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Australian cultures of Botryosphaeriaceae held in Queensland and Victoria plant pathology herbaria revisited

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Abstract

The Botryosphaeriaceae is one of the most widespread and cosmopolitan endophytic group of fungi. However, the species of this group can cause severe disease when the hosts are under stressful conditions. The aim of this study was to identify living cultures from the Botryosphaeriaceae family preserved in the Queensland and Victorian Plant Pathology Herbaria using DNA sequence analyses. The 51 isolates were collected between 1971 and 2017, from 35 different host genera, with the dominant host genera being *Mangifera* (11 isolates), *Acacia* (10), and *Persea* (5). Multilocus sequence analyses resulted in the re-identification of 41 isolates to the genera *Botryosphaeria* (2 isolates), *Diplodia* (4), *Dothiorella* (1), *Lasiodiplodia* (19), and *Neofusicoccum* (15), as well as some that belonged to genera outside of the Botryosphaeriaceae (10). New records for Australia were *Botryosphaeria sinensis*, *Diplodia alatafructa*, *Lasiodiplodia gonubiensis*, *Neofusicoccum cryptoaustrale*, and *N. mangroviorum*. These were identified as a result of a workshop organised by the Subcommittee on Plant Health Diagnostics. The results of this study provide the fundamental information regarding the diversity of Botryosphaeriaceae species present in Australian.

Keywords Biosecurity · Diagnostics · Taxonomy

Introduction

The Botryosphaeriaceae (Dothideomycetes: Botryosphaeriales) includes 24 genera of ecologically diverse fungi that occur as saprobes, endophytes or plant pathogens (Slippers et al. 2017; Yang et al. 2017). Some of these fungi are important pathogens of woody plant species, causing dieback and stem cankers, especially in the tropics and subtropics. Several species of Botryosphaeriaceae can remain as latent pathogens in localised infections for many years, facilitating their global spread through trade in agricultural and forestry products (Burgess et al. 2016; Crous et al. 2016).

The accurate identification of Botryosphaeriaceae by DNA sequence data rather than relying on morphological descriptions, provides the best means to halt their spread and reduce the threat of these fungi. Recent taxonomic changes and the recognition of cryptic species have made the identification of species in the Botryosphaeriaceae challenging. Phillips et al. (2013) recommended that at least two loci, the internal transcribed spacer (ITS) region, and the translation elongation factor 1-alpha (*tef1α*), be used for species separation within Botryosphaeriaceae. However, Slippers et al. (2013) recommended the use of four loci, including the ITS region, *tef1α*,

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beta-tubulin (*tub*), and the RNA polymerase II (*rpb2*), as these loci will provide sufficient resolution to distinguish cryptic species. The amplification of *rpb2* is challenging and subsequently there is lack of data for comparisons (Slippers et al. 2013).

Recent research into grapevine trunk diseases has identified at least 14 Botryosphaeriaceae species that impact Australian viticulture (Pitt et al. 2010, 2013, 2015; Wunderlich et al. 2011). Similarly, in Western Australia, many fungi that belong to Botryosphaeriaceae have been associated with dieback of mango and forest trees (Sakalidis et al. 2011a, 2011b, 2013). Further information about the species of Botryosphaeriaceae elsewhere in Australia must be treated with caution as it predates the recent molecular focussed taxonomic revisions.

Australian plant biosecurity is underpinned by the ability to accurately determine what pathogens are present and established in Australia, in order to recognise pathogens that are exotic. National plant pest reference collections, such as the Queensland and Victorian Plant Pathology Herbaria (BRIP and VPRI, respectively), play a crucial role in diagnostics by providing specimen-based records of Australia's plant pathogens. This information can be rapidly accessed by Australian biosecurity practitioners through the Australian Plant Pathogen with Pest Database (Plant Health Australia 2001). In light of ongoing taxonomic revisions, there is a need for specimens in Australian reference collections to be verified, as well as for the continued professional development of Australian plant biosecurity diagnosticians (Hyde et al. 2010). To this end, a workshop was held at the University of Southern Queensland (26–30 June, 2017) to provide training for 23 professional plant pathologists on the latest developments in morphological and molecular methods for the identification and classification of fungi in the Botryosphaeriaceae.

Materials and methods

Specimens and species identification

Living cultures of 51 specimens were sourced from the Queensland Plant Pathology Herbarium (BRIP) and Victorian Plant Pathology Herbarium (VPRI) (Tables 1 and 2). Identification of the specimens to species level required unambiguous DNA sequence reads that matched data from the ex-type reference specimens on GenBank (Table 3).

DNA extraction, PCR amplification and phylogenetic analyses

Mycelia were collected from cultures grown on potato dextrose agar (Difco™, Becton, Dickinson and Company) and macerated with 0.5 mm glass beads (Daintree Scientific) in a Tissue Lyser (QIAGEN). Genomic DNA was extracted with the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions.

The primers V9G (de Hoog and Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the ITS region of the nrDNA, and the amplification of the partial region of the *tef1α* locus was achieved by either the primer sets EF1-728F (Carbone and Kohn 1999) and EF2 (O'Donnell et al. 1998) or EF1-688F and EF1-1251R (Alves et al. 2008). All loci were amplified with the Phusion High-Fidelity PCR Master Mix with HF Buffer (New England Biolabs). The PCR mix included: 12.5 μL of Phusion Master Mix, 0.5 μL of 10 mM of each primer, and 1 μL of DNA template. Sterile water was used as no-template control. The amplification conditions were as follows: initial denaturation of 98 °C for 30 s, followed by 30 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 mins. The amplified products were purified and sequenced by Macrogen Incorporated (Seoul, Korea).

All sequences generated were assembled using Geneious v.9.1.8 (Biomatters Ltd.) and deposited in GenBank (Table 2). These sequences were aligned with selected sequences of ex-type or authentic representative Botryosphaeriaceae genera (Table 3) using the MAFFT alignment algorithm (Katoh et al. 2009) in Geneious. *Pseudofusicoccum stromaticum* strain CBS 117448 was included as the outgroup (Table 3). The sequences of each locus were aligned separately and manually adjusted as necessary. Alignment gaps were treated as missing character states and all characters were unordered and of equal weight. The Markov chain Monte Carlo (MCMC) algorithm was used to create a phylogenetic tree based on Bayesian probabilities using MrBayes v.3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) in Geneious. To remove the need for *a priori* model testing, the MCMC analysis was set to sample across the entire general time-reversible (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 1000 and the temperature of the heated chain was 0.1. Burn-in was set at 25%, after which the likelihood values were stationary. Maximum likelihood (ML) analysis was run using RAxML v.7.2.8 (Stamatakis and Alchiotis 2010) in Geneious and started from a random tree topology. The nucleotide substitution model used was GTR with a gamma-distributed rate variation.

Results

All 51 isolates were successfully amplified for both ITS and *tef1α* and their sequence datasets were analysed individually and in combination. The dataset contained 650 bp for the ITS region and 420 bp for the *tef1α* locus. The ITS and *tef1α* alignments were trimmed to 525 and 333 bp, respectively,

Table 1 Non-Botryosphaeriaceae re-identified based on DNA analyses

Taxon	Strain [a]	Former identification	Host	State[b], city/town/region
Cladosporium sp.	BRIP 52463	*Fusicoccum* sp.	Cycas sp.	Qld, Townsville
Coniothyrum sp.	VPRI 41605 (=BRIP 65675)	*Diplodia* sp.	*Acacia pycnantha*	Vic, Grampians National Park
	VPRI 41618 (=BRIP 65676)		*Acacia retinodes*	Vic, Grampians National
	VPRI 41620 (=BRIP 65677)		*Acacia retinodes*	Vic, Grampians National
	VPRI 41626 (=BRIP 65678)		*Acacia pycnantha*	Vic, Grampians National
Diaporthe sp.	BRIP 52819b	*Fusicoccum* sp.	*Acacia* sp.	Qld, Brisbane
	BRIP 52820		*Acacia* sp.	Qld, Brisbane
	BRIP 52999b		*Acacia* sp.	Qld, Brisbane
Fusarium sp.	BRIP 52819d	*Botrysphaeria* sp.	*Acacia* sp.	Qld, Brisbane
Huntiella sp.	BRIP 28467	*Fusicoccum luteum*	*Mangifera indica*	Qld, Ayr

[a] BRIP, Queensland Plant Pathology Herbarium, Brisbane, Queensland; VPRI, Victorian Plant Pathology Herbarium, Agribio, Bundoora, Victoria

[b] Qld, Queensland; Vic, Victoria

and combined for phylogenetic analyses. The combined alignment was composed of 859 characters from 144 isolates, of which 99 bp (18.9%), and 99 bp (29.4%) were variable for ITS and *tef1α*, respectively. Species identification was confirmed through careful analyses of the combined ITS and *tef1α* sequence data.

Ten isolates that had been deposited as *Botryosphaeria* (1 isolate), *Diplodia* (4), and *Fusicoccum* (5), were identified as non-Botryosphaeriaceae based on BLASTn search results of the ITS sequences against the GenBank database (Table 1). The remaining 41 isolates that had been deposited as *Botryosphaeria* (5), *Dothiorella* (7), *Fusicoccum* (2), *Lasiodiplodia* (8), *Neofusicoccum* (5), and undetermined (9) were re-identified based on analyses of the combined ITS and *tef1α* sequences (Table 2; Fig. 1).

Seven of these re-identified isolates represent five new species records for Australia. One isolate (BRIP 19781) obtained from *Mangifera indica* (Anacardiaceae) in Ayr, Queensland (Qld), was identified as *Botryosphaeria sinensis* based on 100% identity in the ITS and in the *tef1α* to the ex-paratype strain CGMCC 3.17723. One isolate (BRIP 52819a) obtained from *Acacia* sp. (Fabaceae) in Brisbane, Qld, was identified as *Diplodia alatafructa* based on 100% identity in the ITS, and 99% (1 single nucleotide polymorphism) identity in the *tef1α* to the ex-holotype strain CBS 124931 (Fig. 1). Three isolates (BRIP 54897c, 58861, and 54897) obtained from dead branches of *Acmena smithii* (Myrtaceae) and *Lenwebbia lasioclada* (Myrtaceae) in Brisbane, as well as from *Camellia sinensis* (Theaceae) in northern Qld were identified as *Lasiodiplodia gonubiensis* (Fig. 1). All three BRIP isolates differed from the ex-holotype strain CBS 115812 by 1 single nucleotide polymorphism (SNP) in the ITS region, while the isolates from *C. sinensis* (BRIP 54897c) and *L. lasioclada* (BRIP 58861) differed by 1 SNP in the *tef1α* sequence. An isolate (BRIP 63679) from a leaf of *M. indica* in Western Australia was identified as *Neofusicoccum cryptoaustrale* based on 99% (1 SNP) identity in the ITS region, and 99% (1 SNP) identity in the *tef1α* to the ex-type strain CBS 122813 (Fig. 1). An isolate (BRIP 57901) obtained from *Helianthus annuus* (Asteraceae) in a sunflower screening trial at Gatton, Qld, most likely as an endophyte, was identified as *N. mangroviorum* based on 99% identity (1 SNP) in the ITS, and 99% (2 SNP) identity in the *tef1α* to the ex-type strain CMW 41365 (Fig. 1).

Furthermore, four isolates were clustered in three distinct taxa in the current phylogenetic tree (Fig. 1). These isolates will remain as undescribed species as they require more loci sequences to support their introduction as novel species. One isolate (BRIP 24140) is a sister clade to *B. dothidea* and *B. sinensis*, and differs from both species by 4 bp in *tef1α*. Two other isolates (BRIP 58042b and 58969), *Lasiodiplodia* sp., is a sister clade to *L. iraniensis*, *L. jatrophicola*, and *L. thailandica*. *Lasiodiplodia* sp. differs from *L. iraniensis* by 2 bp in ITS and 7 bp in *tef1α*, from *L. jatrophicola* by 3 bp in ITS and 5 bp in *tef1α*, and from *L. thailandica* by an 8 bp deletion in *tef1α*. The isolate, VPRI 13932, represents a distinct taxon in *Dothiorella*, and differs from the other species by a 26 bp deletion in *tef1α*.

Discussion

Multilocus sequence analyses re-identified 41 isolates from the two herbaria into five genera and 20 species, including 18 known species and three unknown species in Botryosphaeriaceae. Five of these species, *Botryosphaeria*

Table 2 List of isolates identified or re-identified by DNA sequencing in this study. New Australian fungal or host records are in **bold**

Taxon	Strain [a]	Former identification	Host	State[b], city/town/region	GenBank Accessions	
					ITS	*tef1α*
Botryosphaeria sinensis	BRIP 19781	*Fusicoccum* sp.	***Mangifera indica***	Qld, Ayr	MH057165	MH102228
***Botryosphaeria* sp.**	BRIP 24140	*Neofusicoccum parvum*	*Mangifera indica*	Qld, Rita Island	MH057166	MH102229
Diplodia africana	VPRI 41783 (=BRIP 53702)	undetermined	***Pinus muricata***	Vic, Melbourne	MH057169	MH102232
	BRIP 53072	*Zasmidium scaevolicola*	***Scaevola taccada***	Qld, Cape Tribulation	MH057168	MH102231
Diplodia alatafructa	BRIP 52819a	*Botryosphaeria* sp.	***Acacia* sp.**	Qld, Brisbane	MH057167	MH102230
Diplodia seriata	VPRI 42125 (=BRIP 65679)	undetermined	***Araucaria heterophylla***	Vic, Melbourne	MH057170	MH102233
***Dothiorella* sp.**	VPRI 13932 (=BRIP 65673)	*Botryosphaeria sarmentorum*	*Alyxia buxifolia*	Vic, Melbourne	MH057171	MH102234
Lasiodiplodia brasiliensis	BRIP 60182e	undetermined	***Gossypium hirsutum***	Qld, Emerald	MH057184	MH102247
Lasiodiplodia gonubiensis	BRIP 58865	*Lasiodiploda* sp.	***Acmena smithii***	Qld, Brisbane	MH057180	MH102243
	BRIP 54897c	*Lasiodiploda* sp.	***Camellia sinensis***	Qld, Topaz	MH057176	MH102239
	BRIP 58861	*Lasiodiploda* sp.	***Lenwebbia lasioclada***	Qld, Brisbane	MH057179	MH102242
Lasiodiplodia iraniensis	BRIP 63318	undetermined	***Vaccinium* sp.**	Qld, Brisbane	MH057172	MH102235
Lasiodiplodia mahajangana	BRIP 63052	undetermined	***Annona reticulata***	Qld, Alloway	MH057187	MH102250
	BRIP 63346	*Lasiodiplodia* sp.	***Musa* sp.**	Qld, Upper Daradgee	MH057188	MH102251
	BRIP 55402	*Lasiodiplodia theobromae*	***Persea americana***	NSW, Duranbah	MH057177	MH102240
Lasiodiplodia pseudotheobromae	BRIP 64096b	*Lasiodiplodia* sp.	*Annona muricata*	Qld, Tully	MH057189	MH10222
	BRIP 53572	undetermined	*Dimocarpus longan*	Qld, Mareeba	MH057174	MH102237
	BRIP 53606	undetermined	*Macadamia* sp.	Qld, Tolga	MH057175	MH102238
	BRIP 51631	*Lasiodiplodia theobromae*	*Mangifera indica*	Qld, Gumlu	MH057173	MH102236
	BRIP 62846	*Lasiodiplodia theobromae*	*Rosa* sp.	Qld, Tolga	MH057185	MH102248
Lasiodiplodia theobromae	BRIP 58919	*Botryosphaeria* sp.	*Syzygium nervosum*	Qld, Brisbane	MH057182	MH102245
	BRIP 58866	*Botryosphaeria* sp.	*Syzygium wilsonii*	Qld, Brisbane	MH057181	MH102244
	BRIP 62872	*Lasiodiplodia* sp.	*Pinus caribaea*	Qld, Kalpower	MH057186	MH102249
	BRIP 64718	undetermined	*Passiflora edulis*	Qld, Cooktown	MH057190	MH102253
***Lasiodiplodia* sp.**	BRIP 58969	undetermined	*Acacia mangium*	Qld, Mareeba	MH057183	MH102246
	BRIP 58042b	*Lasiodiplodia* sp.	*Vitis vinifera*	Qld, Dimbulah	MH057178	MH102241
Neofusicoccum australe	VPRI 42853 (=BRIP 65680)	*Botryosphaeria* sp.	*Banksia* sp.	Vic, Mornington	MH057204	MH102267
	VPRI 42863 (=BRIP 65681)	undetermined	***Juglans* sp.**	NSW, Leeton	MH057205	MH102268
	BRIP 59728	undetermined	*Persea americana*	WA, Kalamunda	MH057198	MH102261
Neofusicoccum cryptoaustrale	BRIP 63679	*Neofusicoccum* sp.	***Mangifera indica***	WA, Northampton	MH057200	MH102263
Neofusicoccum luteum	BRIP 5016	*Dothiorella aromatica*	*Persea americana*	Qld, Brisbane	MH057191	MH102254
	BRIP 54746	*Neofusicoccum parvum*	*Mangifera indica*	Qld, Mundubbera	MH057194	MH102257
Neofusicoccum mangroviorum	BRIP 57901	*Neofusicoccum luteum*	***Helianthus annuus***	Qld, Gatton	MH057196	MH102259
Neofusicoccum occulatum	BRIP 64094	*Lasiodiplodia theobromae*	***Vaccinium* sp.**	Qld, Tolga	MH057202	MH102265
Neofusicoccum parvum	BRIP 19486	*Dothiorella dominicana*	*Persea americana*	Qld, Maleny	MH057192	MH102255
	BRIP 55401	*Dothiorella* sp.	*Persea americana*	WA, Gingin	MH057195	MH102258
	BRIP 62250a	*Dothiorella* sp.	*Persea americana*	WA, Busselton	MH057199	MH102262
	BRIP 65440	*Dothiorella* sp.	*Mangifera indica*	Qld, Spring Creek	MH057195	MH102266
	BRIP 24083	*Fusicoccum mangiferae*	*Mangifera indica*	Qld, Bowen	MH057193	MH102256
	BRIP 58868	*Botryosphaeria* sp.	***Xanthostemon* sp.**	Qld, Beerburrum	MH057197	MH102260
Neofusicoccum vitifusiforme	BRIP 64010	*Neofusicoccum* sp.	***Geijera salicifolia***	Qld, Kingsthorpe	MH057201	MH102264

[a] BRIP, Queensland Plant Pathology Herbarium, Brisbane, Qld; VPRI, Victorian Plant Pathology Herbarium, Agribio, Bundoora, Vic

[b] NSW, New South Wales; Qld, Queensland; Vic, Victoria; WA, Western Australia

sinensis, *Diplodia alatafructa*, *Lasiodiplodia gonubiensis*, *Neofusicoccum cryoptoaustrale,* and *N. mangroviorum*, are reported for the first time in Australia. New hosts are reported for 14 species, namely *B. sinensis*, *D. africana*, *D. alatafructa*, *D. seriata*, *L. brasiliensis*, *L. gonubiensis*, *L. iraniensis, L. mahajangana*, *N. australe*, *N. cryptoaustrale*, *N. mangroviorum, N. occulatum, N. parvum*, and *N. vitifusiforme*.

Two *Botryosphaeria* species were identified in this study, *B. sinensis* and an undescribed *Botryosphaeria* sp. *Botryosphaeria sinensis* was recently described from *Juglans regia* (Juglandaceae), *Morus alba* (Moraceae), and *Populus* sp. (Salicaceae) in China (Zhou et al. 2016), as a sister taxon to *B. dothidea*. The isolate, BRIP 19781, from *M. indica* represents a new species record for Australia, and a new host association.

Table 3 List of reference sequences included in phylogenetic analyses

Taxon	Strain [a]	Host	Country	GenBank Accessions	
				ITS	*tef1α*
Botryosphaeria dothidea	CBS 115476^{ET}	*Prunus* sp.	Switzerland	AY236949	AY236898
Botryosphaeria fabicerciana	CBS 127193^{HT}	*Eucalyptus* sp.	China	HQ332197	HQ332213
Botryosphaeria fusispora	MFLUCC 10-0098^{HT}	*Entada* sp.	Thailand	JX646789	JX646854
Botryosphaeria ramosa	CBS 122069^{HT}	*Eucalyptus camaldulensis*	Australia	EU144055	EU144070
Botryosphaeria scharifii	CBS 124703^{IS}	*Mangifera indica*	Iran	JQ772020	JQ772057
Botryosphaeria sinensis	CGMCC 3.17723^{PT}	*Populus* sp.	China	KT343254	KU221233
Diplodia africana	CBS 120835^{HT}	*Prunus persica*	South Africa	EF445343	EF445382
Diplodia alatafructa	CBS 124931^{HT}	*Pterocarpus angolensis*	South Africa	FJ888460	FJ888444
Diplodia allocellula	CBS 130408^{HT}	*Acacia karroo*	South Africa	JQ239399	JQ239386
Diplodia crataegicola	MFLU 15-1311^{HT}	*Crataegus* sp.	Italy	KT290244	KT290248
Diplodia estuarina	CMW 41230^{PT}	*Avicennia marina*	South Africa	KP860831	KP860676
Diplodia fraxini	CBS 136010^{NT}	*Fraxinus angustifolia*	Portugal	KF307700	KF318747
Diplodia galiicola	MFLU 15-1310^{HT}	*Galium* sp.	Italy	KT290245	KT290249
Diplodia seriata	CBS 112555^{ET}	*Vitis vinifera*	Portugal	AY259094	AY573220
Dothiorella americana	CBS 128309^{HT}	*Vitis vinifera*	USA	HQ288218	HQ288262
Dothiorella californica	CBS 141587^{HS}	*Umbellularia californica*	USA	KX357188	KX357211
Dothiorella iberica	CBS 115041^{HT}	*Quercus ilex*	Spain	AY573202	AY573222
Dothiorella omnivora	CBS 140349^{HT}	*Corylus avellana*	Italy	KP205497	KP205470
Dothiorella parva	IRAN1579C^{HT} (=CBS 124720^{IS})	*Corylus avellana*	Iran	KC898234	KC898217
Dothiorella sarmentorum	IMI 63581b^{HT}	*Ulmus* sp.	England	AY573212	AY573235
Dothiorella sempervirentis	IRAN1583C^{HT} (=CBS 124718^{IS})	*Cupressus sempervirens*	Iran	KC898236	KC898219
Dothiorella symphoricarposicola	MFLUCC 13-0497^{IS}	*Symphoricarpos* sp.	Italy	KJ742378	KJ742381
Dothiorella vidmadera	DAR 78992^{HT}	*Vitis vinifera*	Australia	EU768874	EU768881
Lasiodiplodia brasiliensis	CMM 4015^{HT}	*Mangifera indica*	Brazil	JX464063	JX464049
Lasiodiplodia bruguierae	CMW 41470^{HT}	*Bruguiera gymnorrhiza*	South Africa	KP860832	KP860677
Lasiodiplodia caatinguensis	CMM 1325^{HT}	*Citrus sinensis*	Brazil	KT154760	KT008006
Lasiodiplodia exigua	CBS 137785^{HT}	*Quercus ilex*	Tunisia	KJ638317	KJ638336
Lasiodiplodia gonubiensis	CBS 115812^{HT}	*Syzygium cordatum*	South Africa	AY639595	DQ103566
Lasiodiplodia gravistriata	CMM 4564	*Anacardium humile*	Brazil	KT250949	KT250950
Lasiodiplodia iraniensis	CBS 124710^{HT}	*Salvadora persica*	Iran	GU945346	GU945334
Lasiodiplodia jatrophicola	CMM 3610^{HT}	*Jatropha curcas*	Brazil	KF234544	KF226690
Lasiodiplodia macrospora	CMM 3833^{HT}	*Jatropha curcas*	Brazil	KF234557	KF226718
Lasiodiplodia mahajangana	CBS 124927^{IS}	*Terminalia catappa*	Madagascar	FJ900597	FJ900643
Lasiodiplodia pseudotheobromae	CBS 116459^{HT}	*Gmelina arborea*	Costa Rica	EF622077	EF622057
Lasiodiplodia subglobosa	CMM 3872^{HT}	*Jatropha curcas*	Brazil	KF234558	KF226721
Lasiodiplodia thailandica	CBS 138760^{HT} (=CPC 22795)	*Mangifera indica*	Thailand	KJ193637	KJ193681
Lasiodiplodia theobromae	CBS 164.96^{NT}	unknown fruit on coral reef coast	Papua New Guinea	AY640255	AY640258
Neofusicoccum australe	CMW 6837 ^{HT}	*Acacia* sp.	Australia	AY339262	AY339270
Neofusicoccum cryptoaustrale	CBS 122813^{HT}	*Eucalyptus* sp.	South Africa	FJ752742	FJ752713
Neofusicoccum eucalypticola	CBS 115766^{IS}	*Eucalyptus grandis*	Australia	AY615143	AY615135
Neofusicoccum eucalyptorum	CBS 115791	*Eucalyptus grandis*	South Africa	AF283686	AY236891
Neofusicoccum luteum	CBS 110299^{HT}	*Vitis vinifera*	Portugal	AY259091	AY573217
Neofusicoccum mangiferae	CBS 118531	*Mangifera indica*	Australia	AY615185	DQ093221
Neofusicoccum mangroviorum	CMW 41365^{HT}	*Avicennia marina*	South Africa	KP860859	KP860702
Neofusicoccum mediterraneum	CBS 121718^{HT}	*Eucalyptus* sp.	Greece	GU251176	GU251308

Table 3 (continued)

Taxon	Strain [a]	Host	Country	GenBank Accessions	
				ITS	*tef1α*
Neofusicoccum occulatum	CBS 128008^{HT}	*Eucalyptus grandis*	Australia	EU301030	EU339509
Neofusicoccum parvum	CMW 9081^{ET}	*Populus nigra*	New Zealand	AY236943	AY236888
Neofusicoccum ursorum	CMW 24480^{HT} (=CBS 122811^{IS})	*Eucalyptus* sp.	South Africa	FJ752746	FJ752709
Neofusicoccum vitisiforme	CBS 110887^{HT}	*Vitis vinifera*	South Africa	AY343383	AY343343
Pseudofusicoccum stromaticum	CBS 117448^{HT}	*Eucalyptus urophylla*	Venezuela	AY693974	AY693975

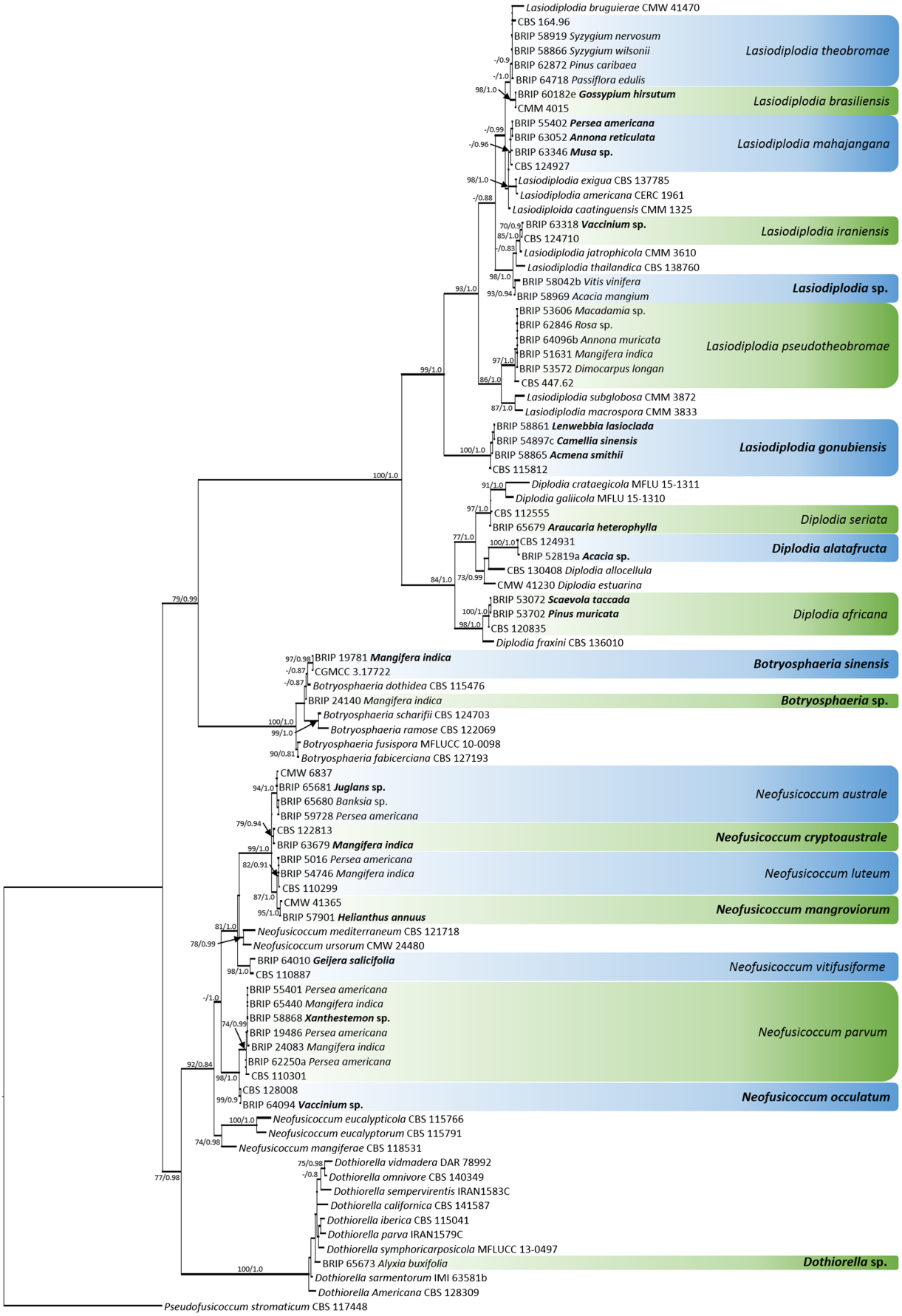
[a] CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CERC, Culture Collection of China Eucalypt Research Centre, Chinese Academy of Forestry, ZhanJiang, GuangDong, China; CGMCC, China General Microbiological Culture Collection Center, Institute of Microbiology, Chinses Academy of Sciences, China; CMM, Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes, Federal Rural University of Pernambuco, Brazil; CMW, Collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DAR, New South Wales Plant Pathology Herbarium, Orange, NSW; GZCC, Guizhou Academy of Agricultural Sciences, Guizhou, China; IMI, CABI Genetic Resource Collection, Surrey, UK; MUCC, Mie University Culture Collection, Tsu City, Mie Prefecture, Japan

Ex-type isolates: ^{ET}, ex-epitype; ^{HT}, ex-holotype; ^{IS}, ex-isotype; ^{NT}, ex-neotype; ^{PT}, ex-paratype

Three *Diplodia* species, including *D. africana*, *D. alatafructa* and *D. seriata*, were identified in this study. *Diplodia africana* was first described as a potential pathogen on *Prunus* spp. in South Africa (Damm et al. 2007), and has since been found on *Juniperus phoenicea* (Cupressaceae) in Italy (Alves et al. 2014). In this study, *D. africana* was identified on *Pinus muricata* (Pinaceae) and *Scaevola taccada* (Goodeniaceae). *Diplodia alatafructa* was first described from a stem wound on *Pterocarpus angolensis* (Fabaceae) in South Africa (Mehl et al. 2011), and has been shown to cause stem lesions and vascular discolouration on *Eriobotrya japonica* (Rosaceae) in Spain (González-Domínguez et al. 2017). The isolate of *D. alatafructa* (BRIP 52819a) from *Acacia* sp. represents a new species record for Australia. *Diplodia seriata* has over 300 host associations and is found worldwide (Farr and Rossman 2017). Despite its plurivorous nature, the identification of *D. seriata* on *Araucaria heterophylla* (Araucariaceae) in Australia represents an extension of its host family. Results of this study not only expand the host associations for these three species, but also a new geographical location for *D. alatafructa*.

Seven *Lasiodiploda* species were identified in this study, *L. brasiliensis, L. gonubiensis, L. iraniensis, L. mahajangana, L. pseudotheobromae, L. theobromae,* and an undescribed *Lasiodiplodia* sp. *Lasiodiplodia brasiliensis* was originally described as a minor pathogen associated with stem-end rot of *Carica papaya* (Caricaceae) and of *M. indica* in Brazil (Marques et al. 2013; Netto et al. 2014). Since then, it has been isolated from other hosts in Brazil, including *Anacardium occidentale* (Anacardiaceae), *Annona squamosa* (Annonaceae), *Cocos nucifera* (Arecaceae), *Spondias purpurea* (Anacardiaceae), and *Vitis vinifera* (Vitaceae) (Cardoso et al. 2017; Correia et al. 2016; Coutinho et al. 2017; Netto et al. 2017; Rosado et al. 2015). It has also been reported from other countries, including in Madagascar from *Adansonia madagascariensis* (Malvaceae), in Thailand from *Tectona grandis* (Lamiaceae) and in Turkey from *Fragaria* × *ananassa* (Rosaceae) (Cruywagen et al. 2017; Doilom et al. 2015). The isolate, BRIP 60182e, from *Gossypium hirsutum* (Malvaceae) represents an extension of its host range. *Lasiodiplodia gonubiensis* was originally described as an endophyte from *Syzygium cordatum* (Myrtaceae) in South Africa (Pavlic et al. 2004), where it has subsequently been isolated from healthy and/or diseased *Bruguiera gymnorrhiza* (Rhizophoraceae), *Ceriops tagal* (Rhizophoraceae), *Sclerocarya birrea* subsp. *caffra* (Anacardiaceae), and *Vachellia karroo* (Fabaceae) in South Africa (Jami et al. 2015, 2017; Osorio et al. 2017; Mehl et al. 2017). *Lasiodiplodia gonubiensis* has also been reported from *Adansonia digitata* (Malvaceae) in Mozambique, *Anacardium humile* (Anacardiaceae) in Brazil, and *Phyllanthus emblica* (Phyllanthaceae) in Thailand (Cruywagen et al. 2017; Netto et al. 2017; Trakunyingcharoen et al. 2015). The isolates in this study represent the first record of *L. gonubiensis* in Australia, as well as new host associations for this species. *Lasiodiplodia iraniensis* has been isolated from various hosts in Iran, namely *Citrus* sp. (Rutaceae), *Eucalyptus* sp. (Myrtaceae), *Juglans* sp. (Jualandaceae), *M. indica, Salvadora persica* (Salvadoraceae), and *Terminalia catappa* (Combretaceae) (Abdollahzadeh et al. 2010; Mohammadi et al. 2013). It has also been reported from *A. digitata* throughout central and southern Africa (Cruywagen

Fig. 1 Phylogenetic tree based on maximum likelihood analysis of the combined ITS and *tef1α* alignment. 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 *Pseudofusicoccum stromaticum* ex-type strain CBS 117448. New species reported in Australia and new host records are in **bold**.


Lasiodiplodia bruguierae CMW 41470
CBS 164.96
BRIP 58919 Syzygium nervosum
BRIP 58866 Syzygium wilsonii
BRIP 62872 Pinus caribaea
BRIP 64718 Passiflora edulis
Lasiodiplodia theobromae
BRIP 60182e Gossypium hirsutum
CMM 4015
Lasiodiplodia brasiliensis
BRIP 55402 Persea americana
BRIP 63052 Annona reticulata
BRIP 63346 Musa sp.
CBS 124927
Lasiodiplodia mahajangana
Lasiodiplodia exigua CBS 137785
Lasiodiplodia americana CERC 1961
Lasiodiploida caatinguensis CMM 1325
BRIP 63318 Vaccinium sp.
CBS 124710
Lasiodiplodia iraniensis
Lasiodiplodia jatrophicola CMM 3610
Lasiodiplodia thailandica CBS 138760
BRIP 58042b Vitis vinifera
BRIP 58969 Acacia mangium
Lasiodiplodia sp.
BRIP 53606 Macadamia sp.
BRIP 62846 Rosa sp.
BRIP 64096b Annona muricata
BRIP 51631 Mangifera indica
BRIP 53572 Dimocarpus longan
CBS 447.62
Lasiodiplodia pseudotheobromae
Lasiodiplodia subglobosa CMM 3872
Lasiodiplodia macrospora CMM 3833
BRIP 58861 Lenwebbia lasioclada
BRIP 54897c Camellia sinensis
BRIP 58865 Acmena smithii
CBS 115812
Lasiodiplodia gonubiensis
Diplodia crataegicola MFLU 15-1311
Diplodia galiicola MFLU 15-1310
CBS 112555
BRIP 65679 Araucaria heterophylla
Diplodia seriata
CBS 124931
BRIP 52819a Acacia sp.
Diplodia alatafructa
CBS 130408 Diplodia allocellula
CMW 41230 Diplodia estuarina
BRIP 53072 Scaevola taccada
BRIP 53702 Pinus muricata
CBS 120835
Diplodia africana
Diplodia fraxini CBS 136010
BRIP 19781 Mangifera indica
CGMCC 3.17722
Botryosphaeria sinensis
Botryosphaeria dothidea CBS 115476
BRIP 24140 Mangifera indica
Botryosphaeria sp.
Botryosphaeria scharifii CBS 124703
Botryosphaeria ramose CBS 122069
Botryosphaeria fusispora MFLUCC 10-0098
Botryosphaeria fabicerciana CBS 127193
CMW 6837
BRIP 65681 Juglans sp.
BRIP 65680 Banksia sp.
BRIP 59728 Persea americana
Neofusicoccum australe
CBS 122813
BRIP 63679 Mangifera indica
Neofusicoccum cryptoaustrale
BRIP 5016 Persea americana
BRIP 54746 Mangifera indica
CBS 110299
Neofusicoccum luteum
CMW 41365
BRIP 57901 Helianthus annuus
Neofusicoccum mangroviorum
Neofusicoccum mediterraneum CBS 121718
Neofusicoccum ursorum CMW 24480
BRIP 64010 Geijera salicifolia
CBS 110887
Neofusicoccum vitifusiforme
BRIP 55401 Persea americana
BRIP 65440 Mangifera indica
BRIP 58868 Xanthestemon sp.
BRIP 19486 Persea americana
BRIP 24083 Mangifera indica
BRIP 62250a Persea americana
CBS 110301
Neofusicoccum parvum
CBS 128008
BRIP 64094 Vaccinium sp.
Neofusicoccum occulatum
Neofusicoccum eucalypticola CBS 115766
Neofusicoccum eucalyptorum CBS 115791
Neofusicoccum mangiferae CBS 118531
Dothiorella vidmadera DAR 78992
Dothiorella omnivore CBS 140349
Dothiorella sempervirentis IRAN1583C
Dothiorella californica CBS 141587
Dothiorella iberica CBS 115041
Dothiorella parva IRAN1579C
Dothiorella symphoricarposicola MFLUCC 13-0497
BRIP 65673 Alyxia buxifolia
Dothiorella sp.
Dothiorella sarmentorum IMI 63581b
Dothiorella Americana CBS 128309
Pseudofusicoccum stromaticum CBS 117448

et al. 2017), *A. occidentale* in Brazil (Netto et al. 2017), *M. indica* in Australia, Brazil and Peru (Netto et al. 2017; Rodriguez-Galvez et al. 2017; Sakalidis et al. 2011b), *S. persica* in Colombia (Úrbez-Torres et al. 2012b), and *Sclerocarya birrea* subsp. *caffra* (Anacardiaceae) in South Africa (Mehl et al. 2017). The isolate, BRIP 63318, from *Vaccinium* sp. (Ericaceae) represents a new host association for *L. iraniensis*. *Lasiodiplodia mahajangana* is predominantly associated with woody hosts in the southern Africa continent (Begoude et al. 2010; Jami et al. 2017; Mehl et al. 2017; Phillips et al. 2013). The isolates in this study represents expansion of its host range to include *Annona reticulata* (Annonaceae) and *Persea americana* (Lauraceae), and an herbaceous host, *Musa* sp. (Musaceae).

Seven *Neofusicoccum* species were identified in this study, including *N. australe, N. cryptoaustrale, N. luteum, N. mangroviorum, N. occulatu, N. parvum* and *N. vitifusiforme*. *Neofusicoccum australe* has been reported from 73 different hosts mainly from countries located in the southern hemisphere (Farr and Rossman 2017). Despite its plurivorous nature, the identification of *N. australe* on *Juglans* sp. in this study represents an extension of its host range. *Neofusicoccum cryptoaustrale* was first described as an endophyte from branches and leaves of *Eucalyptus* trees in South Africa (Pavlic-Zupanc et al. 2013), where it has subsequently been isolated from healthy and/or diseased *Avicennia marina* (Acanthaceae), *Barringtonia racemosa (*Lecythidaceae), *Bruguiera gymnorrhiza (*Rhizophoraceae*)*, *Ceriops tagal (*Rhizophoraceae*)*, *Eucalyptus* spp., *Lumnitzera racemose (*Combretaceae), *Podocarpus henkelii (*Podocarpaceae*)*, *P. latifolius (*Podocarpaceae*)*, and *Rhizophora mucronata (*Rhizophoraceae*)* (Osorio et al. 2017; Pavlic-Zupanc et al. 2017). The isolate in this study represents the first record of *N. cryptoaustrale* in Australia, as well as a new host association. *Neofusicoccum mangroviorum* was isolated from symptomless branches of four genera of mangrove (*Avicennia*, *Bruguiera*, *Lumnitzera*, and *Rhizophora*) and *Mimusops caffra* (Sapotaceae) in South Africa (Osorio et al. 2017, Jami et al. unpublished). The identification of this species on *H. annuus* represents a new species record for Australia, and a new host association. *Neofusicoccum occulatum* was first described from *Eucalyptus* spp. (Myrtaceae) and *Wollemia nobilis* (Araucariaceae), as pathogens on stems of *E. globulus* (Sakalidis et al. 2011a). *Neofusicoccum occulatum* has since been isolated from other woody hosts, such as *Blepharocalyx salicifolius* (Myrtaceae) in Uruguay, *Grevillea* sp. (Proteaceae) in Uganda, *Eucalyptus* spp. in Hawaii, and *V. vinifera* in Australia (Sakalidis et al. 2013). The identification of *N. occulatum* on *Vaccinium* sp. represents a host new host association. *Neofusicoccum parvum* has been reported globally from over 150 different hosts (Farr and Rossman 2017). Despite its plurivorous nature, the identification of *N. parvum* on *Xanthostemon* sp., a tree endemic only to north eastern Qld, represents a new host association. *Neofusicoccum vitifusiforme* has a wide host range having been found to cause, or be associated with, grapevine dieback in South Africa (van Niekerk et al. 2004, who first described and named this species *Fusicoccum vitifusiforme*), Spain (Luque et al. 2009), Mexico (Candolfi-Arballo et al. 2010), USA (Úrbez-Torres 2011) and Italy (Mondello et al. 2013); olive (*Olea europaea*) drupe rot in Italy (Lazzizera et al. 2008; Úrbez-Torres et al. 2012a); dieback of stone-fruit trees (*Prunus* spp.) (Damm et al. 2007) and pome fruit trees (*Malus* and *Pyrus* spp.) in South Africa (Cloete et al. 2011), and blight of blueberry (*Vaccinium corymbosum*) in China (Kong et al. 2010). In this study, *N. vitifusiforme* was identified on leaves of *Geijera salicifolia* (Rutaceae), which is native to dry rainforests in eastern Australia, and represents a new host association.

Species in the Botryosphaeriaceae are spreading around the world, likely facilitated by movement of plant material, including fruits. These fungi are virtually impossible to detect in their endophytic state (Burgess et al. 2016). Even where symptoms are visible, biosecurity measures, including quarantining plant material, must no longer rely on morphological identifications and outdated taxonomy for this group of fungi (Crous et al. 2016). The re-identification of 41 isolates in this study based on phylogenetic analyses of the ITS and *tef1*α loci demonstrates the inadequacy of morphological characters for species level identifications. Ten isolates were identified as not belong to Botryosphaeriaceae, which also illustrates the difficulties faced by plant pathologists and plant diagnosticians even at the generic level. This has also shown to be the case for *Colletotrichum* (Shivas and Tan 2009; Shivas et al. 2016), *Fusarium* (Summerell et al. 2011), *Phytophthora* (Burgess et al. 2009), downy mildew (Shivas et al. 2012), and powdery mildew (Cunnington et al. 2003). Thus, laboratory capability to identify these fungi must be maintained and extensive reference collections supported if effective surveillance and monitoring of the family is to continue.

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