera*	Australia	KC343038	KC343764	KC344006
Diaporthe beckhausii	CBS 138.27	*Viburnum* sp.	unknown	KC343041	KC343767	KC344009
Diaporthe benedicti	CFCC 50062 [T]	*Salix* sp.	USA	KP208847	KP208853	KP208855
Diaporthe bohemiae	CBS 143347 [T]	*Vitis* sp.	Czech Republic	MG281015	MG281536	MG281188
Diaporthe brasiliensis	CBS 133183 [T]	*Aspidosperma tomentosum*	Brazil	KC343042	KC343768	KC344010
Diaporthe caatingaensis	CBS 141542 [T]	*Tacinga inamoena*	Brazil	KY085927	KY115603	KY115600
Diaporthe carpini	CBS 114437	*Carpinus betulus*	Sweden	KC343044	KC343770	KC344012
Diaporthe cassines	CBS 136440 [T]	*Cassine peragua*	South Africa	KF777155	KF777244	
Diaporthe caulivora	CBS 127268 [T]	*Glycine max*	Croatia	KC343045	KC343771	KC344013
Diaporthe cercidis	CFCC 52565	*Cercis chinensis*	China	MH121500	MH121542	MH121582
Diaporthe cf. heveae 2	CBS 681.84	*Hevea brasiliensis*	India	KC343117	KC343843	KC344085
Diaporthe crotalariae	CBS 162.33 [T]	*Crotalaria spectabilis*	USA	KC343056	KC343782	KC344024
Diaporthe cynaroidis	CBS 122676 [T]	*Protea cynaroides*	South Africa	KC343058	KC343784	KC344026
Diaporthe decedens	CBS 109772	*Corylus avellana*	Austria	KC343059	KC343785	KC344027
Diaporthe eugeniae	CBS 444.82	*Eugenia aromatica*	Indonesia	KC343098	KC343824	KC344066
Diaporthe fibrosa	CBS 109751	*Rhamnus cathartica*	Austria	KC343099	KC343825	KC344067
Diaporthe fraxini-angustifoliae	BRIP 54781 [T]	*Fraxinus angustifolia*	Australia	JX862528	JX852534	KF170920
Diaporthe guangxiensis	JZB320087	*Vitis vinifera*	China	MK335765	MK523560	MK500161
	JZB320094 [T]	*Vitis vinifera*	China	MK335772	MK523566	MK500168
Diaporthe hongkongensis	CBS 115448 [T]	*Dichroa febrifuga*	Hong Kong	KC343119	KC343845	KC344087
Diaporthe impulsa	CBS 114434	*Sorbus aucuparia*	Sweden	KC343121	KC343847	KC344089
Diaporthe italiana	MFLUCC 18-0090 [T]	*Morus alba*	Italy	MH846237	MH853686	MH853688
	MFLUCC 18-0091	*Morus alba*	Italy	MH846238	MH853687	MH853689
Diaporthe limonicola	CBS 142549 [T]	*Citrus limon*	Malta	MF418422	MF418501	MF418582
Diaporthe litchicola	BRIP 54900 [T]	*Litchi chinensis*	Australia	JX862533	JX862539	KF170925
Diaporthe lithocarpus	CGMCC 3.15175 [T]	*Lithocarpus glabra*	China	KC153104	KC153095	KF576311
Diaporthe malorum	CAA734 [T]	*Malus domestica*	*Portugal*	*KY435638*	*KY435627*	*KY435668*
Diaporthe melitensis	CBS 142551 [T]	*Citrus limon*	Malta	MF418424	MF418503	MF418584

Species	Strain [1,2]	Host/substrate	Locality	GenBank accessions [3]		
				ITS	*tef1α*	*tub2*
Diaporthe musigena	CBS 129519 [T]	*Musa* sp.	Australia	KC343143	KC343869	KC344111
Diaporthe nothofagi	BRIP 54801 [T]	*Nothofagus cunninghamii*	Australia	JX862530	JX862536	KF170922
Diaporthe ocoteae	CBS 141330 [T]	*Ocotea obtusata*	La Réunion	KX228293		KX228388
Diaporthe oxe	CBS 133186 [T]	*Maytenus ilicifolia*	Brazil	KC343164	KC343890	KC344132
Diaporthe pandanicola	MFLUCC 17-0607 [T]	*Pandanus* sp.	Thailand	MG646974		MG646930
Diaporthe paranensis	CBS 133184 [T]	*Maytenus ilicifolia*	Brazil	KC343171	KC343897	KC344139
Diaporthe pascoei	BRIP 54847 [T]	*Persea americana*	Australia	JX862532	JX862538	KF170924
Diaporthe passiflorae	CBS 132527 [T]	*Passiflora edulis*	South America	JX069860	KY435633	KY435674
Diaporthe perseae	CBS 151.73	*Persea gratissima*	Netherlands Antilles	KC343173	KC343899	KC344141
Diaporthe pescicola	MFLUCC 16-0105 [T]	*Prunus persica*	China	KU557555	KU557623	KU557579
Diaporthe podocarpi-macrophyll	CGMCC 3.18281 [T]	*Podocarpus macrophyllus*	Japan	KX986774	KX999167	KX999207
Diaporthe pseudomangiferae	CBS 101339 [T]	*Mangifera indica*	Dominican Republic	KC343181	KC343907	KC344149
Diaporthe pseudophoenicicola	CBS 462.69 [T]	*Phoenix dactylifera*	Spain	KC343184	KC343910	KC344152
Diaporthe pterocarpicola	MFLUCC 10-0580a [T]	*Pterocarpus indicus*	Thailand	JQ619887	JX275403	JX275441
Diaporthe rudis	CBS 109292 [T]	*Laburnum anagyroides*	Austria	KC343234	KC343960	KC344202
Diaporthe salicicola	BRIP 54825 [T]	*Salix purpurea*	Australia	JX862531	JX862537	KF170923
Diaporthe sennae	CFCC 51636 [T]	*Senna bicapsularis*	China	KY203724	KY228885	KY228891
Diaporthe subcylindrospora	KUMCC 17-0151	*Salix* sp.	China	MG746629	MG746630	MG746631
Diaporthe taoicola	MFLUCC 16-0117 [T]	*Prunus persica*	China	KU557567	KU557635	KU557591
Diaporthe tectonigena	MFLUCC 12-0767 [T]	*Tectona grandis*	China	KU712429	KU749371	KU743976
Diaporthe vawdreyi	BRIP 57887a [T]	*Psidium guajava*	Australia	KR936126	KR936129	KR936128
Diaporthe viniferae	JZB320071 [T]	*Vitis vinifera*	China	MK341551	MK500107	MK500119
	JZB320078	*Vitis vinifera*	China	MK341555	MK500110	MK500124
Diaporthe woodii	CBS 558.93	*Lupinus* sp.	Australia	KC343244	KC343970	KC344212
Diaporthe xishuangbanica	CGMCC 3.18282 [T]	*Camellia sinensis*	China	KX986783	KX999175	KX999216

[1] BRIP, Queensland Plant Pathology Herbarium, Brisbane, Queensland, Australia; CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CFCC, China Forestry Culture Collection Center, Beijing, China; CGMCC, China General Microbiological Culture Collection, Beijing, China; JZB, culture collection of the Institute of Plant and Environment Protect of Beijing Academy of Agriculture and Forestry Sciences, Beijing, China; KUMCC, culture collection of Kunming Institute of Botany, Kunming, China; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; RBG, Royal Botanic Gardens Sydney, Sydney, New South Wales, Australia; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA.

[2] [T], ex-type strain.

[3] ITS, internal transcribed spacer region; *tef1α,* translation elongation factor 1-α; *tub2*, β-tubulin.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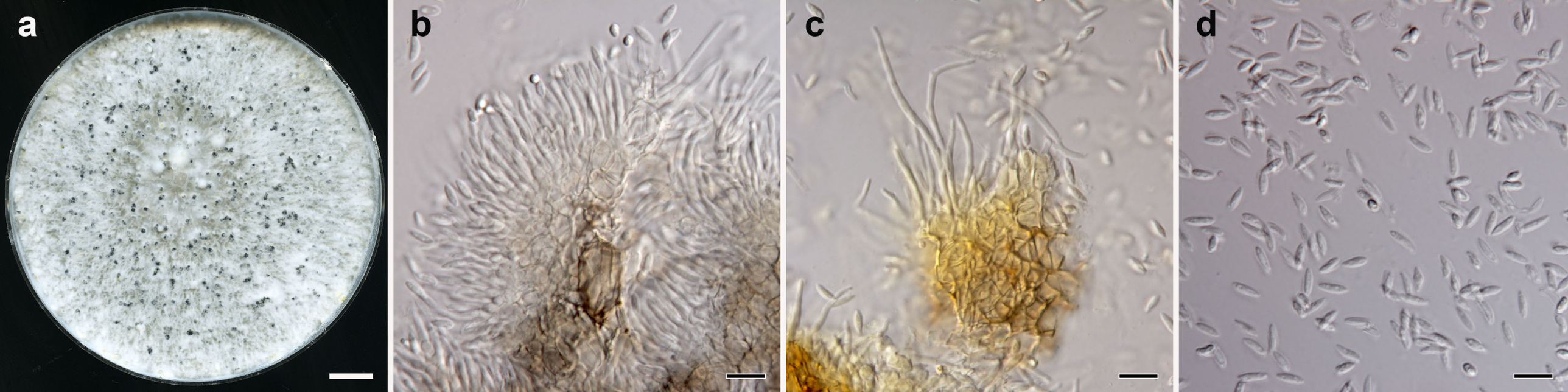
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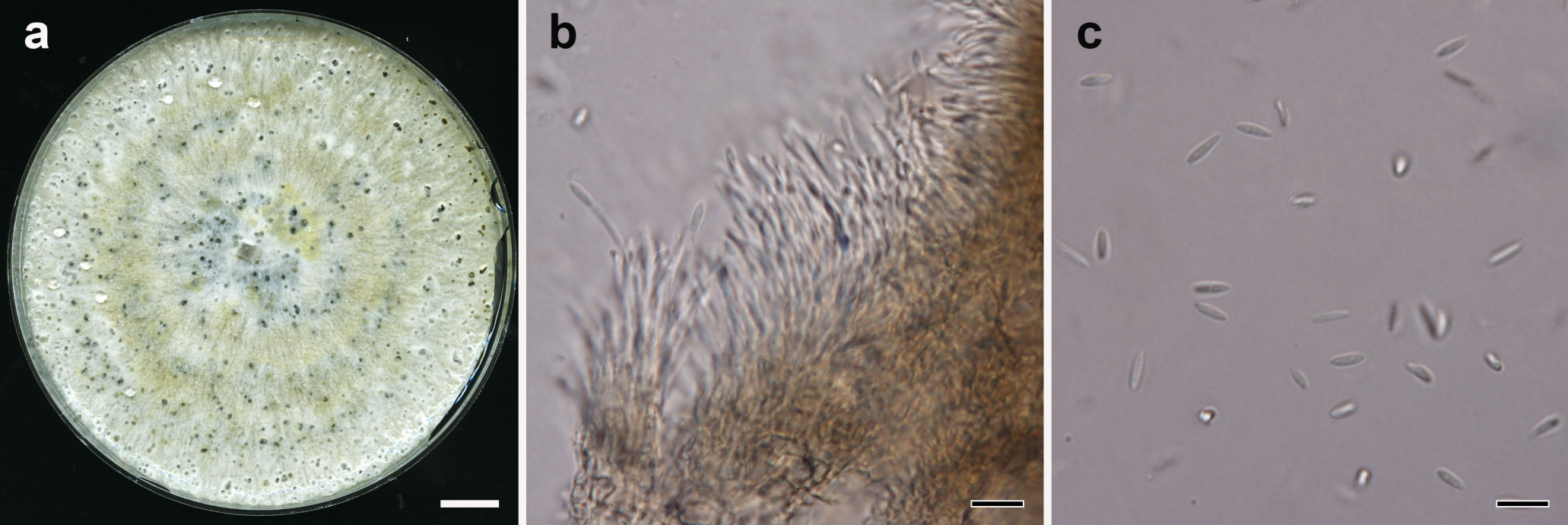
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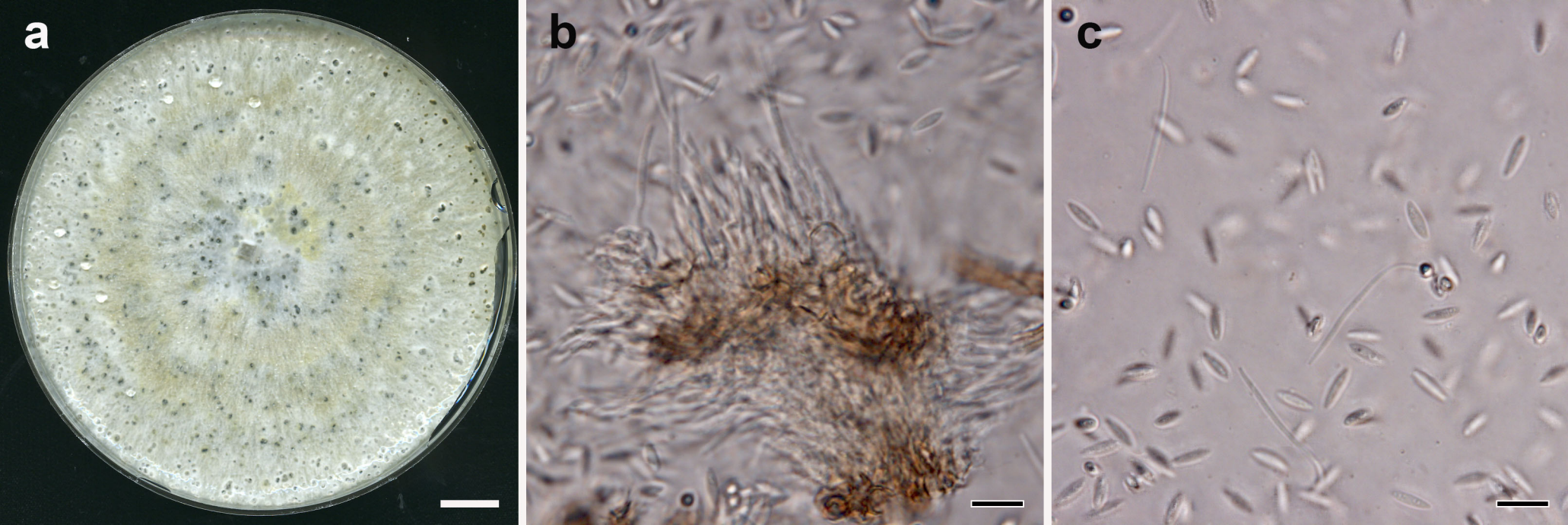
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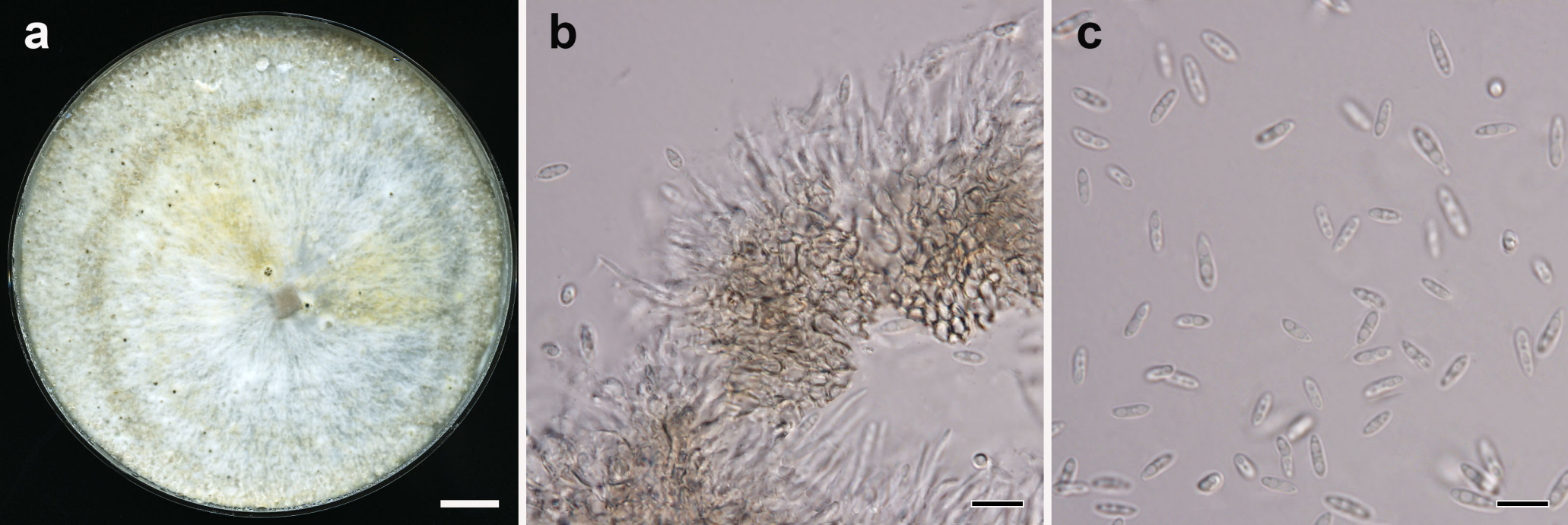
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