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Botryosphaeriaceae partially overlap on asymptomatic and symptomatic tissues of *Anacardiaceae* in agroecosystems and conservation areas in northern South Africa

B. Slippers[1], E. Ramabulana[2], M.P.A. Coetzee[1]

[1]DSI-NRF Centre of Excellence in Plant Health Biotechnology (CPHB), Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa
[2]Department of Plant and Soil Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa

*Corresponding author: bernard.slippers@fabi.up.ac.za

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Abstract: Members of the *Botryosphaeriaceae* are well-known endophytes and stress-related pathogens. We recently characterised the diversity of *Botryosphaeriaceae* in healthy tissues of three tree species in the *Anacardiaceae*, namely *Sclerocarya birrea*, *Mangifera indica* and *Lannea schweinfurthii*. Here we ask how that diversity compares with the *Botryosphaeriaceae* diversity associated with dieback on those tree species. Samples were collected from agroecosystems (Tshikundamalema and Tshipise in Limpopo) and conservation areas (Nwanedi and the Mapungubwe National Park in Limpopo and the Kruger National Park in Mpumalanga) ecosystems. Species were characterised using multigene sequence data and morphological data. *Diplodia allocellula*, *Dothiorella brevicollis*, *Do. viticola*, *Lasiodiplodia crassispora*, *L. mahajangana* and *Neofusicoccum parvum* occurred on both asymptomatic and symptomatic samples. *Dothiorella dulcispinea*, *L. gonubiensis* and *L. exigua*, as well as a previously unknown species described here as *Oblongocollomyces ednahkunjekuae sp. nov*, only occurred in asymptomatic branches. An interesting aspect of the biology of *O. ednahkunjekuaeae* is that it appears to be adapted to higher temperatures, with an optimum growth at 30 °C, and faster growth at 35 °C than at 25 °C. *Lasiodiplodia pseudotheobromae* only occurred in symptomatic branches. *Neofusicoccum parvum* was notably absent from conservation areas, and in agroecosystem it was most common on *M. indica*. Only *L. crassispora* and *L. mahajangana* overlapped on all three tree species and were the dominant species associated with dieback. These results show that not all *Botryosphaeriaceae* occurring asymptomatically in an area contribute equally to disease development on a related group of hosts, and that environmental disturbance plays a significant role in the distribution of *N. parvum*.

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INTRODUCTION

Species of *Botryosphaeriaceae* have been implicated in the decline of numerous woody tree species globally (Golzar & Burgess 2011, Panzavolta *et al.* 2017, Soltaninejad *et al.* 2017, Mahamedi *et al.* 2020). Species of *Botryosphaeriaceae* occur as endophytes and as pathogens on various hosts (Yang *et al.* 2017, Batista *et al.* 2021). In the endophytic phase, the *Botryosphaeriaceae* can remain latent in healthy plant tissues without causing disease symptoms. However, they can cause disease on physically stressed trees caused by injury or harsh environmental conditions (Slippers & Wingfield 2007, Mayorquin *et al.* 2012). *Botryosphaeriaceae*-related diseases manifest as dieback, canker, gummosis and fruit rots (Mehl *et al.* 2013, Adesemoye *et al.* 2014, Netto *et al.* 2014, Valencia *et al.* 2019).

Many species in the *Botryosphaeriaceae* are known to occur in both asymptomatic and symptomatic tissues on numerous hosts worldwide. Examples include *Dothiorella viticola* on *Vachellia karroo* and *Diplodia alatafructa* on *Pterocarpus angolensis* in South Africa (Mehl *et al.* 2011, Jami *et al.* 2013), *Lasiodiplodia margaritaceae* on *Adansonia gregorii* in Australia (Pavlic *et al.* 2008, Sakalidis *et al.* 2011a), *Botryosphaeria mamane* on *Eucalyptus* spp. and *Vachellia mangium* in Venezuela (Mohali *et al.* 2007), among many others. Because of this overlap in their occurrence in asymptomatic and symptomatic tissue, species of *Botryosphaeriaceae* are recognised as opportunistic pathogens with a latent endophytic phase, that can easily spread in healthy plant tissues when plants or plant parts are transported (Mohali *et al.* 2007, Slippers & Wingfield 2007, Jami *et al.* 2013).

Dieback is one of the most commonly reported symptoms associated with infection by species of *Botryosphaeriaceae* (Mehl *et al.* 2013, Lawrence *et al.* 2017, Machado *et al.* 2019). Dieback caused by species of *Botryosphaeriaceae* is characterised by dry twigs and branches, giving the tree an appearance of fire scorch. The disease has been linked to various species in the family, such as *B. dothidea*, *L. theobromae* and *N. parvum* on

Vitis vinifera (Qiu *et al.* 2011, Úrbez-Torres *et al.* 2011, Yan *et al.* 2013, Stempien *et al.* 2017), *Fusicoccum aesculi*, *N. parvum*, *L. crassispora*, *L. iraniensis*, *L. pseudotheobromae*, *L. mahajangana* and *L. theobromae* on *Mangifera indica* (de Oliveira Costa *et al.* 2010, Ismail *et al.* 2012, Marques *et al.* 2013, Trakunyingcharoen *et al.* 2014), *L. mahajangana* and *L. theobromae* on *Euphorbia ingens* (van der Linde *et al.* 2011) and *N. protearum* on *Protea* species (Denman *et al.* 2003, Jami *et al.* 2017), amongst others.

It has been hypothesised that endophytic *Botryosphaeriaceae* are associated with dieback symptoms that have been observed on woody tree species in South Africa (Jami *et al.* 2013). Testing this hypothesis is hampered by the fact that most studies do not sample from both healthy and diseased tissue in a systematic manner. A recent ecological study reported on *Botryosphaeriaceae* associated with asymptomatic samples of three species in the *Anacardiaceae* in South Africa, namely *Sclerocarya birrea*, *Mangifera indica* and *Lannea schweinfurthii* (Ramabulana *et al*. 2022). This study provided the opportunity to compare the overlap in species between symptomatic and asymptomatic tissues on these hosts. We do this in the same agroecosystems and conservation areas in the Limpopo and Mpumalanga provinces of South Africa as Ramabulana *et al*. (2022). These tree species are important agricultural commodities used as raw material in the cosmetic industry, as traditional medicine and as a source of extra income by surrounding rural communities.

MATERIALS AND METHODS

Sample collection, fungal isolation and morpholopical characterisation

Asymptomatic and symptomatic branches (20–30 cm long and 2–5 mm thick) were collected from *S. birrea, M. indica* and *L. schweinfurthii* trees in agroecosystems and conservation areas in the Limpopo and Mpumalanga provinces in 2017 and 2018 (see Ramabulana *et al*. 2022 for a description of collection sites). Two sites in agroecosystems were located at Tshikundamalema and Tshipise in the Limpopo Province, and three sites in conservation areas were found at Nwanedi and the Mapungubwe National Park in Limpopo and the Kruger National Park in Mpumalanga. A single branch showing symptoms of dieback and an asymptomatic branch were collected from each tree and transported to the laboratory for fungal isolations. Isolates from asymptomatic branches were collected from 404 *Anacardiaceae* trees by Ramabulana *et al*. (2022). Here, we analyse isolates from symptomatic branches from 398 of these trees and compare them with the isolates from the asymptomatic branches. The symptomatic branches are from trees at the same sites as those sampled for asymptomatic branches by Ramabulana *et al*. (2022), but not necessarily from the same trees.

Symptomatic branches were surface disinfected in 10 % hydrogen peroxide, rinsed twice in sterile distilled water and de-barked to expose the interface between necrotic and healthy tissue. Small pieces (1–1.5 mm) were isolated from the border zone of necrotic and healthy wood and plated onto 2 % MEA with 0.1 g Streptomycin. Primary isolates from both asymptomatic and symptomatic isolations were incubated at 25 °C and checked regularly for fungal growth. Cultures typical of the *Botryosphaeriaceae* (fast growth, grey-black aerial mycelium) were selected and purified by transferring single hyphal tips onto fresh 2 % MEA. For taxonomic description, selected isolates were grown on Vogel's Minimal Medium (VMM) at 25 °C under constant white light, as described in Oostlander *et al*. (2023). Culture morphology and growth rate for a putatively new species were evaluated on 2 % Potato Dextrose Agar (PDA) at incubation temperatures from 10–35 °C, with increments of 5 °C. Colony colour was evaluated based on Kornerup & Wanscher (1967).

Isolates from symptomatic tissue representing different sites and hosts were grouped based on culture morphology. Twelve isolates were selected as representatives and subjected to DNA extraction for preliminary identification (Suppl. Table S1). Isolates used in this study are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

DNA extraction, amplification and sequencing

Genomic DNA was extracted following the protocol published by Möller *et al.* (1992). DNA was extracted from the mycelium of 7-d-old cultures made from single hyphal tips. DNA concentrations were quantified using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) and adjusted to a working concentration of 50 ng/µL using SABAX water (SABAX; Adcock Ingram, Bryanston, S.A). DNA was stored at -20 °C until further use.

The internal transcribed spacer (ITS) and regions of the translation elongation factor (*tef-1α*), β-tubulin (*β-tub*) and RNA polymerase II second largest subunit (*rpb2*) genes were amplified and sequenced for all the representative isolates. The ITS region, which includes the ITS-1 spacer, 5.8S and ITS-2 spacer, was sequenced using primer pairs ITS-1 & ITS-4 (White *et al.* 1990), the *tef-1α* gene region was sequenced using primer pairs EF1-728F & EF1-986R (Carbone & Kohn 1999), the *β-tub* gene region was sequenced using primers Bt2a & Bt2b (Glass & Donaldson 1995) and the *rpb2* gene region was sequenced using primers rpb2-LasF & rpb2-LasR (Cruywagen *et al.* 2017) and RPB2bot6F & RPB2bot7R (Sakalidis *et al.* 2011b).

Polymerase chain reactions (PCR) were performed in a final volume of 25 µL, containing ~ 40–50 ng genomic DNA, 0.2 µM of each primer, 0.5 U of MyTaq™ DNA polymerase (Bioline, London, UK), 5 µL MyTaq PCR reaction buffer (10 mM Tris-HCL [pH 8.3], 3.0 mM $MgCl_2$, 50 mM KCl, Roche Diagnostics, Mannheim, Germany) and 16.5 µL sterile SABAX water (SABAX; Adcock Ingram, Bryanston, S.A). Amplification reactions were carried out in a thermal cycler (C1000, Bio-Rad, USA) using the following conditions; initial denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C (ITS and *rpb2*) and 56 °C (*tef-1α* and *β-tub*) for 30 s, extension at 72 °C for 1 min and final extension at 72 °C for 7 min. The resulting amplicons were separated by electrophoresis on a 1 % agarose gel stained with GelRed and purified using Exosap (Mixture of Exonuclease I and FastAP Alkaline Phosphatase) (Thermo Fisher Scientific Inc. Waltham, MA, USA) following the manufacturer's instructions.

Sequencing reactions were conducted with the ABI Prism® Big Dye™ cycle sequencing kit following the protocol outlined by the manufacturer (Applied Biosystems, Foster City, CA, USA). Sequencing of the amplicons was conducted at the sequencing facility of the University of Pretoria, South Africa. Sequence reads were obtained in both directions (forward and reverse) using the same primers to amplify the respective gene regions. Consensus

Fig. 1. *Mangifera indica* trees with severe dieback surrounded by healthy *M. indica* trees at Tshipise.

sequences from the forward and reverse sequence reads were generated using CLC Main Workbench v. 7.9 (QIAGEN, Aarhus, Denmark).

Phylogenetic analyses and identification of isolates

ITS, *tef-1α*, *β-tub* and *rpb2* sequences generated in this study were identified by comparing them with sequences of previously published species on GenBank (http://www.ncbi.nlm.nih.gov/genbank) using BLASTn searches. Reference sequences showing similarity to query sequences on BLASTn searches were retrieved from GenBank (Suppl. Table S2).

Eighteen isolates obtained from asymptomatic branches were selected based on their host association and grouping in the phylogenetic trees presented in Ramabulana *et al*. (2022) (Suppl. Table S1). Sequences from these isolates together with 12 selected isolates from symptomatic branches and reference sequences from GenBank were aligned using the online version of MAFFT v. 7 (Katoh *et al.* 2019). Manual adjustments of sequence alignment were carried out using BioEdit Sequence Alignment Editor v. 7.2.5 (Hall 1999). Sequence datasets for the four loci were analysed individually and in combination.

Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian (BI) methods. The ML phylogenetic analyses were performed using raxmlGUI v. 2.0 (Edler *et al.* 2021), with the nucleotide substitution models HKY+G+I, selected for the ITS, and TN93+G for the *tef-1α*, *β-tub, rpb2*, respectively, using jModelTest v. 2.1.7 (Darriba *et al.* 2012) within raxmlGUI. A TN93+G+I model was applied to the combined dataset in raxmlGUI, but the data was partitioned, allowing model parameters to vary among the partitions. Bootstrap analyses were done using 1 000 replicates to determine the robustness of the trees.

Bayesian inference (BI) of phylogenetic trees was performed using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003). Four simultaneous Markov chains were run for 5 × 10^6 generations and trees were sampled every 100th generation. The first 25 % of the trees, representing the burn-in phase of the analyses, were discarded and the remaining trees were used to calculate posterior probabilities (PP) based on a majority rule consensus tree. Effective sampling size (ESS) values were assessed using Tracer v. 1.7.1 (http://tree.bio.ed.ac.uk/software/tracer/). Trees were viewed in FigTree v. 1.3.1 (Rambaut 2009). The trees were rooted to sequences of *Melanops tulasnei* (*Botryosphaeriales*; *Melanopsaceae*) as the outgroup taxa.

All the isolates from asymptomatic and symptomatic tissue not included in the phylogenetic analyses were assigned to a species based on their grouping within morphogroups and the identity of the representative isolates included in the phylogenetic analyses. Isolates that looked different from other isolates in the morphogroups were also sequenced to confirm their identity.

RESULTS

Sample collection and tree health status

Most trees with symptoms of dieback did not appear to be seriously affected, except *M. indica* trees at Tshikundamalema and Tshipise which showed severe symptoms of dieback (Fig. 1). It is not clear whether the dieback and severe decline symptoms on *M. indica* trees are only due to infection by species of the *Botryosphaeriaceae* or other factors such as nutrient deficiency and unfavourable abiotic conditions.

Fungal isolates

A total of 573 isolates with typical culture morphology and colour characteristics of the *Botryosphaeriaceae* were obtained from asymptomatic and symptomatic samples. Isolations from asymptomatic samples yielded 402 isolates (see Ramabulana *et al*. 2022), while isolations from symptomatic samples yielded 171 isolates. Most isolates from both asymptomatic and symptomatic samples were obtained from *S. birrea*, followed by *L. schweinfurthii* and then *M. indica* (Table 1).

DNA extraction, amplification and sequencing

DNA amplification and sequencing were successful for the ITS, *tef-1α* and *β-tub* loci of all isolates. The *rpb*2 gene region could not be amplified for some isolates. BLASTn analyses of the sequences showed the highest similarity to species of *Botryosphaeriaceae.*

Phylogenetic analyses and identification of isolates

Alignment of sequences yielded 584, 537, 451, 537 and 2 109 bp for the ITS, *tef-1α*, *β-tub*, *rpb2* and combined dataset, respectively. The topology of the trees from ML and BI analyses of the ITS, *tef-1α*, *β-tub*, and *rpb2*, as well as the combined datasets (Fig. 2; Suppl. Figs S1–S4), were generally similar in the separation of clades representing species identified. Isolates included in phylogenetic analyses were separated into five main clades corresponding to five genera of *Botryosphaeriaceae*; *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neofusicoccum* and *Oblongocollomyces*.

Based on the phylogenetic concordance of the five datasets, the isolates obtained from symptomatic tissue were identified as *D. allocellula*, *Do. brevicollis*, *Do. viticola*, *L. crassispora*, *L. mahajangana*, *L. pseudotheobromae*, and *N. parvum* (Figs 2, 3; Suppl. Figs S1–S4). With the exception of *L. pseudotheobromae*, these species, together with *Do. dulcispinae*, *L. exigua*, *L. gonubiensis* and an *Oblongocollomyces* species (described below) are known from asymptomatic tissue (Ramabulana *et al*. 2022) (Figs 2, 3; Suppl. Figs S1–S4).

Table 1. Number of *Botryosphaeriaceae* isolates obtained from asymptomatic and symptomatic branches of *Sclerocarya birrea*, *Lannea schweinfurthii* and *Mangifera indica*.

Host	Isolates from asymptomatic tissue	Isolates from symptomatic tissue	Total
L. schweinfurtii	112	65	177
M. indica	100	35	135
S. birrea	190	71	261
Total	402	171	573

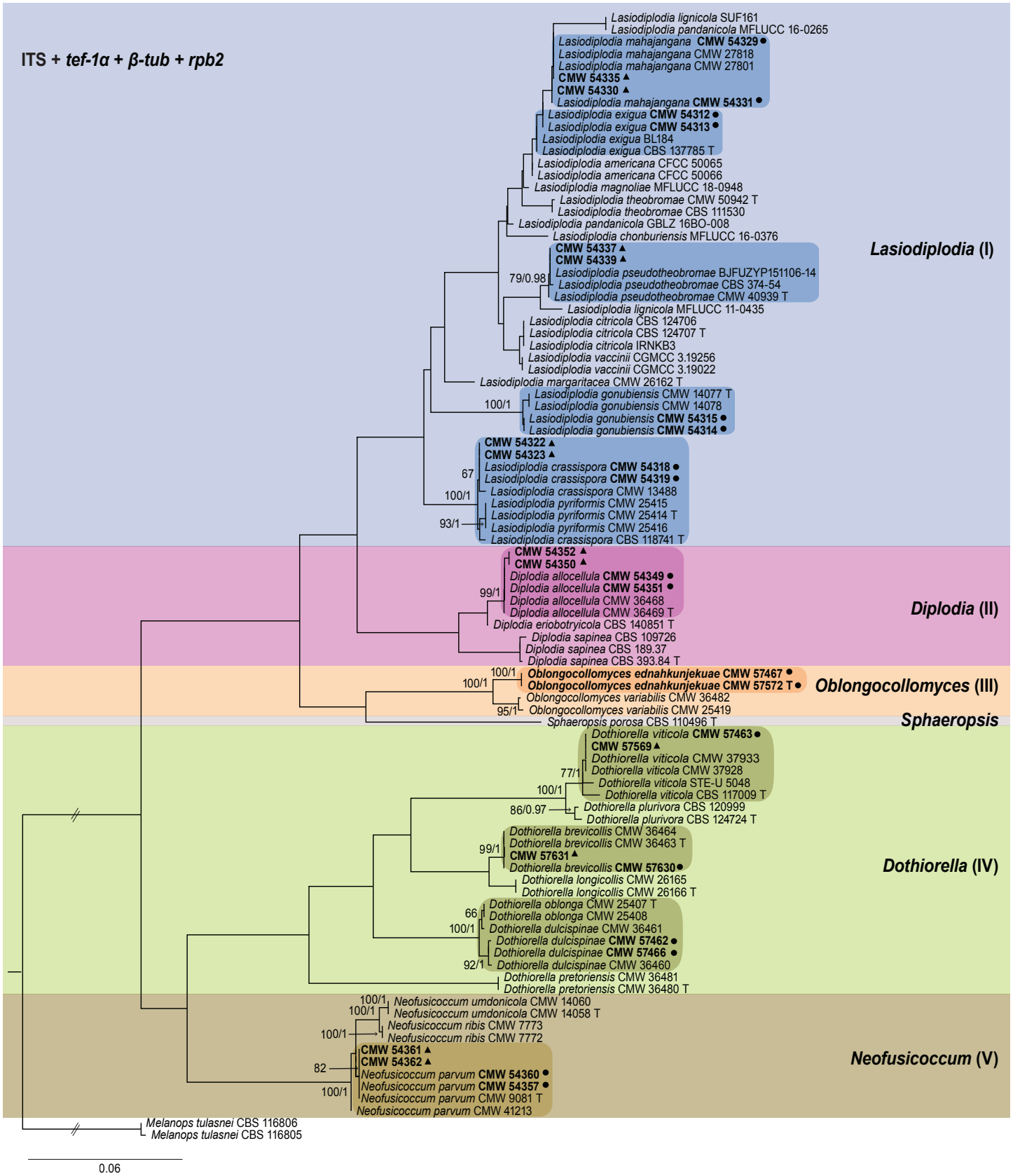

Fig. 2. Maximum likelihood phylogenetic tree of previously described *Botryosphaeriaceae* species and isolates from tree species of *Anacardiaceae* based on combined partial ITS, *tef-1α*, *β-tub* and *rpb2* gene regions. Isolates from asymptomatic branches are indicated with (●) and those from symptomatic branches are indicated with (▲). Bootstrap support (> 60 %) and PP values (≥ 0.95) are shown on the nodes. The tree was rooted to *Melanops tulasnei.* Isolates with only strain numbers were isolated in this study.

Species diversity and co-occurrence on asymptomatic and symptomatic tissues

Eleven species of *Botryosphaeriaceae* (*D. allocellula*, *Do. brevicollis*, *Do. dulcispinae*, *Do. viticola*, *L. crassispora*, *L. exigua*, *L. gonubiensis*, *L. mahajangana*, *L. pseudotheobromae*, *N. parvum* and *O. ednahkunjekuaeae*) were identified as endophytes and as potential pathogens on the three tree species of *Anacardiaceae* in the Limpopo and Mpumalanga provinces.

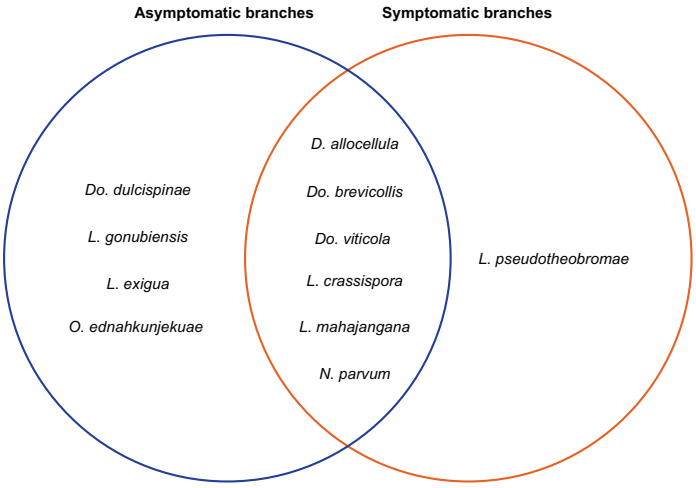

Fig. 3. Venn diagram showing species of *Botryosphaeriaceae* occurring in both asymptomatic and symptomatic tree species of *Anacardiaceae.*

Six species, namely *D. allocellula*, *Do. brevicollis*, *Do. viticola*, *L. crassispora*, *L. mahajangana* and *N. parvum* occurred in both asymptomatic and symptomatic branches of the three tree species (Fig. 3). Four species, *Do. dulcispinae*, *L. exigua*, *L. gonubiensis* and *O. ednahkunjekuae* were exclusive to asymptomatic branches. It is noteworthy that *L. pseudotheobromae* was the only species unique to symptomatic branches.

There was variation in the number of species identified as endophytes and as pathogens on the three tree species sampled (Fig. 5). Eleven species were identified on *S. birrea* trees, but they differed in the disease status of the tissues from which they were isolated. *Dothiorella dulcispinae*, *L. exigua*, *L. gonubiensis*, *O. ednahkunjekuaeae* occurred in asymptomatic branches only, *D. allocellula*, *Do. brevicollis*, *Do. viticola*, *L. crassispora*, *L. mahajangana* and *N. parvum* occurred in both asymptomatic and symptomatic branches, and *L. pseudotheobromae* occurred in symptomatic branches only. Five species, *D. allocellula*, *Do. brevicollis*, *L. crassispora*, *L mahajangana* and *L. pseudotheobromae* were isolated from *L. schweinfurthii. Diplodia allocellula*, *Do. brevicollis* and *L. crassispora* occurred in both asymptomatic and symptomatic branches, *L. mahajangana* occurred in asymptomatic branches only and *L. pseudotheobromae* occurred in symptomatic branches. *Lasiodiplodia crassispora*, *L. mahajangana* and *N. parvum* occurred in both asymptomatic and symptomatic branches of *M. indica*.

Species diversity and dominance of the *Botryosphaeriaceae* occurring in both asymptomatic and symptomatic branches of the same tree species varied across the different sampling sites in agroecosystems and conservation areas. In the absence of more replicates of these two contrasting types of sites, conclusions from results are tentative, but there were some notable differences that would be worth future study. For example, only three species occurred in agroecosystems, namely *L. mahajangana*, *L. crassispora* and *N. parvum*. It is notable that *N. parvum* only occurred in agroecosystem sites, where it was fairly commonly isolated, especially from *M. indica*. All species, except *N. parvum*, occurred in conservation areas.

Taxonomy

Oblongocollomyces ednahkunjekuae Slippers, Ramabulana & M.P.A. Coetzee, ***sp. nov.*** MycoBank MB 853978. Fig. 4.

Etymology: Named after Prof. Ednah Kunjeku, who has played an instrumental role in training postgraduate students at the University of Venda and enabling their sampling in unique habitats in the northern parts of South Africa.

Asexual morph: *Conidiomata* pycnidial, globose to subglobose, rarely with a short neck, superficial, immersed or semi-immersed, scattered on the medium, rarely in clusters; wall of 3–6 layers of brown *textura angularis*. *Paraphyses* intermingled among conidiogenous cells, hyaline, smooth, flexuous, aseptate, rarely branched, with obtuse apices. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, holoblastic, cylindrical to ampulliform, proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently. *Conidia* hyaline to honey coloured to brown, 0–1-septate, smooth, hilum truncate, moderately thick-walled, ellipsoidal to obtuse; 18.8–25.5 × 11–14 µm (av. 22.1 × 12.5 µm) in size.

Culture characteristics: Mycelium on PDA at 25 °C was fluffy with abundant aerial mycelium, appearing white to grey in the centre (4A1–C1). Reverse greyish beige to light olive brown (4B4–D5), sometimes darkening to a light blue-grey from the centre (4B1–D1). Cultures grown at 30 or 35 °C developed pale red to pink rose (11A3–A5) patches around the edges, and appeared greyish-ruby from the bottom (12C3–D4).

Growth rate: The optimal growth rate was on PDA at 30 °C (6.3 cm/d), followed by 35 °C (5.3 cm/d) and 25 °C (5 cm/d). Growth rapidly decreased below these temperatures to 2.3 cm/d at 20 °C, 8 mm/d at 15 °C and less than 1 mm/d at 10 °C.

Typus: **South Africa**, Mapungubwe National Park, on *Sclerocarya birrea*, 2018, *E. Ramabulana* (**holotype** PRU(M) 4586, preserved as dried culture; culture ex-holotype CMW 57572).

Additional material examined: **South Africa**, Mapungubwe National Park, on *Sclerocarya birrea*, 2018, *E. Ramabulana* (culture CMW 57467).

Notes: Sporulation was induced on VMM using the methods described in Oostlander *et al*. 2023. The dried, sporulating culture serves as holotype.

DISCUSSION

Eleven species of *Botryosphaeriaceae* were identified as endophytes and as possible pathogens on *S. birrea*, *M. indica* and *L. schweinfurthii* trees in the Limpopo and Mpumalanga provinces. Ten species identified in this study, *D. allocellula*, *Do. brevicollis*, *Do. dulcispinae*, *Do. viticola*, *L. crassispora*, *L. exigua*, *L. gonubiensis*, *L. mahajangana*, *L. pseudotheobromae* and *N. parvum* represent known taxa of *Botryosphaeriaceae.* The remaining species is new to science and is described here as *Oblongocollomyces ednahkunjekuae*. Six species, *D. allocellula*,

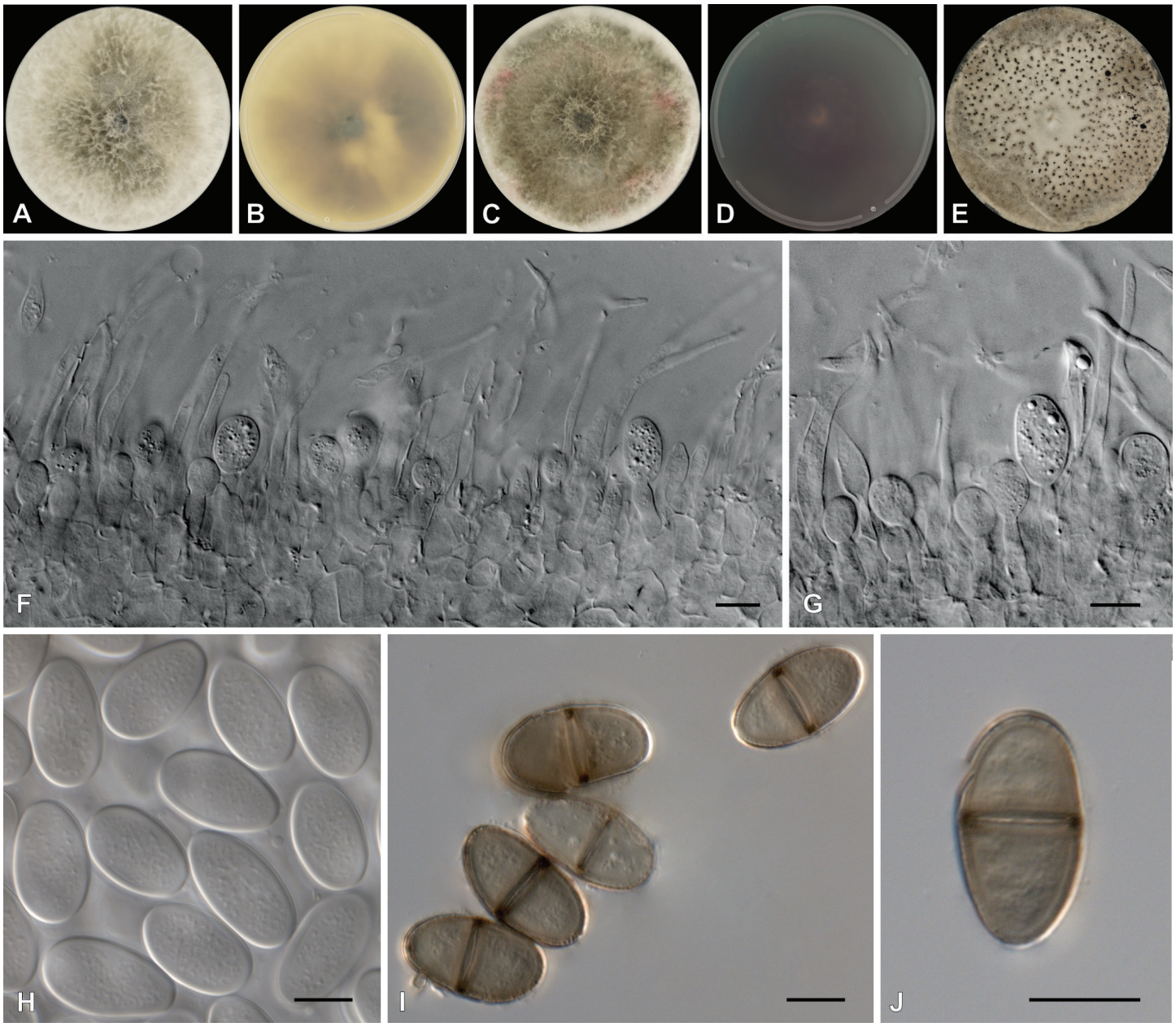

Fig. 4. *Oblongocollomyces ednahkunjekuae*. **A.** Top view of a colony on PDA at 25 °C after 5 d. **B.** Bottom view of a colony on PDA at 25 °C after 5 d. **C.** Top view of a colony on PDA at 35 °C after 4 d. **D.** Bottom view of a colony on PDA at 35 °C after 4 d. **E.** Sporulating culture on VMM after 21 d. **F, G.** Conidiogenous cells, developing conidia and paraphyses. **H.** Immature conidia. **I.** Mature conidia. **J.** Mature conidium, showing the thickened cell wall and septum. Scale bars = 10 µm.

Do. brevicollis, *Do. viticola*, *L. crassispora*, *L. mahajangana* and *N. parvum,* occurred in both asymptomatic and symptomatic branches. *Dothiorella dulcispinae*, *L. exigua*, *L. gonubiensis* and *O. ednahkunjekuae* were only isolated from asymptomatic branches, while *L. pseudotheobromae* was the only species unique to symptomatic branches of *Anacardiaceae*. There were also notable differences between species diversity in sites in agroecosystems and conservation areas, with conservation areas having a higher number of species, while *N. parvum* only occurred in agroecosystem sites.

A new species of *Oblongocollomyces* is described in this study. The genus was introduced by Yang *et al*. (2017) to accommodate an anomalous "sphaeropsis/diplodia-like" species, originally named *Sphaeropsis variabilis* (Slippers *et al*. 2014), that was distinct from other *Botryosphaeriaceae* genera based on phylogeny and morphology. *Oblongocollomyces variabilis* was isolated from *Vachellia erioloba* (= *Acacia erioloba*; *Fabaceae*) in Namibia (Slippers *et al*. 2014). The only other species described in the genus is *O. zhivanae* from north Queensland, Australia, interestingly also from a *Vachellia* species (*V. farnesiana*) (Limbongan *et al*. 2023). *Oblongocollomyces ednahkunjekuae* was isolated in this study from a different plant family (*S. birrea*; *Anacardiaceae*) in the Mapungubwe National Park in the north of South Africa. These sites from which all three species were isolated are all on a similar latitude below 20th parallel south and are in hot, dry and fairly remote areas. It would appear that this genus is adapted to such conditions. This was also evident in the optimal growth rate of the *O. ednahkunjekuae* at 30 and 35 °C, compared to many other *Botryosphaeriaceae* that typically grow fastest at 20 to 25 °C.

Most species described in this study were isolated from asymptomatic samples, demonstrating how common species of *Botryosphaeriaceae* occur as endophytes in healthy plant material (Slippers & Wingfield 2007, Jami *et al.* 2013, Ramabulana *et al*. 2022). The discrepancy in the number of isolates and species obtained between asymptomatic and

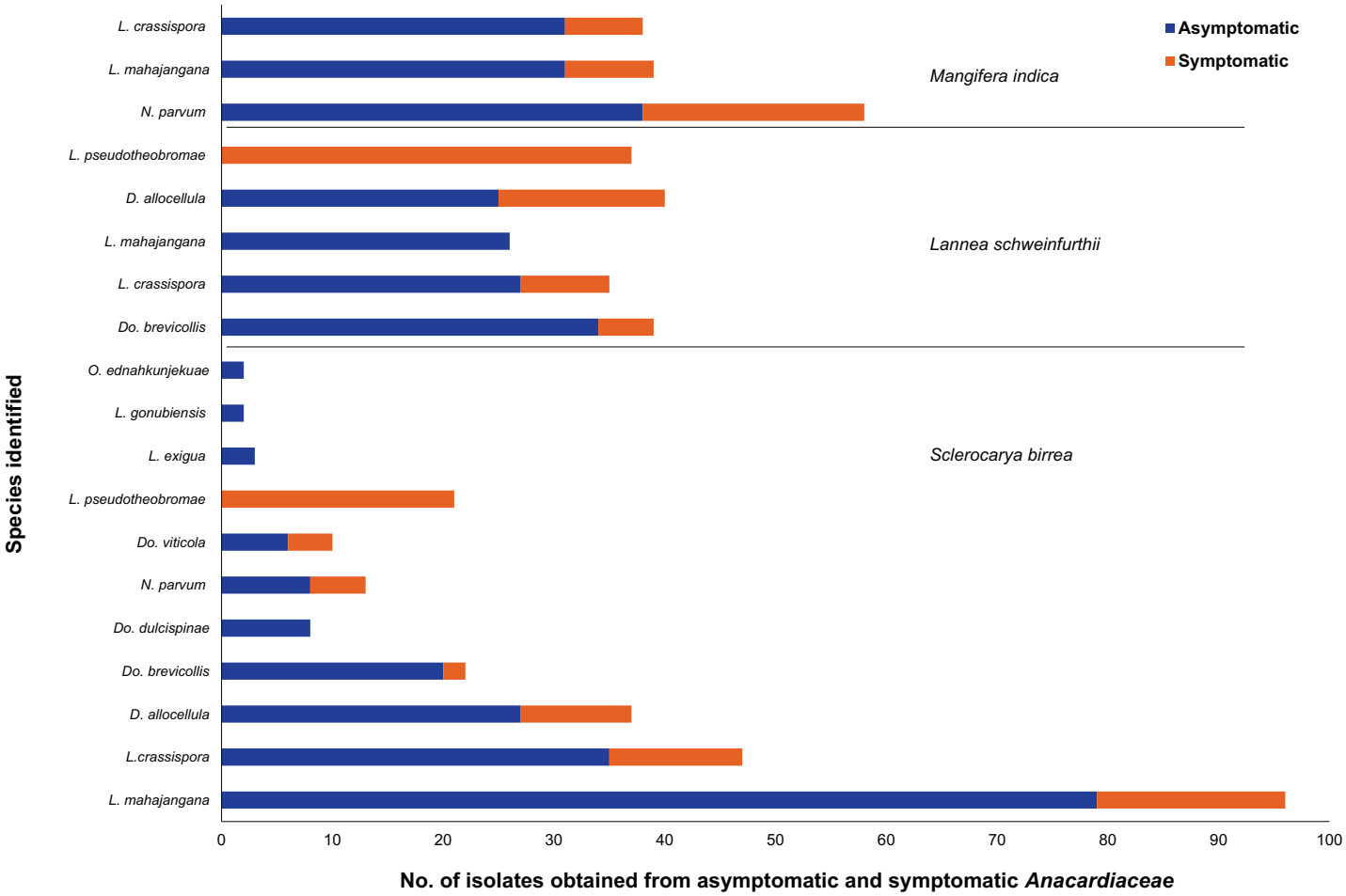

Fig. 5. *Botryosphaeriaceae* species occurring in asymptomatic and symptomatic branches of *Sclerocarya birrea*, *Lannea schweinfurthii* and *Mangifera indica.* Isolates not sequenced were assigned to a specific species based on their grouping within the different morphogroups from which representative isolates were sequenced.

symptomatic branches might be because other fungi (including saprophytes) outcompete the *Botryosphaeriaceae* once the dead tissue ages or decays, while these fungi are amongst the more common endophytes (Slippers & Wingfield 2007, Begoude *et al.* 2010, Heath *et al.* 2011, Jami *et al.* 2013, Marques *et al.* 2013, Mehl *et al.* 2017).

The six species, *D. allocellula*, *Do. brevicollis*, *Do. viticola*, *L. crassispora*, *L. mahajangana* and *N. parvum*, occurring in both asymptomatic and symptomatic samples indicate their ability to occupy healthy tissue as latent opportunistic pathogens. Such pathogens are predicted to become important in the era of climate change (Desprez-Loustau *et al.* 2007, Piskur *et al.* 2011). The pathogenicity of some species identified, including *Do. viticola*, *L. crassispora*, *L. mahajangana* and *N. parvum,* has been well established (van der Linde *et al.* 2011, Ismail *et al.* 2012, Mayorquin *et al.* 2012, Correia *et al.* 2016). For example, *Lasiodiplodia crassispora* was first described as a canker pathogen on *Santalum album* in Australia (Burgess *et al.* 2006). The fungus has also been isolated from infected *Eucalyptus urophylla,* grapevine and mango in Uruguay, California and Brazil (Pérez *et al.* 2010, Úrbez-Torres *et al.* 2011, Marques *et al.* 2013, Correia *et al.* 2016). In South Africa, the fungus is known to cause dieback on *Pterocarpus angolensis* and *S. birrea* (Mehl *et al.* 2011, 2017). In this study, *L. crassispora* was isolated from asymptomatic and symptomatic branches of *S. birrea*, *L. schweinfurthii* and *M. indica*.

Dothiorella viticola occurred in both asymptomatic and symptomatic *S. birrea* trees at Mapungubwe National Park, but was not present at other sites or in other hosts. *Dothiorella viticola* was first described as a saprophyte from declining *V. vinifera* in Spain (Luque *et al.* 2005). Since its description, the fungus has been reported as an endophyte and a pathogen causing dieback, canker and gummosis on various hosts including citrus in California and Tunisia, *Populus cathayana* in China, and *V. vinifera* in Chile, Australia and USA (Úrbez-Torres *et al.* 2007, Zhang *et al.* 2009, Adesemoye *et al.* 2011, Qiu *et al.* 2011, Díaz *et al.* 2013, Valencia *et al.* 2015, Hamrouni *et al.* 2018). In South Africa, *Do. viticola* has been reported as an endophyte and as a pathogen on *Celtis africana*, *Gymnosporia buxifolia*, *Prunus persica*, *Podocarpus henkelii*, *Senegalia mellifera*, *V. karroo* and *V. vinifera* (Jami *et al.* 2017).

Lasiodiplodia mahajangana was the dominant endophyte occurring on tree species of *Anacardiaceae*, which is consistent with the study of Cruywagen *et al.* (2017) who reported *L. mahajangana* as the dominant species isolated from baobabs in Africa (Benin, Namibia, Senegal, South Africa and Zimbabwe). *Lasiodiplodia mahajangana* was first described as an endophyte on healthy *Terminalia catappa* in Madagascar (Begoude *et al.* 2010). The second report of the fungus was in South Africa on *Euphorbia ingens* trees with blue stain symptoms (van der Linde *et al.* 2011). The fungus appears to be widely distributed in Africa, with a diverse host range. Other hosts previously reported for this fungus in South Africa include *S. birrea* and *M. indica* (Mehl *et al.* 2017).

Neofusicoccum parvum was the dominant species isolated from symptomatic *M. indica* branches. *Neofusicoccum parvum*

has previously been reported to cause diseases on mango in many parts of the world (*e.g.* de Oliveira Costa *et al.* 2010, Trakunyingcharoen *et al.* 2014, Li *et al.* 2021). *Neofusicoccum parvum* is a common endophyte and a well-known pathogen associated with dieback, canker, fruit rot and many other diseases of woody tree species in plantations, orchards and natural ecosystems (Iturritxa *et al.* 2011, Pillay *et al.* 2013, Sakalidis *et al.* 2013). This fungus has been recorded as the most widespread species in a broad range of native and non-native hosts in South Africa (Jami *et al.* 2017), but it has a global or near-global distribution (Slippers *et al.* 2017, Burgess *et al.* 2018, Batista *et al.* 2021). The global distribution of this fungus suggests that it is invasive in some regions, but its native distribution range is not known. It is notable that *N. parvum* was absent from conservation areas and less common on native hosts in agroecosystems than on the agricultural species, *M. indica*. This fungus clearly thrives in human managed and agricultural systems, as has been noted before (Pavlic-Zupanc *et al*. 2015).

The results obtained from this study indicate that not all species of *Botryosphaeriaceae* are associated with dieback of the three tree species of *Anacardiaceae* sampled. *Dothiorella dulcispinae*, *L. exigua*, *L. gonubiensis*, *O. ednahkunjekuaeae* were not associated with disease symptoms on any tree species at the time when samples were collected. This indicates that the capacity to colonise living wood does not translate to causing disease under all circumstances. Furthermore, some species identified were only associated with disease symptoms on specific hosts and not on others. For example, *L. mahajangana* was associated with dieback of *S. birrea* and *M. indica*, but not on *L. schweinfurthii* trees. The reason for this phenomenon has not yet been investigated, but the pattern of infection observed in this study could be due to factors associated with host stress responses, host defence mechanisms or traits of the fungus. These results provide useful baseline data for further studies seeking to understand host-pathogen interactions between species of *Botryosphaeriaceae* and their hosts.

Lasiodiplodia pseudotheobromae was the only species unique to symptomatic branches. The fungus was the dominant species isolated from symptomatic branches of *S. birrea* and *L. schweinfurthii* at Kruger National Park. *Lasiodiplodia pseudotheobromae* was thought to have a narrow host range and limited geographic distribution, but recent studies have reported a broad host range and occurrence in tropical environments, like most other species in the genus (Mehl *et al.* 2011, Begoude *et al.* 2012, Machado *et al.* 2014, Netto *et al.* 2014). *Lasiodiplodia pseudotheobromae* has been reported as a pathogen on hosts in Australia, Brazil, Cameroon, China, Egypt and Mexico (Begoude *et al.* 2010, Mehl *et al.* 2011, Ismail *et al.* 2012, Machado *et al.* 2014, Netto *et al.* 2014, Coutinho *et al.* 2016, Pipattanapuckdee *et al.* 2019, Chen *et al.* 2021). The fungus is also common in South Africa where it has been reported to cause dieback and canker on *Adansonia digitata, P. angolensis*, *Sclerocarya birrea, Syzygium cordatum*, *Senegalia mellifera, Terminalia sericea, T. catappa* and *V. karroo* (Jami *et al.* 2017).

Lasiodiplodia pseudotheobromae is associated with different symptoms on the various agriculture and forestry hosts (Ismail *et al.* 2012, Marques *et al.* 2013, Pipattanapuckdee *et al.* 2019, Chen *et al.* 2021). For example, *L. pseudotheobromae* has been reported to cause stem-end rot and dieback on mango (Kwon *et al.* 2017), papaya stem-end rot (Netto *et al.* 2014), English walnut stem canker (Li *et al.* 2016), blueberry dieback (Wang *et al.* 2016), pedicel and peduncle discoloration of grapes (Dissanayake *et al.* 2015) and cankers on *Anacardium occidentale*, *Eucalyptus*, *Citrus, Coffea* and *Gmelina* species (Phillips *et al.* 2008, Pérez *et al.* 2010, Chen *et al.* 2011, Slippers *et al.* 2014, Trakunyingcharoen *et al.* 2015, Coutinho *et al.* 2016, Cruz *et al.* 2019).

The decline symptoms of *M. indica* trees at Tshipise might reflect a combination of biotic and abiotic factors. Mango decline is a serious problem in mango-growing countries around the world (Sandhu & Gill 2013, Kumar & Kumar, 2016). The complexity of events leading to mango decline makes it difficult to ascertain the cause to a specific pathogen, pest or abiotic condition. Other than severe dieback from which we isolated species of *Botryosphaeriaceae*, other symptoms, including stunted growth and leaf necrosis were observed on *M. indica* trees. These symptoms are often associated with nutrient deficiency and other abiotic stressors (Sandhu & Gill 2013, Kumar & Kumar 2016). *Mangifera indica* is a non-native, domesticated tree species and is not always grown in areas with optimal growing conditions. Mango trees thrive well in areas characterised by clay-loamy soil with good water and nutrient-holding capacity (Normand *et al.* 2015). The sites sampled in this study are characterised by sandy soil with limited nutrient and water-holding capacity and we assume that the non-optimal growing conditions might have contributed to the decline of *M. indica* trees.

This study demonstrates the common occurrence of the *Botryosphaeriaceae* as latent opportunistic pathogens on native and non-native tree species of *Anacardiaceae.* The presence and dominance of known pathogens in the *Botryosphaeriaceae* such as *L. mahajangana, L. pseudotheobromae* and *N. parvum* on symptomatic samples supports suggestions by Slippers & Wingfield (2007) that the most damaging species of *Botryosphaeriaceae* have a broad host range and a wide distribution. The information is useful for screening for resistance in commercial species, such as mango. The results also raise concerns about the future health status of these trees and other woody tree species in native and commercial sites in the context of climate change. It is important to implement monitoring of these trees to understand the potential impact of climate change on disease expression by species of *Botryosphaeriaceae*.

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Supplementary Material: http://fuse-journal.org/

Fig. S1. Maximum likelihood phylogenetic tree generated from the ITS sequence dataset. Isolates from asymptomatic branches are indicated with (●) and those from symptomatic branches are indicated with (▲). T = ex-type. Bootstrap support values above 60 % and PP values equal or above 0.95 are shown at the nodes. The tree was rooted to sequences of *Melanops tulasnei.*

Fig. S2. Maximum likelihood phylogenetic tree generated from analyses of the *tef-1α* dataset. Isolates from asymptomatic branches are indicated with (●) and those from symptomatic branches are indicated with (▲). T = ex-type. Bootstrap support values (> 60 %) and PP values (≥ 0.95) are shown on the nodes. The tree was rooted to *Melanops tulasnei*

Fig. S3. Maximum likelihood phylogenetic tree based on analyses of the *β-tub* dataset. Isolates from asymptomatic branches are indicated with (●) and those from symptomatic branches are indicated with (▲). T = ex-type. Bootstrap support values (> 60 %) and PP values (≥ 0.95) are shown on the nodes. The tree was rooted to *Melanops tulasnei.*

Fig. S4. Maximum likelihood phylogenetic tree based on analyses of the *rpb*2 dataset. Isolates from asymptomatic branches are indicated with (●) and those from symptomatic branches are indicated with (▲). T = ex-type. Bootstrap support (> 60 %) and PP values (≥ 0.95) are shown on the nodes. The tree was rooted to *Melanops tulasnei.*

Table S1. Strain numbers, origin and GenBank accession number for reference strains used for phylogenetic analyses.

Table S2. Representative fungal isolates obtained from asymptomatic and symptomatic branches of *Anacardiaceae* included in phylogenetic analyses. Isolate numbers in bold indicate isolates sequenced in this study. Isolates from asymptomatic tissue are from Ramabulana *et al*. (2022).