Botryosphaeriaceae associated with *Acacia heterophylla* (La Réunion) and *Acacia koa* (Hawaii)

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ABSTRACT

Acacia koa and *A. heterophylla* are commonly occurring native trees on the Hawaiian Islands and La Réunion, respectively. A recent phylogenetic study suggested that *A. heterophylla* renders *A. koa* paraphyletic, and that the former likely arose from the Hawaiian Islands around 1.4 million years ago. An intriguing question is whether their microbiota is similar, although they occur naturally in two very distant geographical locations. In this study, we compared the fungi in the *Botryosphaeriaceae* isolated from natural populations of *A. koa* and *A. heterophylla*. These fungi were chosen because they commonly occur on woody plants and some are important pathogens. They are also known to have been moved globally on asymptomatic plant materials. Isolates were identified based on comparisons of DNA sequence data for the rDNA-ITS, *TEF1-α* and β-tubulin loci. Ten *Botryosphaeriaceae* species were identified, of which four species were specific to *A. koa* from the Hawaiian Islands and five to *A. heterophylla* in La Réunion. Only one species, *Neofusicoccum parvum*, which is known to have a wide global distribution, was common to both hosts. The overall results of this study suggest that although *A. koa* and *A. heterophylla* share a recent evolutionary history, they have established independent microbiota, at least in terms of the *Botryosphaeriaceae*.

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1. Introduction

The *Botryosphaeriaceae* Theiss. & Syd. (*Botryosphaeriales*, *Dothideomycetes*) includes 24 genera and over 100 species (Slippers et al., 2017; Yang et al., 2017) and is one of the most cosmopolitan groups of fungi occurring on woody plants. The *Botryosphaeriaceae* are typically endophytes that have evolved in close association with their hosts on which they can cause disease, usually under conditions of stress (Slippers and Wingfield, 2007; Mehl et al., 2013; Marsberg et al., 2017). Host stress factors are key issues in emerging diseases caused by *Botryosphaeriaceae* (Slippers and Wingfield, 2007) and climate change is increasing the risk of diseases caused by this group of fungi (Desprez-Loustau et al., 2006; Sturrock et al., 2011).

It is not clear if there are general patterns of host specificity in the *Botryosphaeriaceae*. While they are usually known to have wide host ranges and geographical distributions, some species have been found to display strong host specificity and limited geographic distributions (Slippers et al., 2017). These fungi are also known to have been moved extensively by humans due to global trade in plant material (Crous et al., 2016a; Burgess and Wingfield, 2017; Sakalidis et al., 2011a). Thus, even where the *Botryosphaeriaceae* have been isolated from native plants, they are not necessarily native to those plants. For example, Mehl et al. (2017) showed movement of these fungi between planted mango (*Mangifera indica* L.) trees and native marula trees (*Sclerocarya birrea* (A.Rich.)) Hochst. subsp. *caffra* (Sond.).

Acacia heterophylla Willd. and *Acacia koa* A. Gray are indigenous to La Réunion and Hawaiian Islands, respectively (Brown et al., 2012). These trees are particularly interesting because *A. heterophylla* is phylogenetically most closely related to *A. koa* (Le Roux et al., 2014), but they occur in areas very distant to each other in the Hawaiian Islands and La Réunion. Their unusual close relatedness relative to

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their areas of origin led Le Roux et al. (2014) to suggest that they represent a single taxon that have been separated for *ca.* 1.4 million years. Their hypothesis was that *A. heterophylla* was most likely introduced to La Réunion from Hawaiian Islands via a rare extreme long-distance dispersal event (Van Zandt et al., 2014).

There have been few studies conducted on the fungal community of *A. heterophylla* and *A. koa* trees based on molecular data. Only two fungi, *Colletotrichum karstii* You L. Yang et al. (Hernandez-Restrepo et al., 2016), and *Vermiculariopsiella acaciae* Crous & M.J. Wingf. have been reported on leaves of *A. heterophylla* in La Réunion (Crous et al., 2016b). While there are more fungal reports from leaves of *A. koa* in Hawaii, such as *Beltraniella acacia* Crous, *Dactylaria acacia* Crous, *Rhexodenticula acaciae* Crous, *Rubikia evansii* Crous, *Torula acaciae* Crous (Crous et al., 2017), *Calonectria ilicicola* Boedijn & Reitsma (Crous et al., 2004), and rust fungi such as *Endoraecium acaciaei* Hodges & D.E. Gardner, *E. hawaiiense* Hodges & D.E. Gardner and *E. koae* (Arthur) Scholler and Aime, (2006), there has never been a study on endophytic fungi of these trees in the areas where they occur naturally.

The aim of this study was to identify and compare the *Botryosphaeriaceae* species diversity on *A. heterophylla* and *A. koa*, and more broadly, to consider whether these fungi reflect the historical biogeography of these trees as documented by Le Roux et al. (2014).

2. Materials and methods

2.1. Collection of samples and isolations

Branch samples were collected from asymptomatic *A. heterophylla* on La Réunion Island during 2014 and from *A. koa* in the Hawaiian Islands during 2015. Five branches from each of 20 *A. heterophylla* and 25 *A. koa* trees were randomly chosen for sampling. The collected branches (*A. heterophylla* = 100 and *A. koa* = 125) were placed in paper bags and transferred to the laboratory for further study. After storage at 4 °C for one week, 12 pieces (0.5 cm) were randomly selected from each branch, surface-disinfested in 10 % hydrogen peroxide for two minutes, and rinsed three times in sterile water. All branch samples were placed on 2 % malt extract agar (MEA) (Biolab, Midrand, South Africa) in Petri dishes with four samples per plate. Petri dishes were incubated at 24 °C for seven days and fungal growth from each sample showing morphological characteristics (fast growing, white to black cultures with fluffy aerial hyphae) of the *Botryosphaeriaceae* were transferred to fresh MEA plates. After 4–5 d, all isolates showing typical growth were transferred to 15 % water agar (WA) in order to make single hyphal tip subcultures. These isolates are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. A sub-set of live cultures and dried cultures representing novel taxa were lodged at the South African National Collection of Fungi at Agricultural Research Centre, Plant Protection Research Institute (ARC-PPRI), Roodeplaat, South Africa.

2.2. DNA sequence analyses

DNA was extracted using a PrepMan® Ultra kit (Applied Biosystems) from mycelia taken from 5-d-old pure cultures. DNA sequences were generated for the internal transcribed spacer region of the ribosomal RNA (rRNA) operon amplified with primers ITS-1F (Gardes and Bruns, 1993) and ITS-4 (White et al., 1990), the translation elongation factor 1-α (TEF1-α) gene amplified with primers EF1-728F and EF1-986R (Carbone and Kohn, 1999), as well as the β-tubulin (*TUB2*) gene amplified with primers BT2a and BT2b (Glass and Donaldson, 1995). The

conditions for the PCR and DNA sequencing were the same as those described by Jami et al. (2012).

Phylogenetic analyses for all the datasets were performed using Maximum Likelihood (ML). The best-fit nucleotide substitution models for each dataset were identified separately using Modeltest v.3.7 (Posada and Buckley, 2004). The GTR model was identified for all datasets of ITS, *TEF-1α* and TUB2 as well as the combined datasets. The ML analyses were performed in PAUP v.4.0b10 and confidence levels were determined with 1000 bootstrap replications. All the sequences of this study were deposited in GenBank (Table 1).

2.3. Morphological characteristics

To induce sporulation, cultures were inoculated onto sterilized twigs of *Salix* sp, placed on the surface of 2 % MEA and incubated at 25 °C under near-UV light. Conidiomata harvested from hardwood twigs were dissected using a Cryomicrotome (Leica, Germany). Fungal structures were mounted in Tissue Freezing Medium® (Leica, Germany) and cut in 12–14 μm thickness. Sections were mounted in 85 % lactic acid for observation. All measurements and images were taken using a Nikon Eclipse Ni compound and SMZ18 dissecting microscopes (Nikon, Japan). Images were captured with a Nikon DS-Ri camera and with the imaging software NIS-Elements BR. Up to 50 measurements were made for spores and other morphologically characteristic structures where these were available.

Growth in culture was studied in the dark at temperatures between 10 °C and 35 °C at 5 °C intervals. An agar disc taken from the edge of an actively growing culture was placed at the centre of 90 mm Petri dishes containing 2 % MEA. Three replicate plates were used for each isolate per temperature. Cultures were allowed to grow until the fastest growing isolate reached the edge of a Petri dish at which point the experiment was terminated and two measurements perpendicular to each other of colony diameters were made. The averages of the colony diameters were then calculated. Colony colours were assigned using the designations of Rayner (1970).

3. Results

3.1. Isolates and DNA sequence analyses

A total of 16 isolates were obtained from seven asymptomatic *A. koa* (25 sampled trees) and eight isolates from seven asymptomatic *A. heterophylla* (20 sampled trees) (Table 1). The sequence datasets for the ITS, *TUB2* and *TEF-1α* were analysed individually and in combination (Figs. 1, S1–S3). The ITS sequence dataset contained 579 characters, *TUB2* dataset contained 412 characters, *TEF-1α* dataset contained 409 characters and combined dataset contained consisted of 1400 characters (TreeBASE Accession No. 22704).

The topologies of the trees emerging from the ML analyses were similar for the individual gene regions (Figs. S1–S3), as well as in the combined analyses, with regards to the clades representing species isolated in this study. Ten clades were identified in all the analyses, representing (*Neofusicoccum occulatum* Sakalidis & T.I. Burgess, *Neofusicoccum parvum* Pennycook & Samuels) Crous et al., *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Lasiodiplodia exigua* Linald. et al., (=*Lasiodiplodia americana* S.F. Chen et al.), *Lasiodiplodia iraniensis* Abdollahz. et al., (=*Lasiodiplodia jatrophicola* A.R. Machado & O.L. Pereira), *Lasiodiplodia rubropurpurea* T.I. Burgess et al., *Botryosphaeria ramosum* (Pavlic et al.) A.J.L. Phillips & A. Alves and three unknown groups in the genus *Dothiorella* Sacc. (Fig. 1). Five species were specific to samples from *A. koa* in the Hawaiian

Table 1
The Botryosphaeriaceae isolates from *Acacia heterophylla* and *A. koa* of this study used in the phylogenetic analyses. Type isolates are indicated in bold.

Isolate No.	Identity	Location	Host	GenBank		
				ITS	TEF1-α	TUB2
CMW 46460	*Botryosphaeria ramosaum*	La Réunion	*Acacia heterophylla* (tree no. 2)	MH447651	–	MH548326
CMW 46457[T]	***Dothiorella reunionis* sp. nov.**	La Réunion	*A. heterophylla* (tree no. 3)	MH447649	MH548347	–
CMW 46458[T]	***Dothiorella heterophyllae* sp. nov.**	La Réunion	*A. heterophylla* (tree no. 5)	MN103794	MH548348	MH548324
CMW 48017[T]	***Dothiorella koae* sp. nov.**	Hawaii	*A. koa* (tree no. 12)	MH447652	MH548338	MH548327
CMW 48018		Hawaii	*A. koa* (tree no. 1)	MH447653	MH548337	MH548328
CMW 48019		Hawaii	*A. koa* (tree no. 1)	MH447654	MH548336	MH548329
CMW 48020		Hawaii	*A. koa* (tree no. 1)	MH447655	MH548335	–
CMW 48033		Hawaii	*A. koa* (tree no. 12)	MH447660g	–	–
CMW 48034	*Lasiodiplodia exigua* (=*L. americana*)	Hawaii	*A. koa* (tree no. 22)	MH447661	MH548342	MH548333
CMW 46452	*L. iraniensis* (=*L. jatrophicola*)	La Réunion	*A. heterophylla* (tree no. 12)	MH447645	MH548344	MH548322
CMW 46453		La Réunion	*A. heterophylla* (tree no. 12)	MH447646	–	–
CMW 46456		La Réunion	*A. heterophylla* (tree no. 15)	MH447648	MH548346	MH548323
CMW 46459	*L. rubropurpurea*	La Réunion	*A. heterophylla* (tree no. 2)	MH447650	–	MH548325
CMW 48036	*L. theobromae*	Hawaii	*A. koa* (tree no. 13)	MH447662g	MH548339	MH548334
AK13 3.2a	*L. theobromae*	Hawaii	*A. koa* (tree no. 13)	MN103789	MN119480	MN119475
AK13 3.2b	*L. theobromae*	Hawaii	*A. koa* (tree no. 13)	MN103790	–	MN119476
CMW 48021	*Neofusicoccum occulatum*	Hawaii	*A. koa* (tree no. 28)	MH447656	MH548343	MH548330
CMW 48023	*N. occulatum*	Hawaii	*A. koa* (tree no. 20)	MH447658	MH548340	MH548332
CMW 48024	*N. occulatum*	Hawaii	*A. koa* (tree no. 28)	MH447659	–	–
AK24 2.4	*N. occulatum*	Hawaii	*A. koa* (tree no. 24)	MN103793	MN119483	MN119479
CMW 48022	*N. parvum*	Hawaii	*A. koa* (tree no. 20)	MH447657	MH548341	MH548331
CMW 46455	*N. parvum*	La Réunion	*A. heterophylla* (tree no. 10)	MH447647	MH548345	–
AK20 3.6a	*N. parvum*	Hawaii	*A. koa* (tree no. 20)	MN103791	MN119481	MN119477
AK20 3.6c	*N. parvum*	Hawaii	*A. koa* (tree no. 20)	MN103792	MN119482	MN119478

Islands and four species from *A. heterophylla* from La Réunion (Fig. 2). *N. parvum* was the only species shared between La Réunion and the Hawaiian Islands (Fig. 3).

3.2. Morphological characteristics

The isolates in the clades corresponding to new species of *Dothiorella* had pale to dark olivaceous colonies with short aerial hyphae. The cultures produced rosette-like growth patterns and had dense mycelium with irregular edges.

3.3. Taxonomy

Three unidentified species emerging from the phylogenetic analyses are described as follows:

Dothiorella reunionis Marinc., Jami & M.J. Wingf., **sp. nov.** (Fig. 4). MycoBank No.: MB 825260.

Etymology: Name refers to La Réunion island where this species was collected.

Conidiomata formed on hardwood twigs placed in 2 % MEA at 25 °C under near-UV light for 2 weeks, stromatic, uni- or multi-loculate, immersed, erumpent, superficial, covered abundantly with pigmented hyphae, 220–455 μm tall, 205–660 μm wide. *Conidiophores* hyaline, generally unbranched, septate, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, hyaline or slightly pigmented, clavate to cylindrical, some with annellations, 4–14 μm long, 2–6.5 μm wide. *Conidia* hyaline when young, becoming brown with age, verruculous (noticeable in higher magnification, ×100), ellipsoidal with truncate base, tapering towards the base, 1(–3)-septate, septum median, supra- or sub-median, 18.5–31.5 × 8.5–13.5 μm (avg. 24.9 × 10.6 μm). *Spermatiaproducing cells* hyaline, clavate to cylindrical, straight to curved, 7–15.5 μm long, 2–7 μm wide. *Spermatia* hyaline, shape variable, ellipsoidal to cylindrical and intermediate forms, mostly aseptate, with base truncate, straight or curved, 6–15.5 × 2.5–5.5 μm (avg. 9.7 × 4.0 μm).

Culture characteristics on 2 % MEA in the dark showing optimum growth at 25 °C reaching 75.5 mm diam in 4 d, followed by at 20 °C reaching 70.2 mm, no growth at 35 °C, showing circular growth with even margin, aerial mycelium not dense, becoming abundant and fluffy at lower temperatures, culture texture and colour varying at different temperatures, at 25 °C in 4 d culture covered with white aerial hyphae with a tint of pale greenish brown in an inner circle.

Specimen examined: **La Réunion**, asymptomatic branch of *Acacia heterophylla*, Mar. 2015, M.J. Wingfield, PREM 62202, holotype, culture ex-holotype CMW 46457 = PPRI 25142.
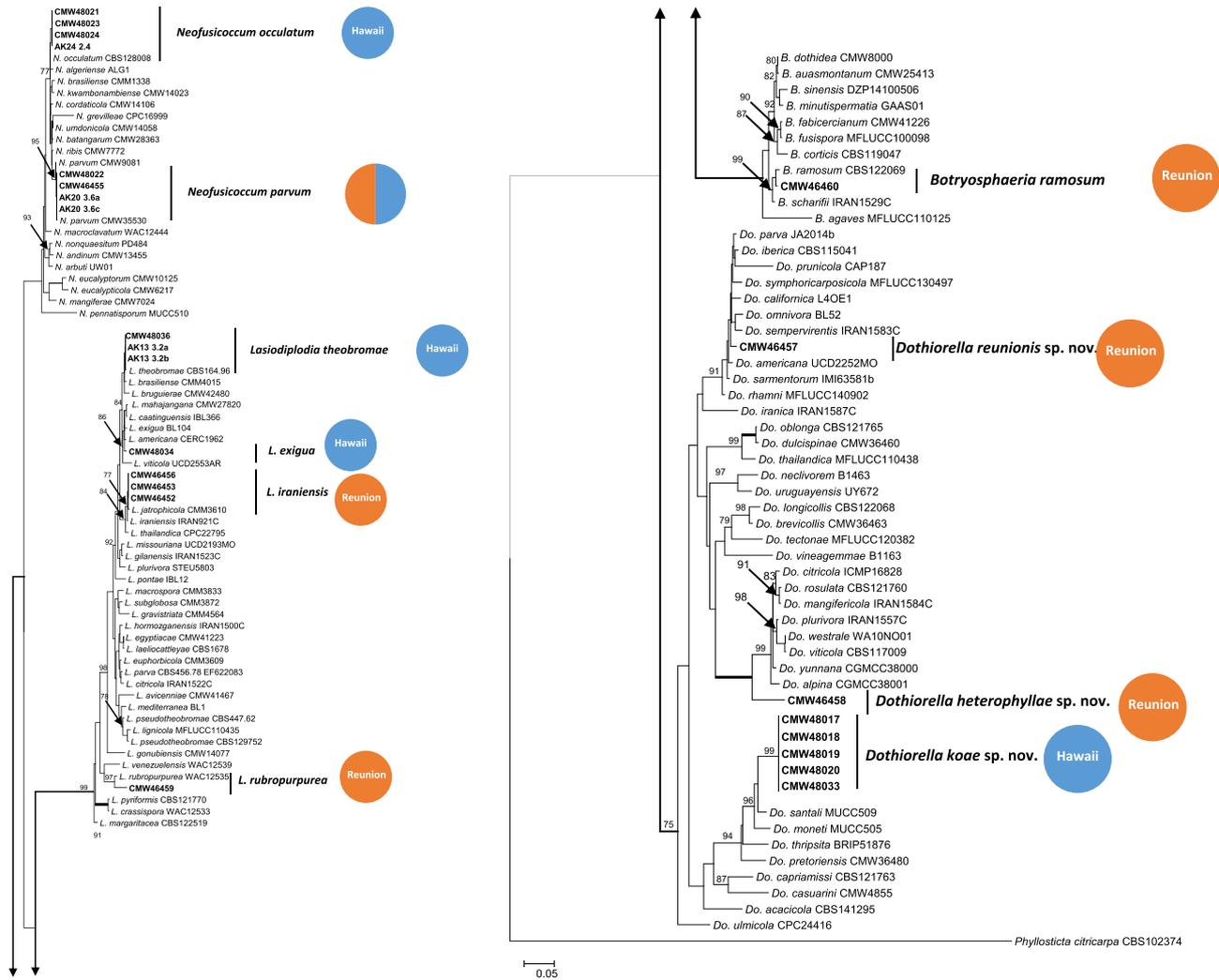
Note: Although only a single isolate was obtained for this species, we have described it as new taxon, because it forms in a distinct lineage among other *Dothiorella* species. *D. reunionis* differs with *Do. sarmentorum* (Fr.) A.J.L. Phillips et al., by 1 bp in ITS and 7 bp in *TEF-1α*.

Dothiorella heterophyllae Marinc., Jami & M.J. Wingf., **sp. nov.** (Fig. 5.). MycoBank No.: MB 825261.

Etymology: Name refers to the host species from which it was isolated, *Acacia heterophylla*.

Conidiomata formed on hardwood twig placed in 2 % MEA at 25 °C under near-UV light for 2 weeks, stromatic, immersed, erumpent, covered with numerous pigmented hyphae, 225–345 μm tall, 210–440 μm wide. *Conidiophores* lining inside the conidiomatal wall, mostly reduced to conidiogenous cells. *Conidiogenous cells* discrete, blastic, hyaline, cylindrical to clavate, with annellations, 7–16.5 μm long, 2.5–6 μm wide. *Conidia* hyaline when young, becoming brown when mature, verruculous, ellipsoidal, tapering towards a truncated base, 1-septate, septum median, 18–26 × 8.5–11.5 μm (av. 22.6 × 9.8 μm).

Culture characteristics on 2 % MEA in the dark showing optimum growth at 20 °C reaching 80.5 mm in 7 d, followed by at 15 °C reaching 69.3 mm, no growth at 30 °C and 35 °C, showing circular growth with undulate margin, aerial mycelium sparse, olivaceous brown, culture texture and colour relatively uniform at different temperatures, at 10 °C and 25 °C more aerial hyphae present and less pigmentation.

Fig. 1. Maximum Likelihood (ML) tree of the combined data set of ITS, *TEF1-α* and β-tubulin loci sequences. Bootstrap values above 75 % are given at the nodes. The tree was rooted to *Phyllosticta citricarpa* (CBS 102374). Isolates of this study are indicated as bold.

S. examined: **La Réunion**, asymptomatic branch of *Acacia heterophylla*, Mar. 2015, M.J. Wingfield, PREM 62203, holotype, culture ex-holotype CMW 46458 = PPRI 25141.

Note: Although only a single isolate was obtained for this species we have described it as new taxon, because it forms as a distinct lineage based on ITS, *TUB2* and *TEF-1α* sequences among other *Dothiorella* species.

Dothiorella koae Marinc., Jami & M.J. Wingf., **sp. nov.** (Fig. 6). MycoBank No.: MB 825262.

Etymology: Name refers to the host species from which it was isolated, *A. koa*.

Conidiomata formed on hardwood twig placed in 2 % MEA at 25 °C under near-UV light for 2 weeks, stromatic, uni- or multi-loculate, immersed, erumpent or superficial, moderately covered with pigmented hyphae, 120–480 μm tall, 165–470 μm wide. *Conidiophores* lining inside the wall, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, determinate, blastic, hyaline, cylindrical to clavate, 6–11 μm long, 2–4.5 μm wide. *Conidia* hyaline when young, becoming brown with maturity, smooth becoming verruculose, fusiform to cylindrical with a round apex and a truncated base, sometimes abruptly tapering towards the

base, mostly straight, 1–2-septate, septum median, supra- or submedian, septa not evenly distributed when more than one, 24.5–35 × 6.5–9.5 μm (avg. 29.5 × 8.1 μm).

Culture characteristics on 2 % MEA in the dark showing optimum growth at 25 °C reaching 74.5 mm in 7 d, followed by at 20 °C reaching 54.5 mm, no growth at 30 °C and 35 °C, showing circular growth with undulate margin, aerial mycelium moderately dense, at 20 °C creamy aerial mycelium densely packed near the centre, culture morphology similar at different temperature, tint of greenish brown in culture.

Specimens examined: **Hawaii**, asymptomatic branches of *Acacia koa*, Apr 2015, M.J. Wingfield, PREM 62204, holotype, culture ex-holotype CMW 48017 = PPRI 25139; additional cultures CMW 48018 = PPRI 25140, CMW 48019, CMW 48020, CMW 48033.

Notes: This species not only formed a distinct phylogenetic clade among *Dothiorella* species and differs with *Dothiorella santali* K.M. Taylor et al., based on unique fixed alleles in ITS (3 bp), *TUB2* (5 bp) and *TEF-1α* (12 bp) sequences data, but also had very distinct conidia. The conidia are distinguishable from other species of *Dothiorella* by being narrow and being predominantly 2-septate.
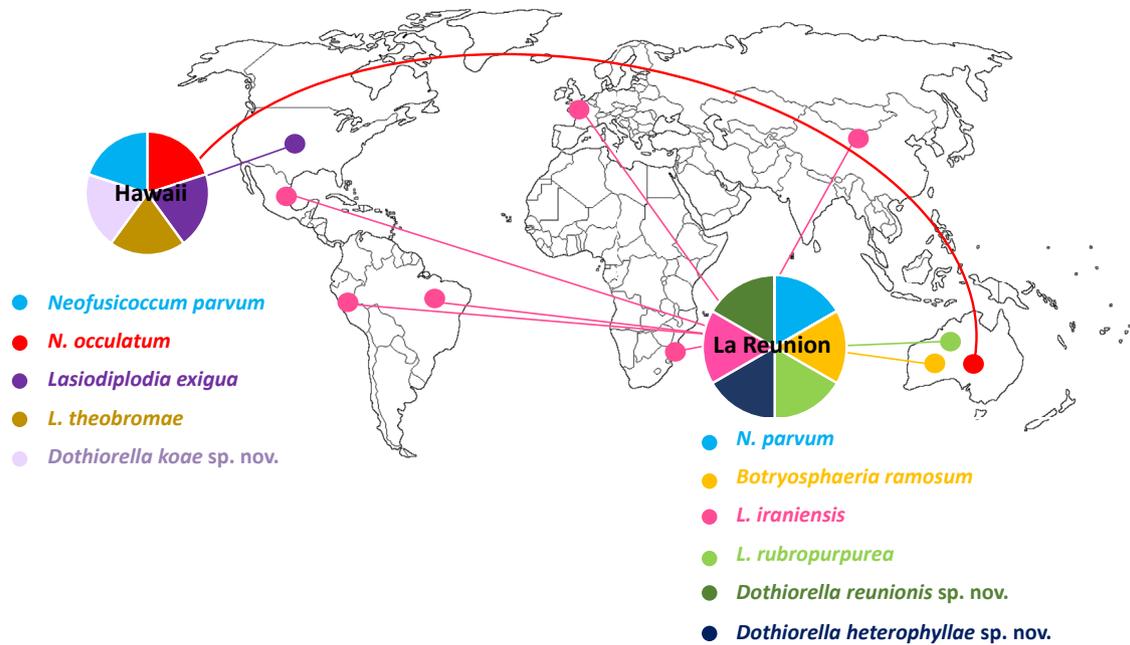
Fig. 2. The distribution of ten *Botryosphaeriaceae* species of this study in Hawaii and La Réunion Islands and across the world. *These two species exist all over the world.
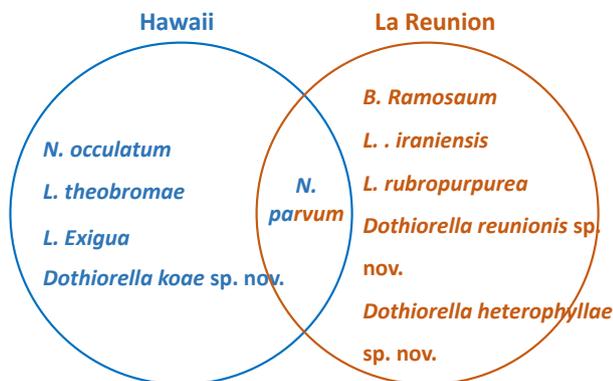

Fig. 3. The pattern of overlapping *Botryosphaeriaceae* species between Hawaii and La Réunion Islands.

4. Discussion

Ten *Botryosphaeriaceae* species were identified from branches of asymptomatic *A. koa* and *A. heterophylla* in Hawaiian Islands and La Réunion, respectively. With only one exception, these *Botryosphaeriaceae* were unique to the islands from which they were sampled. Four unique species were identified from *A. koa* and five species from *A. heterophylla*. Only *N. parvum* occurred in association with trees from both regions. Three other known species, *N. occulatum*, *L. exigua* (=*L. americana*), *L. theobromae*, and a new *Dothiorella* species were identified on *A. koa*. Similarly, three known species including *L. iraniensis* (=*L. jatrophicola*), *L. rubropurpurea* and *B. ramosum*, and two new *Dothiorella* species were identified in association with *A. heterophylla*. Overall, our results expand the geographical and host range of *Botryosphaeriaceae* and provide the first *Botryosphaeriaceae* records for both the locations and hosts studied.

All three new species of this study resided in the genus *Dothiorella*. They include *Do. reunionis* and *Do. heterophyllae* described on *A. heterophylla* in La Réunion and *Do. koae* on *A. koa* in Hawaiian Islands. Another *Dothiorella* sp., *Do. acacicola* Crous & M.J. Wingf. has been described from La Réunion where it was isolated from leaves of *Acacia mearnsii* De Wild. (Crous et al., 2016b). *Acacia mearnsii* is the most widespread invasive woody plant in La Réunion, which is very competitive with *A. heterophylla* and has negatively impacted on the regeneration of *A. heterophylla* (Tassin, 2002). *Dothiorella acacicola* was not found on *A. heterophylla* in this study even though these trees grow in close proximity to each other. This could reflect host specificity in the fungus or an artefact of sampling.

It was not surprising that *N. parvum* was found on both *A. koa* in Hawaii and *A. heterophylla*, despite their geographic separation. *N. parvum* has a wide global distribution and host range (Slippers and Wingfield, 2007) and has been reported from 29 countries on 90 hosts (Sakalidis et al., 2013). In South Africa *N. parvum* has been reported from 30 hosts across 12 locations, making it the second most widespread species of the *Botryosphaeriales* with the highest host diversity in that country (Jami et al., 2017). All evidence suggests that this species has been moved widely through the global trade in plants and plant products. It is likely that its wide host range, and evident adaptability to a wide climatic range, contributes to its ability to establish and spread in new areas after introduction.

An interesting result of this study was that *L. theobromae* was found only on *A. koa* in Hawaii. This was contrary to the fact that this is one of the most widespread *Botryosphaeriaceae* species globally, having been reported on large number of hosts (https://nt. ars-grin.r gov/fungaldatabases) and on all continents except Antarctica (Mehl et al., 2017). The species tends to occur on trees in warmer parts of the world and its occurrence in Hawaiian Islands might reflect this fact. Although, La Réunion is a tropical region too, however, the absent of *L. theobromae* from there is likely a sampling effect.

Three species, *B. ramosum*, *L. iraniensis* and *L. rubropurpurea*, were found only on *A. heterophylla* in La Réunion. These species are known on various other woody plants such as *Eucalyptus* in Australia in the case of *B. ramosum* and *L. rubropurpurea* (Burgess et al., 2006; Pavlic et al., 2008) and a wide diversity of woody
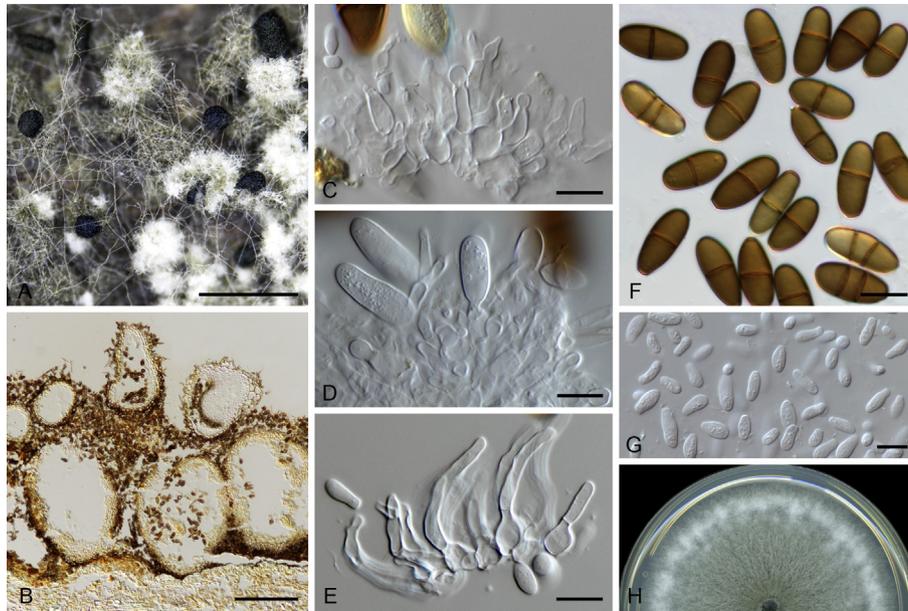
Fig. 4. Microscopic features of *Dothiorella reunionis* (ex-holotype, CMW 46457 = PPRI 25142). (**A**). Conidiomata formed on hardwood twig with dark conidial mass gathered at the tip. (**B**). Vertical section of clustered conidiomata. (**C, D**). Conidiogenous cells of brown conidia. (**E**). Spermatia producing cells. (**F**). Conidia. (**G**). Spermatia. (**H**). Colony on 2 % MEA showing optimum growth in the dark at 25 °C in 4 d. Scale bars: A = 1 mm; B = 250 μm; C–G = 10 μm.
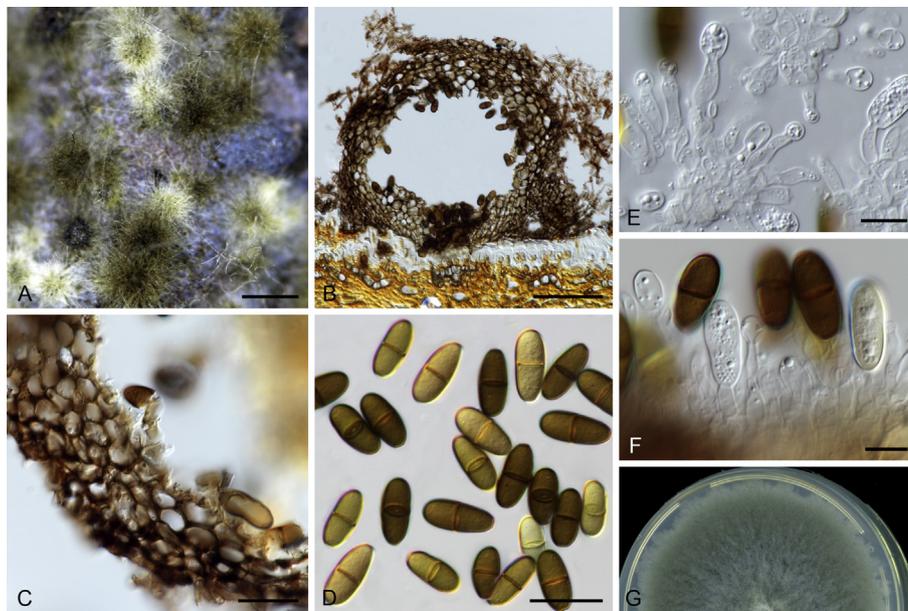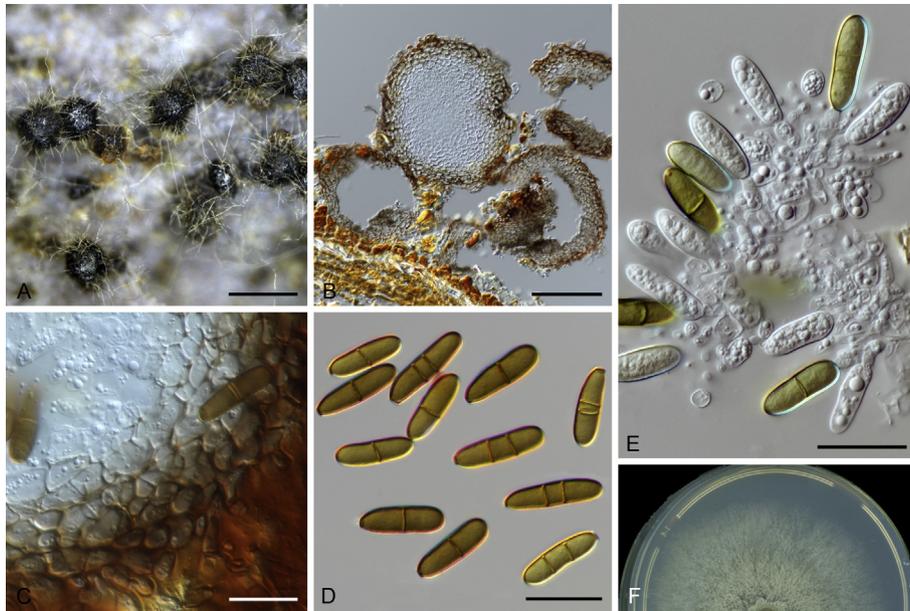

Fig. 5. Microscopic features of *Dothiorella heterophyllae* (ex-holotype, CMW 46458 = PPRI 25141). (**A**). Conidiomata formed on hardwood twig. (**B**). Vertical section of conidiomata. (**C**). Conidiomatal wall. (**D**). Conidia. (**E, F**). Conidiogenous cells and young conidia attached. (**G**). Colony on 2 % MEA showing optimum growth in the dark at 20 °C in 7 d. Scale bars: A = 500 μm; B = 100 μm; C, D = 25 μm; E, F = 10 μm.

plants in many different parts of the world for *L. iraniensis* (Abdollahzadeh et al., 2010; Correia et al., 2016; Cruywagen et al., 2017; Machado et al., 2014; Netto et al., 2017; Rodríguez-Gálvez et al., 2017; Sakalidis et al., 2011b; Yang et al., 2017; Zhu et al., 2015). This species could have been accidently co-introduced into La Réunion with other woody plants.

Lasiodiplodia exigua and *N. occulatum* were isolated only from *A. koa* (Hawaiian Islands). *Lasiodiplodia exigua* has been reported on *Pistacia vera* L. and *Retama raetam* (Forssk.) Webb in USA and Tunisia, respectively, as well as from *Adansonia* sp. L. in Cameroon and Senegal (Chen et al., 2015; Cruywagen et al., 2017; Linaldeddu

et al., 2015). *Neofusicoccum occulatum* has been reported from *Wollemia nobilis* W.G.Jones et al., and *Eucalyptus* L'Hérit. species in Australia (Sakalidis et al., 2011c), as well as from non-native *Psidium guajava* L. in Hawaii (Yang et al., 2017). Our results represent a new host association for both *L. exigua* and *N. occulatum* but their wide distribution elsewhere in the world suggests that they have probably been introduced into the Hawaiian Islands.

The origin of the *Botryosphaeriaceae* on *A. koa* in Hawaiian Islands and *A. heterophylla* in La Réunion is unknown. The *Botryosphaeriaceae* are clearly promiscuous and they are not known to be transferred vertically i.e. internally with seed (Bihon et al., 2011).

Fig. 6. Microscopic features of *Dothiorella koae* (ex-holotype, CMW 48017 = PPRI 25139). (**A**). Conidiomata formed on hardwood twig. (**B**). Vertical section of clustered conidiomata. (**C**). Conidiomatal wall. (**D**). Conidia. (**E**). Conidiogenous cells and young conidia attached. (**F**). Colony on 2 % MEA showing optimum growth in the dark at 25 °C in 7 d. Scale bars: A = 500 μm; B = 100 μm; C—E = 25 μm.

Consequently, if seed provided the first source of *A. heterophylla* in La Réunion, the *Botryosphaeriaceae* are unlikely to have been transferred at the same time. Most of the species detected in this study have been found elsewhere in the world and anthropogenic movement of plant material could have brought most of them to these islands. Some of the species could be native to their respective hosts or islands but sampling of a wide range of native trees in these areas would be needed to support this notion. Overall, the results of this study show that two closely related trees, *A. koa* and *A. heterophylla*, have been colonised by numerous, most likely invasive, *Botryosphaeriaceae* in the two areas where these trees occur naturally, without any associations relating to the origin of these trees.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.07.001.

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