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Overlap of latent pathogens in the Botryosphaeriaceae on a native and agricultural host

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ABSTRACT

Some species of the Botryosphaeriaceae are capable of infecting a broad range of host plants. We studied the species diversity of Botryosphaeriaceae associated with marula (*Sclerocarya birrea* subsp. *caffra*, Anacardiaceae) trees in South Africa over two seasons, as well as species common to both *S. birrea* and adjacent mango (*Mangifera indica*, Anacardiaceae) trees in a subset of sites. Gene flow amongst populations of Botryosphaeriaceae shared on these tree species was tested using microsatellite markers. Twelve species were identified from *S. birrea* and eleven species were found on *M. indica* trees. From isolations done in 2006, the dominant species on *S. birrea* was *Neofusicoccum vitifusiforme*, while *N. parvum* was the dominant species isolated from *M. indica*. *Neofusicoccum parvum* was dominant in isolations from both hosts in 2012. Isolates of *Botryosphaeria fabicerciana*, *Lasiodiplodia maha-jangana*, *L. pseudotheobromae*, *L. theobromae*, *N. mediterraneum*, and *N. umdonicola* were also collected from both hosts. Population genetic analyses on isolates of *N. parvum* suggested that three populations were present, each comprising isolates from both hosts. There was significant gene flow between *N. parvum* populations on these hosts. This ability to infect multiple hosts and to migrate amongst them facilitates the establishment and spread of species and genotypes of the Botryosphaeriaceae, such as *N. parvum*, in new areas.

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Introduction

Fungi in the Botryosphaeriaceae are well known as endophytic and opportunistic pathogens of woody plants. These fungi

infect plants via wounds or through natural plant openings such as buds, lenticels, and stomata (Slippers & Wingfield 2007). Many species in the family have a wide range of plant hosts, including commercial fruit crops (van Niekerk et al.

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2004; Slippers et al. 2005; Chen et al. 2014), forest trees (Burgess et al. 2006b; Slippers et al. 2009), and plants in native woody ecosystems (Pavlic et al. 2007; Mehl et al. 2011; Jami et al. 2014). These fungi occur in healthy plant tissues as latent pathogens and persist endophytically until stress occurs, after which disease symptoms can manifest (Slippers & Wingfield 2007).

The spores (sexual and asexual) of *Botryosphaeriaceae* are principally dispersed by wind or rain splash (Swart et al. 1987; Mehl et al. 2013). Since many of the *Botryosphaeriaceae* have broad host ranges (Slippers & Wingfield 2007; Jami et al. 2014), these fungi can spread to and infect both related and unrelated plants. There are many examples of inter-host exchanges of the *Botryosphaeriaceae*, and these include those amongst and between native and non-native trees. For example, species of the *Botryosphaeriaceae* have been shown to move between trees in native stands of *Eucalyptus* (*Myrtaceae*) and adjacent plantations of these trees (Burgess et al. 2006b), between native waterberry trees (*Syzygium cordatum*; *Myrtaceae*) and related eucalypt plantations (*Myrtaceae*) (Pavlic et al. 2007), from *Pinus resinosa* windbreaks to pine nurseries (Stanosz et al. 2007), among various tree hosts in the *Casuarinaceae*, *Cupressaceae*, *Fabaceae*, *Myrtaceae*, *Proteaceae*, *Santalaceae* (Sakalidis et al. 2011), and among native *Terminalia* spp. (*Combretaceae*) and between these trees and *Theobroma cacao* (*Malvaceae*) (Begoude et al. 2012), amongst others.

The ability of fungi such as the *Botryosphaeriaceae* to infect multiple hosts, increases the threat that they pose as potential economic and ecological important pathogens of native and cultivated trees globally. In South Africa, two related tree species, the native *Sclerocarya birrea* subsp. *caffra* known locally as marula, and non-native mango (*Mangifera indica*), in the *Anacardiaceae* commonly occur in close proximity to each other.

Mangifera indica is native to India and is an important subtropical crop cultivated in various countries, including South Africa (Snyman 1998). Species of the *Botryosphaeriaceae* are associated with two important diseases on *M. indica* globally. These include stem-end rot on fruit which occurs when these fungi gain entrance via the peduncle (Johnson & Kotzé 1994) causing disease when fruits ripen or are harvested (Menge & Ploetz 2003). The *Botryosphaeriaceae* can also infect *M. indica* via wounds that occur during fruit abscission, pruning or hand-picking, or via lenticels on the fruit surface (Menge & Ploetz 2003). Another important disease known as blossom blight occurs when *Botryosphaeriaceae* infect the *M. indica* inflorescences (Ploetz 2003).

Sclerocarya birrea is an iconic native African tree with a broad geographic range that extends from Senegal through Ethiopia to South Africa and into Angola and Namibia (Peters 1988). It is extensively used by local communities and is prominent in the production of well-known liqueur (Shackleton et al. 2002). Little is known regarding the diseases of *S. birrea* but a few fungi (7 species) have been recorded, and none of these include the *Botryosphaeriaceae*. This is likely due to a very limited number of studies that have considered the fungi associated with this tree species (Doidge 1950; Crous et al. 2000; Farr & Rossman 2016).

The aims of this study were to determine which species of the *Botryosphaeriaceae* infect *S. birrea* trees in South Africa. Since *S. birrea* and *M. indica* trees are taxonomically related

and grow in close proximity to each other, *M. indica* trees were also sampled. This was principally to determine whether species of the *Botryosphaeriaceae* might be common to both trees. A subsequent aim was to seek evidence of gene flow in specific species of the *Botryosphaeriaceae* that occur on both *S. birrea* and *M. indica*.

Materials and methods

Sample collections and isolations

Two sample collections were made in 2006 and 2012. In 2006, branches from *Sclerocarya birrea* trees were sampled at three locations: Skukuza/Pretoriuskop area in the Kruger National Park (Mpumalanga Province), Hans Merensky estate close to Hoedspruit (Limpopo Province), and Lakelands, Mfolozi Village in the KwaZulu-Natal Province. One hundred and forty four branches from 130 *Mangifera indica* trees were also sampled at the Hans Merensky estate from two orchards; one orchard where trees were chemically treated and a second where trees were organically grown. For the former, 15 branches were sampled from the central tree and then four trees in a 10 m diagonal to this tree were sampled (one branch each). This was followed by sampling one branch from 15 trees in the vicinity of each of the four trees, making up 79 branches from 65 trees. In the organic orchard, the same strategy was used except that a single branch was sampled from the central tree, resulting in 65 branches sampled. In 2012, three to five branches per tree were collected from neighbouring *S. birrea* and nearby *M. indica* trees alongside the road between Hoedspruit and Klaserie (Limpopo Province). Two sites along this road, less than 10 km apart, were sampled and these included six *M. indica* trees and three *S. birrea* trees at the first site, and 13 *M. indica* trees and 14 *S. birrea* trees at the second site.

Isolations were made from discoloured pith tissue, leaf samples, edges of visible lesions, and from asymptomatic twigs following the method described by Pavlic et al. (2004). Isolations were made one and four weeks after sampling for the 2006 samples and two, four, six, and eight weeks after sampling for the 2012 samples. Resulting cultures were purified and isolates resembling the *Botryosphaeriaceae* retained for further study.

Isolates from the 2006 collections were transferred to 2 % water agar (Biolab, South Africa) overlaid with sterile pine needles and incubated under near ultraviolet light (Smith et al. 1996) at 25 °C. Fruiting structures were sectioned and spores examined microscopically to group isolates into genera. Isolates collected in 2012 were purified using single hyphal tip transfers (Mehl et al. 2011). Cultures used in this study have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, Pretoria, South Africa.

DNA extractions

DNA was extracted from all isolates collected in both 2006 and 2012 for identification using DNA sequence data comparisons. For the 2006 isolates, DNA was extracted using the method of

van Wyk et al. (2006) while the method of Wright et al. (2010), with the exception that DNA pellets were suspended in 50 μ l TE buffer, was used for the 2012 isolates.

PCR and DNA sequencing reactions

Isolate identification was done using data from the ITS rDNA (which included the ITS1, 5.8S nrRNA gene, and ITS2), translation elongation factor 1 α (*tef1*), and β -tubulin-2 (*tub2*) loci. Primer sets ITS1 and ITS4 (White et al. 1990), EF1-728F and EF1-986R (Carbone & Kohn 1999) and EF1F and EF2R (Jacobs et al. 2004), and Bt-2a and Bt-2b (Glass & Donaldson 1995) were used to amplify the ITS rDNA, *tef1*, and *tub2* loci, respectively.

For PCR amplifications, ~ 5–30 ng template DNA was combined with one of three different mixtures to successfully amplify loci for DNA sequencing. The first mix consisted of 1.5 \times FastStart PCR buffer (with 3 mM added MgCl₂) (Roche Molecular Biochemicals, Alameda, California), 0.2 μ M of each primer, 2.5 μ M of each dNTP, and 0.5 U FastStart Taq Polymerase (Roche). The second mix consisted of 1 \times KAPA Taq Buffer A (KAPA Biosystems, Cape Town, South Africa), 0.4 μ M of each primer, 2.5 mM of each dNTP, and 1 U KAPA Taq Polymerase (KAPA Biosystems). The third mix consisted of 1 \times MyTaq Reaction Buffer (Bioline, Germany), 0.2 mM of each primer, and 0.5 U MyTaq DNA Polymerase (Bioline). Sterile Sabax water (Adcock Ingram, Johannesburg, South Africa) was added to adjust mixtures to a volume of 25 μ l per reaction.

One of two PCR cycling conditions were used to successfully amplify loci of isolates collected. The first set of cycling conditions consisted of an initial denaturation step of 95 $^{\circ}$ C for 2 min followed by 40 cycles of 95 $^{\circ}$ C for 20 s, 55 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 1 min 30 s, followed by a final extension step of 72 $^{\circ}$ C for 7 min. PCR products were visualized using 1.5 % agarose-ethidium bromide gels run in 1 \times TAE buffer and product sizes estimated using a Lambda DNA/EcoRI + HindIII marker 3 (Fermentas Life Sciences, USA). The second set of PCR cycling conditions and the method used to visualize products were the same as those described by Mehl et al. (2014).

PCR products were purified and sequenced using the methods described by Mehl et al. (2011). Sequences generated during this study were deposited in GenBank (Table 1) and datasets and phylogenetic trees submitted to TreeBase (S19055 – <http://purl.org/phylo/treebase/phyloids/study/TB2:S19055>). Two SSR products (amplified using unlabelled primers) representing the same individual allele per locus were also purified and sequenced to confirm scores. Sequences were visually assessed and edited using MEGA v. 5 (Tamura et al. 2011) and additional sequences sourced from GenBank as required.

Phylogenetic analyses

Isolates were identified by subjecting their respective DNA sequences to BLASTn analysis. To confirm identifies, sequence datasets were constructed and phylogenetic analyses made. Species were represented by sequences from the ex-type strain and one or two ex-paratype strains.

Two groups of sequence datasets were generated in this study. The first consisted of all Botryosphaeriaceae species with a representative group of isolates from each species, and a selection of isolates from the *Neofusicoccum parvum-ribis* complex. For this first group, only sequence data for the ITS and *tef1* loci were generated and analyzed. Two isolates of *Melanops tulasnei* (Phillips & Alves 2009) were used as outgroup taxa for these analyses. The second group consisted of isolates and species identified as members of the *N. parvum-ribis* complex. For the latter group, sequence datasets for ITS, *tef1*, and *tub2* were generated and analyzed, with no outgroup taxon selected. In all cases, sequence datasets were aligned using MAFFT v. 6 (Kato & Toh 2008) by applying the G-INS-i algorithm and checked visually. Maximum parsimony (MP), and maximum likelihood (ML) phylogenetic analyses were undertaken on the datasets for individual loci sequenced as well as on the combined dataset. MP analyses were done in PAUP* (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) with the same settings used by Mehl et al. (2014). Additionally, a partition homogeneity test (PHT) was done for each combined dataset with the same settings as those used by Mehl et al. (2014). For ML analyses, datasets were parsed through jModelTest v. 2.1.3 (Darrida et al. 2012) with the corrected Akaike Information Criterion selected to determine the best nucleotide substitution model. Analyses of each dataset, as well as the combined dataset were then done using PhyML v. 3.0.1 (Guindon et al. 2010) with the relevant model parameters selected. Bootstrap analyses were used to determine the robustness of trees resulting from the MP and ML analyses. Trees were visualized using TreeGraph v. 2 (Stöver & Müller 2010).

SSR amplifications

Genotypes of *Neofusicoccum parvum* isolates were determined using eight microsatellite (SSR) markers (Slippers et al. 2004). Loci were amplified with primers labelled with the same dyes as those used by Slippers et al. (2004). In cases where amplification proved unsuccessful, sequence data were generated using unlabelled primers. Two reaction mixtures were used. The first mix consisted of ~20 ng template DNA, 1.5 \times PCR buffer (with 3 mM added MgCl₂), 1.25 μ M of each dNTP, 0.1 μ M of each primer, and 0.2 U FastStart Taq Polymerase. The second mix consisted of 1 \times MyTaq Reaction Buffer, 0.2 μ M of each primer, and 0.5 U MyTaq DNA Polymerase. The PCR cycling conditions and method of visualization of the SSR products were the same as those used by Mehl et al. (2014). Dilutions (1:25) were made of SSR products in sterile Sabax water and 1 μ l of this mix was combined with 10 μ l of a suspension of LIZ-labelled Genescan 500 size standard (Applied Biosystems, Life Technologies) mixed with formamide (14 μ l LIZ ml⁻¹ formamide). The products were then run on an Applied Biosystems 3500 Genetic Analyzer. Alleles were scored using GeneMapper[®] Software (Applied Biosystems).

Population genetic analyses

Isolates collected in 2012 and identified as *Neofusicoccum parvum*, a species common to both *Sclerocarya birrea* and *Mangifera indica*, were grouped according to their host in the analyses. Null alleles were treated as missing data. Population

Table 1 – Isolates used in the phylogenetic analyses. Culture numbers in boldface indicate ex-type isolates. Sequence data sourced from GenBank italicized.

Species	Culture number	Other numbers	Host	Location	Collector(s)	ITS	<i>tef1</i>	<i>tub2</i>
<i>Botryosphaeria fabicerciana</i>	CMW 27094	CBS 127193	<i>Eucalyptus</i> sp.	Fujian Province, China	M. J. Wingfield	HQ332197	HQ332213	
<i>B. fabicerciana</i>	CMW 27121	CBS 127194	<i>E. grandis</i> hybrid	Fujian Province, China	M. J. Wingfield	HQ332198	HQ332214	
<i>B. fabicerciana</i>	CMW 25215		<i>Mangifera indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997394	KU997130	KU997568
<i>B. fabicerciana</i>		MAN2132	<i>M. indica</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997460	KU997237	KU997579
<i>B. fabicerciana</i>		MAN25238	<i>M. indica</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997558	KU997319	
<i>B. fabicerciana</i>		MAR28238	<i>Sclerocarya birrea</i> subsp. <i>caffra</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997549	KU997310	KU997616
<i>Diplodia allocellula</i>	CMW 36468	CBS 130408	<i>Acacia karroo</i>	Pretoria, S. Africa	F. Jami & M. Gryzenhout	JQ239397	JQ239384	
<i>D. allocellula</i>	CMW 36469	CBS 130409	<i>Ac. karroo</i>	Pretoria, S. Africa	F. Jami & M. Gryzenhout	JQ239398	JQ239385	
<i>D. allocellula</i>	CMW 24131		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997376	KU997114	
<i>Lasiodiplodia crassispora</i>	CMW 13488		<i>Eucalyptus urophylla</i>	Venezuela	S. Mohali	DQ103552	DQ103559	
<i>L. crassispora</i>	CMW 14688	WAC12534	<i>Santalum album</i>	Ord River, Kununurra, W.A.	T. Burgess	DQ103551	DQ103558	
<i>L. crassispora</i>	CMW 14691	WAC12533	<i>San. album</i>	Ord River, Kununurra, W.A.	T. Burgess	DQ103550	DQ103557	
<i>L. crassispora</i>	CMW 24111		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997362	KU997103	
<i>L. gonubiensis</i>	CMW 14077	CBS 115812	<i>Syzygium cordatum</i>	Gonubie, Eastern Cape, S. Africa	D. Pavlic	AY639595	DQ103566	
<i>L. gonubiensis</i>	CMW 14078	CBS 116355	<i>Syz. cordatum</i>	Gonubie, Eastern Cape,	D. Pavlic	AY639594	DQ103567	
<i>L. gonubiensis</i>	CMW 24123		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997370	KU997109	
<i>L. gonubiensis</i>	CMW 24127		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997374	KU997113	
<i>L. iraniensis</i>	CBS 124710	IRAN1520C	<i>Salvadora persica</i>	Hormozgan, Iran	J. Abdollahzadeh/A. Javadi	GU945348	GU945336	
<i>L. iraniensis</i>	CBS 124711	IRAN1502C	<i>Juglans</i> sp.	Golestan, Iran	A. Javadi	GU945347	GU945335	
<i>L. iraniensis</i>	CMW 25232		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997384	KU997119	
<i>L. mahajangana</i>	CMW 27801	CBS 124925	<i>Terminalia catappa</i>	Mahajanga, Madagascar	J. Roux	FJ900595	FJ900641	
<i>L. mahajangana</i>	CMW 27818	CBS 124926	<i>Ter. catappa</i>	Mahajanga, Madagascar	J. Roux	FJ900596	FJ900642	
<i>L. mahajangana</i>	CMW 27820	CBS 124927	<i>Ter. catappa</i>	Mahajanga, Madagascar	J. Roux	FJ900597	FJ900643	
<i>L. mahajangana</i>	CMW 25199		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997387	KU997121	KU997563
<i>L. mahajangana</i>	CMW 25202		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997388	KU997122	KU997564
<i>L. mahajangana</i>		MAR1212	<i>S. birrea</i> subsp. <i>caffra</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997455	KU997231	
<i>L. pseudotheobromae</i>	CBS 447.62		<i>Citrus aurantium</i>	Suriname	C. Smulders	EF622081	EF622060	
<i>L. pseudotheobromae</i>	CBS 116459	KAS2	<i>Gmelina arborea</i>	San Carlos, Costa Rica	J. Carranza-Velásquez	EF622077	EF622057	
<i>L. pseudotheobromae</i>	CMW 25203		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997389	KU997123	
<i>L. pseudotheobromae</i>	CMW 28517		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997123	KU997226	KU997576
<i>L. pseudotheobromae</i>		MAR25328	<i>S. birrea</i> subsp. <i>caffra</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997547	KU997307	
<i>L. theobromae</i>	CMW 10130		<i>Vitex donniana</i>	Uganda	J. Roux	AY236951	AY236900	
<i>L. theobromae</i>	CBS 164.96		Fruit on coral reef coast	Papua New Guinea	A. Aptroot	AY640255	AY640258	

<i>L. theobromae</i>	CMW 24125		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997372	KU997111	
<i>L. theobromae</i>	CMW 25212		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997392	KU997128	KU997566
<i>Melanops tulasnei</i>	CBS 116805		<i>Quercus robur</i>	Bavaria, Munich, Germany	P. A. Saccardo	FJ824769	FJ824774	
<i>M. tulasnei</i>	CBS 116806		<i>Q. robur</i>	Bavaria, Munich, Germany	P. A. Saccardo	FJ824770	FJ824775	
<i>Neofusicoccum australe</i>	CBS 112872	STE-U4425	<i>Vitis vinifera</i>	Stellenbosch, Western Cape, S. Africa	F. Halleen	AY343388	AY343347	
<i>N. australe</i>	CBS 112877	STE-U4415	<i>V. vinifera</i>	Stellenbosch, Western Cape, S. Africa	F. Halleen	AY343385	AY343346	
<i>N. australe</i>	CMW 25211		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997391	KU997127	
<i>N. kwambonambiense</i>	CMW 14023	CBS 123639	<i>Syzygium cordatum</i>	Kwambonambi, S. Africa	D. Pavlic	EU821900	EU821870	EU821840
<i>N. kwambonambiense</i>	CMW 14123	CBS 123643	<i>Syz. cordatum</i>	Kwambonambi, S. Africa	D. Pavlic	EU821924	EU821894	EU821864
<i>N. kwambonambiense</i>	CMW 25198		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997386	KU997120	KU997562
<i>N. kwambonambiense</i>	CMW 28412		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997418	KU997143	KU997572
<i>N. kwambonambiense</i>		MAN210316	<i>M. indica</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997532	KU997290	KU997605
<i>N. mediterraneum</i>	CBS 121558	PD311	<i>Olea europaea</i>	Lepre, Scorrano, Italy	C. Lazzizera	GU799463	GU799462	
<i>N. mediterraneum</i>	CBS 121718	CPC13137, PD312	<i>Eucalyptus</i> sp.	Rhodes, Greece	P. Crous, M. J. Wingfield, A. Phillips	GU251176	GU251308	
<i>N. mediterraneum</i>	CMW 24080		<i>S. birrea</i> subsp. <i>caffra</i>	Kruger National Park, Mpumalanga Province, S. Africa	B. Hinze	KU997339	KU997094	
<i>N. mediterraneum</i>	CMW 24083		<i>S. birrea</i> subsp. <i>caffra</i>	Kruger National Park, Mpumalanga Province, S. Africa	B. Hinze	KU997341	KU997095	
<i>N. mediterraneum</i>	CMW 24122		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997369	KU997108	
<i>N. mediterraneum</i>	MAN21312		<i>M. indica</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997470	KU997247	KU997588
<i>N. parvum</i>	CMW 9081	ICMP8003, ATCC58191	<i>Populus nigra</i>	TePuke/BP, New Zealand	G. Samuels	AY236943	AY236888	AY236917
<i>N. parvum</i>	CBS 110301	CAP074	<i>V. vinifera</i>	Palmella, Portugal	A. Phillips	AY259098	AY573221	EU673095
<i>N. parvum</i>	CMW 28377		<i>M. indica</i>	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997395	KU997131	KU997569
<i>N. parvum</i>		MAR11328	<i>S. birrea</i> subsp. <i>caffra</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997539	KU997293	KU997607
<i>N. parvum</i>		MAR21022	<i>S. birrea</i> subsp. <i>caffra</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997456	KU997232	KU997577
<i>N. parvum</i>		MAR2134	<i>S. birrea</i> subsp. <i>caffra</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997474	KU997252	KU997593
<i>N. umdonicola</i>	CMW 14058	CBS 123645	<i>Syz. cordatum</i>	Kosi Bay, S. Africa	D. Pavlic	EU821904	EU821874	EU821844
<i>N. umdonicola</i>	CMW 14106	CBS 123644	<i>Syz. cordatum</i>	Sodwana Bay, S. Africa	D. Pavlic	EU821905	EU821875	EU821839
<i>N. umdonicola</i>		MAN210236	<i>M. indica</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997531	KU997289	KU997604
<i>N. vitifusiforme</i>	CBS 110880	STE-U5050	<i>V. vinifera</i>	Stellenbosch, Western Cape, S. Africa	J. van Niekerk	AY343382	AY343344	
<i>N. vitifusiforme</i>	CBS 110887	STE-U5252	<i>V. vinifera</i>	Stellenbosch, Western Cape, S. Africa	J. van Niekerk	AY343383	AY343343	
<i>N. vitifusiforme</i>	CMW 24068		<i>S. birrea</i> subsp. <i>caffra</i>	Kruger National Park, Mpumalanga Province, S. Africa	B. Hinze	KU997329	KU997092	

(continued on next page)

Table 1 – (continued)

Species	Culture number	Other numbers	Host	Location	Collector(s)	ITS	tef1	tub2
<i>N. vitifusiforme</i>	CMW 24077		<i>S. birrea</i> subsp. <i>caffra</i>	Kruger National Park, Mpumalanga Province, S. Africa	B. Hinze	KU997336	KU997093	
<i>N. vitifusiforme</i>	CMW 24112		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997363	KU997104	
<i>N. vitifusiforme</i>	CMW 24117		<i>S. birrea</i> subsp. <i>caffra</i>	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997367	KU997107	
<i>Pseudofusicoccum olivaceum</i>	CMW 20881	CBS 124939	<i>Pterocarpus angolensis</i>	Mawewe Nature Reserve, S. Africa	J. Mehl & J. Roux	FJ888459	FJ888437	
<i>Ps. olivaceum</i>	CMW 22637	CBS 124940	<i>Pt. angolensis</i>	Pretoriuskop, Kruger National Park, S. Africa	J. Roux	FJ888462	FJ888438	
<i>Ps. olivaceum</i>		MAN22138	<i>M. indica</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997555	KU997316	
<i>Ps. olivaceum</i>		MAN22312	<i>M. indica</i>	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997461	KU997238	

subdivision was tested using the Bayesian clustering algorithm implemented in STRUCTURE v. 2.3.4 (Hubisz et al. 2009) on the dataset for all isolates. Burnin was set at 300 000 and the number of MCMC repeats done after burnin was set at 900 000. An admixture model was selected, allele frequencies were set as correlated and lambda was set at one. Twenty iterations were done for each prior of $K = 1$ to $K = 10$. Results were then parsed through STRUCTURE HARVESTER (Earl & vonHoldt 2012) and the DeltaK (Evanno et al. 2005) output used to identify the number of potential subpopulations. To confirm the result, K-means clustering ($K = 1$ to $K = 10$) was done in GenoDive v. 2b24 (Meirmans & van Tienderen 2004) based on both allele frequencies and an Analysis of Molecular Variance (AMOVA) using 50 000 steps and 20 replicates.

The data were clone-corrected by identifying identical genotypes using the Assign Clones option in GenoDive. Clone correction was done because the presence of clonal genotypes is known to affect several measures of population statistics (Halkett et al. 2005). Genotypic diversity was calculated prior to clone-correction, also using GenoDive. Non-random association or linkage disequilibrium of loci in the combined dataset of isolates from *S. birrea* and *M. indica* was determined using the Index of Association (I_A) and rBarD and calculated using MultiLocus v. 1.3b (Agapow & Burt 2001). To determine where most of the variation originated within the dataset, an AMOVA (Excoffier et al. 1992) test was done in GenoDive. Allelic frequencies, expected gene diversity, and measures of population differentiation (F_{ST} , Hedrick's G'_{ST} and Jost's D_{EST}) were computed, also using GenoDive.

Gene flow was determined using two methods. The private alleles method computes the effective number of migrants (N_m) based on rare alleles present (Barton & Slatkin 1986), and is implemented in the Genepop web service (Rousset 2008). A second method (implemented in BIMr) uses Bayesian Inference to estimate migration rates from the previous generation to that sampled while factoring in environmental factors that can influence these rates (Faubet & Gaggiotti 2008). For the second method, ten iterations were done with burnin set at 300 000 followed by 900 000 runs.

Results

Isolate collections

Sixty-two isolates were obtained from the *Sclerocarya birrea* samples collected in 2006 and identified based on sequence data (Fig 1, Table S1). Thirty-eight of these originated from samples collected in the Kruger National Park (KNP), 23 from Lakelands, Mfolozi Village, and one from the Hans Merensky estate. Most isolates produced *Neofusicoccum*-like conidia, but some had pigmented conidia typical of *Lasiodiplodia* species. One hundred and forty-one isolates resulted from the *Mangifera indica* samples collected from the Hans Merensky estate from the 144 branches sampled.

Isolations from samples collected in September 2012 from the Hoedspruit area resulted in a collection of 196 isolates, of which 34 were obtained from branches of the 17 *S. birrea* trees sampled and 163 from the 19 *M. indica* trees sampled (Fig 1, Table S1).

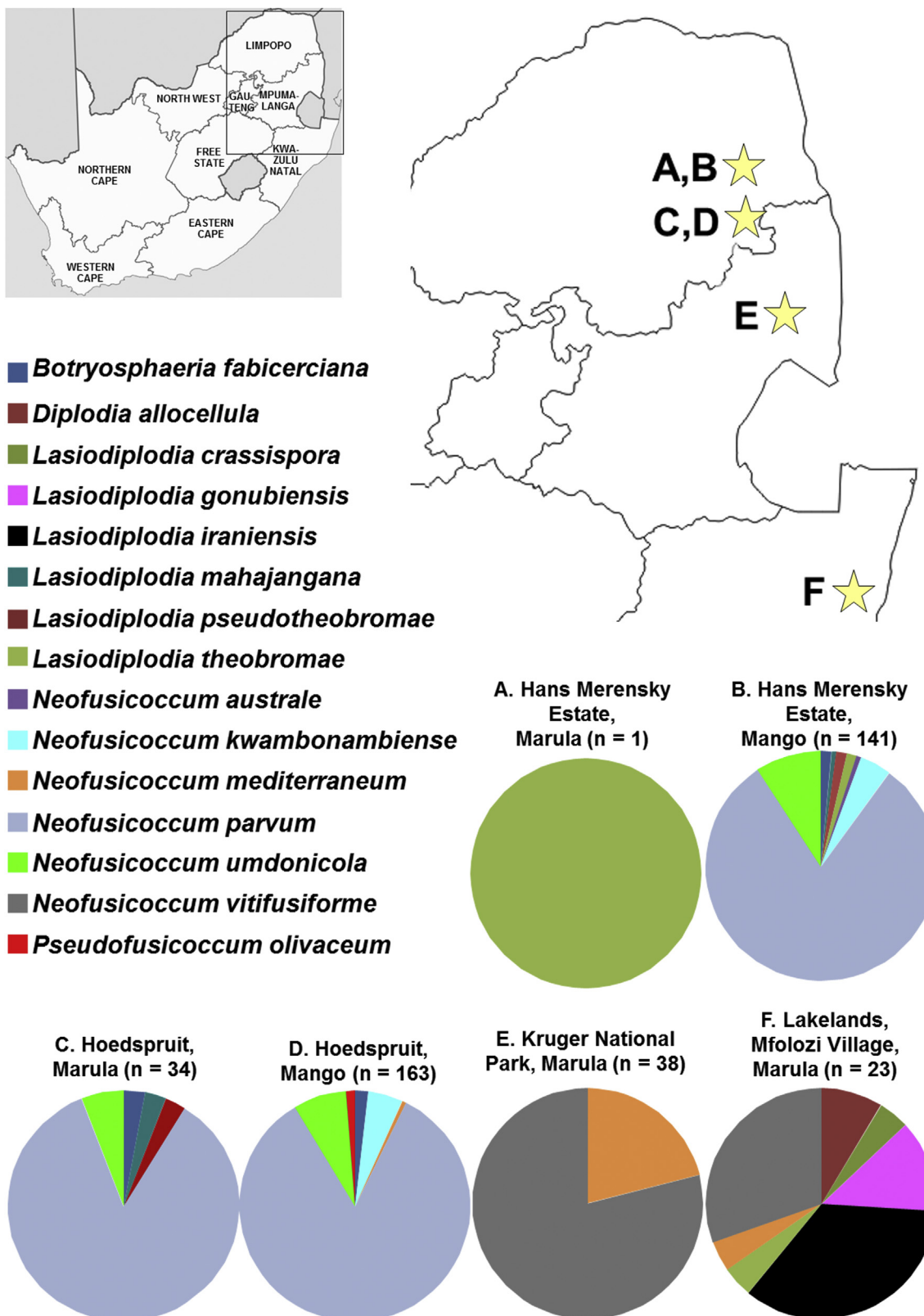


Fig 1 – Map representing sites sampled (indicated by stars). A–F designate either one of both tree hosts sampled at a site, and are represented by pie charts that show species sampled at a site as well as the proportion of isolates obtained for that species. Map sources: https://en.wikipedia.org/wiki/File:Map_of_South_Africa_with_English_labels.svg, http://www.d-maps.com/carte.php?num_car=23733&lang=en.

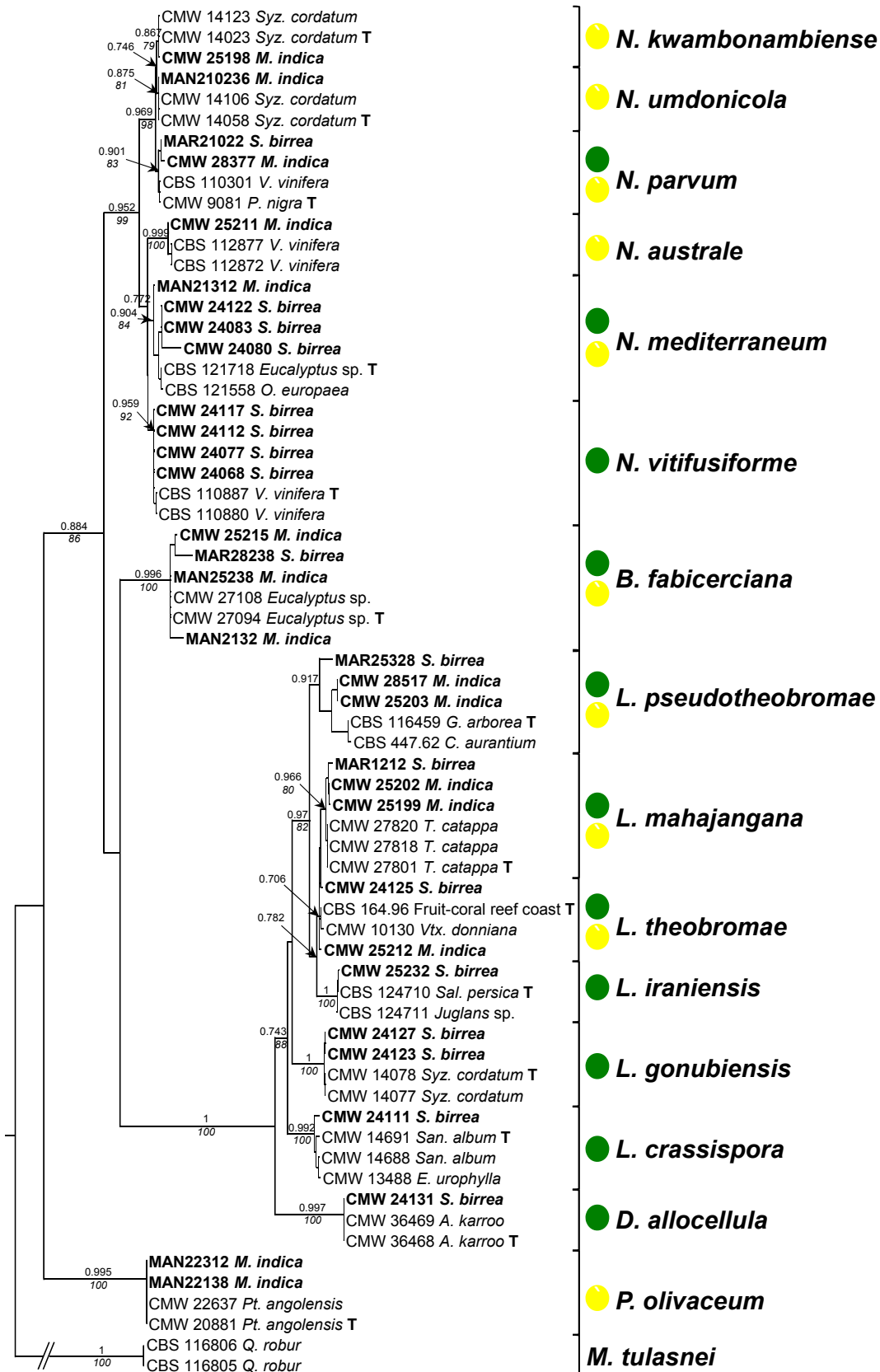


Fig 2 – Maximum likelihood (ML) tree resulting from analysis on the combined dataset of ITS and *tef1* sequence data for the first group. The tree is rooted to two isolates of *Melanops tulasnei* (CBS 116805, CBS 116806). Bootstrap values above 70 % for the ML analysis (normal) and maximum parsimony analysis (italicized) appear at the relevant nodes. A bold T after an isolate designates an ex-type isolate for the respective species. Isolates in bold were obtained during this study. Species obtained from either *S. birrea* (green) or *M. indica* (yellow) are designated with circles before the species name. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DNA sequence analyses and species identifications

Sequence datasets generated for the ITS, *tef1*, and *tub2* loci were analyzed both individually and in combination. For the first group, the ITS dataset consisted of 540 characters (153 parsimony informative, 374 constant, 13 parsimony uninformative), and yielded 194910 most parsimonious trees (TL = 270, CI = 0.73, RI = 0.953, RC = 0.696). The model selected for ML analysis was TPM1uf ($\gamma = 0.295$). The *tef1* dataset consisted of 309 characters (191 parsimony informative, 107 constant, 11 parsimony uninformative), and yielded 738939 most parsimonious trees (TL = 469, CI = 0.663, RI = 0.943, RC = 0.625). The model selected for ML analysis was HKY (ti/tv = 1.534, $\gamma = 0.789$). The combined analysis consisted of 849 characters (344 parsimony informative, 481 constant, 24 parsimony uninformative), and yielded 1034389 most parsimonious trees (TL = 756, CI = 0.672, RI = 0.943, RC = 0.633). The model TVM ($\gamma = 0.679$, p-inv = 0.261) was selected for ML analysis. The PHT value was 0.001.

For the second group of isolates and species that grouped within the *Neofusicoccum parvum-ribis* complex, the ITS dataset consisted of 502 characters (4 parsimony informative, 490

constant, 8 parsimony uninformative), and yielded a single most parsimonious tree (TL = 4, CI = 1, RI = 1, RC = 1). The model selected for ML analysis was K80 (ti/tv = 3.014). The *tef1* dataset consisted of 266 characters (7 parsimony informative, 257 constant, 2 parsimony uninformative), and yielded four most parsimonious trees (TL = 7, CI = 1, RI = 1, RC = 1). The model selected for ML analysis was HKY (ti/tv = 8.088). The *tub2* dataset consisted of 420 characters (9 parsimony informative, 404 constant, 7 parsimony uninformative), and yielded a single most parsimonious tree (TL = 10, CI = 0.9, RI = 0.974, RC = 0.876). The model selected for ML analysis was HKY (ti/tv = 1.855). The combined analysis consisted of 1188 characters (20 parsimony informative, 1151 constant, 17 parsimony uninformative), and yielded two most parsimonious trees (TL = 27, CI = 0.741, RI = 0.915, RC = 0.678). The model HKY (ti/tv = 3.470, $\gamma = 0.023$) was selected for ML analysis. The PHT value was 0.001.

Tree topologies emerging from the MP and ML analyses were similar for each analysis. For the first group, differences occurred where some clades for *Lasiodiplodia* species collapsed in the ITS dataset (Fig. S1), but were easily resolved when analyzing *tef1* (Fig. S2). While the two sequence datasets were

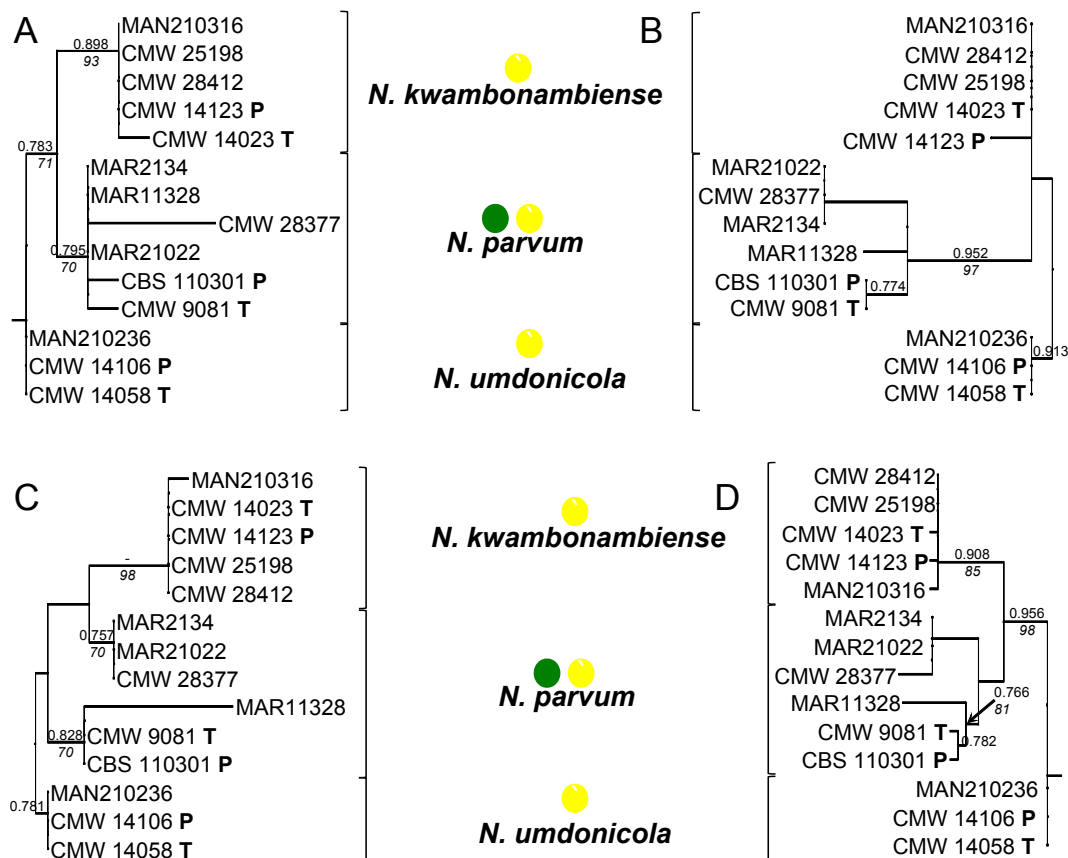


Fig 3 – Unrooted maximum likelihood trees resulting from analysis of the ITS (A), *tef1* (B), *tub2* (C) and combined (D) sequence datasets for isolates grouping with known species within the *N. parvum-ribis* species complex. Bootstrap values above 70 % for the ML analysis (normal) and maximum parsimony analysis (italicized) appear at the relevant nodes. A bold T after an isolate designates an ex-type isolate for the respective species while a bold P designates a paratype isolate. Isolates in bold were obtained during this study. Species obtained from either *S. birrea* (green) or *M. indica* (yellow) are designated with circles above the species name. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

separately interpreted given the results of the PHT tests, both datasets were combined to illustrate all species identified (Fig 2) as the incongruence reflected the interpretation of the individual trees. For the second group, isolates grouped into distinct clades representing species, but two subclades (based on the *tef1* and *tub2* analyses) emerged for isolates grouping together as *Neofusicoccum parvum*. These sub-clades were not evident when analyzing the ITS dataset. All three gene phylogenies are shown along with the combined phylogeny resulting from concatenation of the three datasets (Fig 3). Isolates collected in both 2006 and 2012 grouped with known species of the Botryosphaeriaceae, in five genera, specifically Botryosphaeria, Diplodia, Lasiodiplodia, Neofusicoccum, and Pseudofusicoccum.

Seven species of Botryosphaeriaceae were obtained from the 2006 isolations made from *Sclerocarya birrea* trees (Table S1, Fig 1). The dominant taxon emerging from these isolations was *Neofusicoccum vitifusiforme* that occurred at all three sites (KNP: $n = 36$, Lakelands: $n = 9$, Hans Merensky: $n = 1$). *Neofusicoccum mediterraneum* was the only other species isolated from the KNP ($n = 8$) and Lakelands ($n = 1$). Single isolates of *Lasiodiplodia theobromae* were obtained from the Hans Merensky and Lakelands samples. The remaining four species, including *Diplodia allocellula* ($n = 2$), *Lasiodiplodia crassispora* ($n = 1$), *Lasiodiplodia gonubiensis* ($n = 3$), and *Lasiodiplodia iranensis* ($n = 8$), were isolated exclusively from the Lakelands samples.

Eight species of Botryosphaeriaceae were isolated from the 2006 *Mangifera indica* samples. The most common species was *N. parvum* ($n = 114$). Other species obtained included *Neofusicoccum umdonicola* ($n = 13$), *Neofusicoccum kwambonambiense* ($n = 6$), *Botryosphaeria fabicerciana* ($n = 2$), *Lasiodiplodia*

pseudotheobromae ($n = 2$), *Lasiodiplodia theobromae* ($n = 2$), *Lasiodiplodia mahajangana* ($n = 1$), and *Neofusicoccum australe* ($n = 1$).

Five species of Botryosphaeriaceae were recovered from the 2012 isolations made from *S. birrea* trees in the Hoedspruit area. Isolates of *N. parvum* ($n = 29$) were most common. In addition, two isolates of *N. umdonicola* and single isolates of *B. fabicerciana*, *L. mahajangana*, and *L. pseudotheobromae* were also identified from these samples.

Six species of Botryosphaeriaceae were obtained from the 2012 isolations made from *M. indica* trees in the Hoedspruit area. Again, the most commonly isolated taxon was *N. parvum* ($n = 137$). Isolates of *N. umdonicola* ($n = 12$), *N. kwambonambiense* ($n = 8$), *B. fabicerciana* ($n = 3$), *Pseudofusicoccum olivaceum* ($n = 2$) and *N. mediterraneum* ($n = 1$) were also identified from these samples.

Six species of Botryosphaeriaceae were common to both *S. birrea* and *M. indica* trees sampled in 2012. These included *B. fabicerciana*, *L. mahajangana*, *L. theobromae*, *N. mediterraneum*, *N. parvum*, and *N. umdonicola*. Low isolate numbers precluded all, except *N. parvum* from further study. Since *N. parvum* was most common on both tree species, isolates were further studied using microsatellite markers.

Population genetic analyses on *N. parvum*

A set of 94 *Neofusicoccum parvum* isolates (29 from *Sclerocarya birrea* and 65 from *Mangifera indica*) were selected. These were representative of both sites where branches were collected, and included isolates obtained at each of the four time-points (two, four, six, and eight weeks) when isolations were made. Of the eight SSR loci tested, two (*BotF15* and *BotF37*)

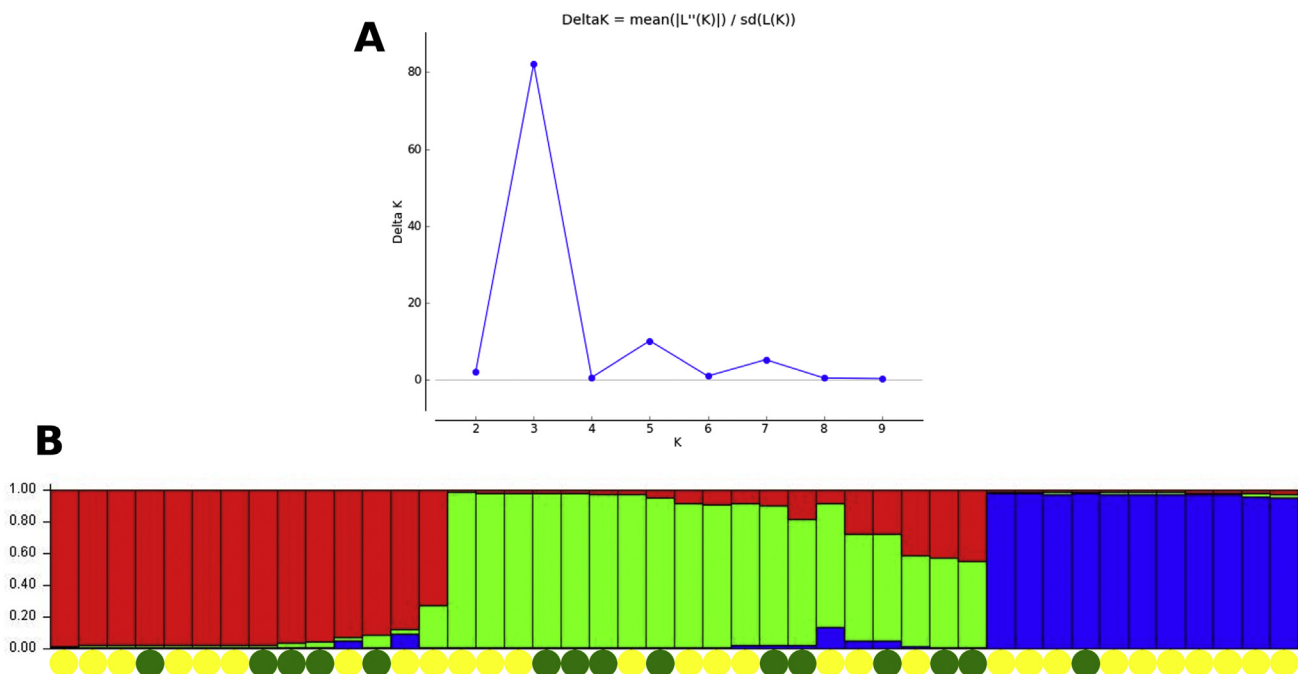


Fig 4 – Results of the STRUCTURE analyses done for *Neofusicoccum parvum* isolates. The optimal number of populations present (3) is signalled by the highest DeltaK peak. The corresponding barplot shows individual isolates, grouped according to population assigned, in order of Q, with populations assigned colours of red, green and blue. Circles beneath designate isolates from *S. birrea* (green) or *M. indica* (yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were monomorphic and not used. Eight allele variants were detected in one locus (*BotF17*), seven variants in a second locus (*BotF35*), three variants in two other loci (*BotF11* and *BotF18*), and two variants in the last two loci (*BotF21* and *BotF23*) studied (Table S2).

Results from STRUCTURE and STRUCTURE HARVESTER indicated that three populations were represented among the 94 isolates (Fig 4). All populations consisted of a mix of isolates from both tree hosts, and from both sites sampled (Fig 4), and isolates shared alleles at multiple loci (Table S2). These results were confirmed by the K-means output in GenoDive (Meirmans & Van Tienderen 2004) that indicated three populations, with the same isolates clustering together as in STRUCTURE, except that one isolate grouped with a different population. Additional STRUCTURE analyses on each sub-population indicated no further substructure in the data. Low genotypic diversity (0.383) was detected on the full dataset, although this did not differ significantly from what was expected ($P = 0.159$, Table 2). Genotypic diversity values for the *S. birrea* and *M. indica* populations differed slightly (not significant), with the *S. birrea* population more diverse than that from *M. indica* (Table 2).

Following clone-correction, the dataset consisted of 78 isolates (27 from *S. birrea* and 51 from *M. indica*). For both the Index of Association and rBarD tests, values obtained for the population of *S. birrea* isolates fell within the range of expected values for those resulting from random datasets, indicating sexual outcrossing and linkage equilibrium amongst loci. However, the values obtained for the *M. indica* isolates, and for the combined dataset of isolates from both tree hosts, fell outside the range of expected values based on random datasets generated (Table 2). This indicated linkage disequilibrium amongst the loci of isolates of *M. indica* sampled. The AMOVA showed that most of the genetic variation was accounted for within the two populations (*S. birrea* and *M. indica*), and not between them (Table 3).

Six alleles were unique to the *M. indica* population (Table 2). Gene diversity was slightly higher for *M. indica* than *S. birrea*. Moderate levels of genetic differentiation were detected

Table 3 – Analysis of variance (AMOVA) for *N. parvum* populations obtained from *S. birrea* and *M. indica*.

Source of variation	Sum of squares	Variance components	Percentage variation
Among populations	467.158	4.674	6.4
Among individuals within populations	10 418.431	68.542	93.6
Total	10 885.589	73.216	100

between the *S. birrea* and *M. indica* populations ($F_{ST} = 0.065$, $G'_{ST} = 0.136$, $D_{EST} = 0.076$) (Wright 1978). For gene flow, the effective number of migrants inferred by Genepop was 0.522. Per population migration rates, as calculated by BIMr, indicated that there was more movement from *M. indica* to *S. birrea* populations (source: *S. birrea* = 0.875 ± 0.006 , source: *M. indica*: 0.617 ± 0.007) than the other way round (source: *S. birrea* = 0.126 ± 0.006 , source: *M. indica*: 0.383 ± 0.007) (Table S3).

Discussion

At least 11 species of the Botryosphaeriaceae are associated with native *Sclerocarya birrea* trees in South Africa and all of these represent previously described taxa. Of these, *Botryosphaeria faberciana*, *Lasiodiplodia iraniensis*, and *Neofusicoccum mediterraneum* are recorded from South Africa for the first time. The remaining eight species (*Diplodia allocellula*, *Lasiodiplodia crassispora*, *Lasiodiplodia gonubiensis*, *Lasiodiplodia mahajangana*, *Lasiodiplodia pseudotheobromae*, *Lasiodiplodia theobromae*, *Neofusicoccum parvum*, *Neofusicoccum umdonicola*, *Neofusicoccum viti-fusiforme*) have been isolated previously from other hosts in the country, including both native (Pavlic et al. 2007; Mehl et al. 2011; Jami et al. 2012, 2014) and non-native (van Niekerk et al. 2004; Damm et al. 2007; Pavlic et al. 2007; Begoude et al. 2010; Mehl et al. 2014) trees. This is, however, the first time that any of them have been recorded from *S. birrea*.

Table 2 – Statistics resulting from the population genetic analyses of *Neofusicoccum parvum* isolates on each host (*S. birrea* and *M. indica*) as well as the combined dataset. Included are gene and genotypic diversities, and two measures of linkage disequilibrium (I_A and rBarD).

Locus	<i>S. birrea</i>	<i>M. indica</i>	Combined
Number of isolates	27	51	78
Alleles observed	19	25	25
Private alleles observed	0	6	
Gene diversity (H_s)	0.513	0.526	0.519
Genotypic diversity ^a			
Observed	0.493, $P = 0.419$	0.330, $P = 0.07$	0.383, $P = 0.159$
Expected	0.535	0.554	0.554
Index of association (I_A)			
Observed	0.422, $P = 0.001$	0.384, $P < 0.001$	0.380, $P < 0.001$
Range	–0.298–0.483	–0.217–0.275	–0.157–0.206
Linkage disequilibrium (rBarD)			
Observed	0.109, $P = 0.001$	0.083, $P < 0.001$	0.080, $P < 0.001$
Range	–0.077–0.125	–0.047–0.059	–0.033–0.043

a Genotypic diversities were computed prior to clone-correction.

Ten species of the *Botryosphaeriaceae*, including *B. fabicerciana*, *L. mahajangana*, *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum australe*, *Neofusicoccum kwambonambiense*, *N. mediterraneum*, *N. parvum*, *N. umdonicola*, and *Pseudofusicoccum olivaceum*, were isolated from *Mangifera indica* in this study. Of these, only *L. theobromae*, *N. parvum*, and *Ps. olivaceum* have previously been found on *M. indica* trees in South Africa (Trakunyingcharoen et al. 2014) while the remaining seven species represent first reports on this host in the country. Additionally, *L. mahajangana*, *N. australe*, *N. kwambonambiense*, and *N. umdonicola* add to the assemblage of *Botryosphaeriaceae* associated with *M. indica* globally (Trakunyingcharoen et al. 2014). Some of the species in the list compiled by Trakunyingcharoen et al. (2014) are known pathogens of *M. indica* and occur in South Africa, but were not recovered in this study. These include *L. crassisporea* (van Niekerk et al. 2010) and *Neofusicoccum mangiferae* (Pavlic et al. 2007).

Several of the species isolated from *S. birrea* or *M. indica* trees in this study are known only from South Africa and might be native to the country. These species include *D. allocellula*, *L. gonubiensis*, and *P. olivaceum*, all of which previously occupied a limited distribution in the country. *Diplodia allocellula* was isolated from *Acacia karroo* trees in Pretoria, Gauteng Province (Jami et al. 2012), *L. gonubiensis* from *Syzygium cordatum* in Gonubie, Eastern Cape Province (Pavlic et al. 2007), and *P. olivaceum* from *Pterocarpus angolensis* and *Terminalia sericea* at several sites in the Mpumalanga Province (Mehl et al. 2011). Results of this study indicate that these fungi occupy broader distributions in the country and probably infect a greater number of hosts, both native and non-native.

A number of species isolated from either *S. birrea* and/or *M. indica* trees are possibly aliens in South Africa. Examples include *B. fabicerciana* that was first described in China from *Eucalyptus* species (Chen et al. 2011) and has been recorded from *M. indica* trees in Brazil (Marques et al. 2013), and *L. theobromae* that has been recovered from various native tree hosts and non-native fruit trees in South Africa (Jami et al. 2014). Apart from these, *L. crassisporea* is possibly also alien as it was first described from *Santalum album* in Western Australia and *Eucalyptus urophylla* in Acarigua, Venezuela (Burgess et al. 2006a), and is also known to infect grapevine in California, USA (Úrbez-Torres et al. 2010). In South Africa, it has been isolated from *Pt. angolensis* in the Mpumalanga Province (Mehl et al. 2011) and grapevines in the Western Cape Province (van Niekerk et al. 2010) and likely infects other plant species in the country. These fungi illustrate an increasingly alarming pattern where many tree pathogens are being moved around the world unknowingly via infected plant tissue. They are consequently being introduced into novel areas where they can then infect a broad range of native and non-native hosts, potentially with negative consequences (Desprez-Loustau et al. 2007; Liebhold et al. 2012; Gladieux et al. 2015; Wingfield et al. 2015).

Neofusicoccum vitifusiforme was the dominant taxon amongst the 2006 isolations from *S. birrea* trees in the Kruger National Park (Mpumalanga Province) and Lakelands (KwaZulu Natal Province) areas. This fungus has been associated in South Africa with various cultivated plants in the Western

Cape and Gauteng Provinces, including grapevines (van Niekerk et al. 2004), plum and peach trees (Damm et al. 2007), and ornamental *Schizolobium parahyba* trees (Mehl et al. 2014). Its occurrence on *S. birrea* trees at all three sites sampled (located in the Limpopo, Mpumalanga, and KwaZulu-Natal Provinces) suggests that it could either be native to South Africa or that it originated from other cultivated plants established in close proximity to *S. birrea* trees. It was not isolated in 2012, probably due to sampling having been restricted to Hoedspruit.

Neofusicoccum parvum was the dominant *Botryosphaeriaceae* species isolated from *S. birrea* and *M. indica* trees sampled in the Hoedspruit area in 2012, as well as from *M. indica* trees at the Hans Merensky estate in 2006. This was not unexpected as the fungus is a dominant *Botryosphaeriaceae* species on various woody hosts. These include almond trees in Spain (Gramaje et al. 2012), grapevines in Algeria and Uruguay (Abreo et al. 2013; Berraf-Tebbal et al. 2015), *M. indica* in Australia (Slippers et al. 2005), olives in Italy (Carlucci et al. 2013), *Sch. parahyba* in South Africa (Mehl et al. 2014), *Terminalia catappa* in South Africa (Begoude et al. 2010), and ornamental *Tibouchina* spp. in Australia, New Zealand, and South Africa (Heath et al. 2011). More recently, the abundance of *N. parvum* in some areas has been linked to environmental disturbance and host composition linked to human activity (Pavlic-Zupanc et al. 2015). This could provide an explanation for the dominance of this pathogen from the samples obtained in this study.

Similar levels of genetic diversity were found within both the *S. birrea* and *M. indica* populations of *N. parvum* sampled in this study. The AMOVA on these data showed that the genetic variation observed was represented within the two populations, as opposed to diversity amongst the populations. Gene diversity was slightly higher in the *M. indica* population, possibly because more isolates were obtained from that host and because more *M. indica* trees were available to sample. Isolates obtained in this study had similar levels of genetic diversity as compared to previous studies considering this fungus in South Africa. Gene diversity ($H_S = 0.519$) was slightly lower than that reported by Sakalidis et al. (2013, $H_S = 0.574$), who considered isolates sampled from *Eucalyptus* sp., *Syz. cordatum*, and *M. indica* trees, and Pavlic-Zupanc et al. (2015, $H_S = 0.579$), who sampled from *Syz. cordatum* trees across the country. The high levels of gene diversity in all these studies adds weight to the suggestion (Sakalidis et al. 2013) that the fungus is native to South Africa, although the possibility that the fungus was introduced cannot be overruled.

Three populations of *N. parvum* were identified by STRUCTURE analysis, comprising isolates from both *S. birrea* and *M. indica*. A distinct population signifies a unique, ancestral lineage of the fungus (de Queiroz 1999). Each population comprised isolates from both tree hosts, indicating movement of the fungus between them. Other studies have also noted the presence of multiple lineages of *N. parvum* in South Africa. Pavlic-Zupanc et al. (2015) identified three populations and Sakalidis et al. (2013) showed that South African isolates grouped within nine of the 12 lineages of this fungus. These

lineages could represent additional cryptic species that are closely related. High levels of genetic variation and subclades within the species hinting at potential cryptic relatives have been noted in several studies (Baskarathevan *et al.* 2012; Abdollahzadeh *et al.* 2013; Chen *et al.* 2014). Three cryptic species have previously been identified by Pavlic *et al.* (2009) in South Africa and it is possible that the three lineages identified in this study also represent cryptic species. Analyses of multi-locus sequence data would be required to confirm or refute this possibility.

Results from analyses of linkage disequilibrium on the combined population of *N. parvum* isolates from *S. birrea* and *M. indica* indicated that clonal reproduction is the dominant mode of reproduction. This is underscored by low genotypic diversity for both the complete dataset as well as for datasets from each host. Examination of allele frequencies also indicated that individual alleles dominated the profile at four of the six loci sampled (BotF11, BotF18, BotF21, and BotF23) in isolates from both hosts. While the sexual state of *N. parvum* has not been recorded in South Africa, the fungus probably reproduces both sexually and asexually, as alluded to by Sakalidis *et al.* (2013), and the high genetic diversity observed in this study may be due to unobserved outcrossing.

Low genetic differentiation and extensive gene flow characterized *N. parvum* isolates derived from the *S. birrea* and *M. indica* populations in this study. Similar results have been obtained in other studies on the Botryosphaeriaceae where neighbouring hosts have been sampled. Burgess *et al.* (2006b) sampled *N. australe* from native eucalypts and two adjacent plantations of non-native *Eucalyptus globulus* and showed that low differentiation and high gene flow were characteristic of these populations. Sakalidis *et al.* (2011) also demonstrated high levels of gene flow amongst populations of this fungus from native woody hosts. Likewise, Begoude *et al.* (2012) showed movement of both *L. theobromae* and *L. pseudotheobromae* between *Terminalia* species and *Theobroma cacao*. Cumulatively, results from all of these studies illustrate the ease with which the Botryosphaeriaceae can move between both native and non-native woody plants.

The global dissemination of plants by people (Liebhold *et al.* 2012; Bebbler *et al.* 2014) has enabled the introduction and establishment of both *M. indica* and likely most of the Botryosphaeriaceae isolated in this study, into areas outside of their original geographic range. While several species of these fungi are possibly native, many have likely been unintentionally introduced, probably due to their endophytic persistence in infected, albeit asymptomatic, plant material (Slippers & Wingfield 2007). Most of the Botryosphaeriaceae isolated in this study are capable of infecting both *S. birrea* and *M. indica* simultaneously, including *Botryosphaeria dothidea*, *L. crassispora*, *L. theobromae*, *N. mediterraneum*, and *N. parvum*. Previous studies have noted the ability of these fungi to infect multiple tree species concurrently, including natives and non-natives (Sakalidis *et al.* 2011; Begoude *et al.* 2012; Jami *et al.* 2014). Added to this is the concern of introducing novel genotypes of a species already present in an area, which can become established and/or recombine with other genotypes already present to produce novel genotypes capable of infecting naïve hosts (Gladieux *et al.* 2015). The ability of these fungi to migrate among host plants can facilitate infections of

novel hosts and threaten the sustainability of both commercially important plants as well as native ecosystems (Desprez-Loustau *et al.* 2007; Fisher *et al.* 2012).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funbio.2016.07.015>.

REFERENCES

- Abdollahzadeh J, Zare R, Phillips AJL, 2013. Phylogeny and taxonomy of *Botryosphaeria* and *Neofusicoccum* species in Iran, with description of *Botryosphaeria scharifii* sp. nov. *Mycologia* 105: 210–220.
- Abreo E, Martinez S, Bettucci L, Lupo S, 2013. Characterization of Botryosphaeriaceae species associated with grapevines in Uruguay. *Australasian Plant Pathology* 42: 241–249.
- Agapow PM, Burt A, 2001. Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* 1: 101–102.
- Barton NH, Slatkin M, 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* 56: 409–415.
- Baskarathevan J, Jaspers MV, Jones EE, Cruickshank RH, Ridgway HJ, 2012. Genetic and pathogenic diversity of *Neofusicoccum parvum* in New Zealand vineyards. *Fungal Biology* 116: 276–288.
- Bebber DP, Holmes T, Gurr SJ, 2014. The global spread of crop pests and pathogens. *Global Ecology and Biogeography* 23: 1398–1407.
- Begoude BAD, Slippers B, Perez G, Wingfield MJ, Roux J, 2012. High gene flow and outcrossing within populations of two cryptic fungal pathogens on a native and non-native host in Cameroon. *Fungal Biology* 116: 343–353.
- Begoude BAD, Slippers B, Wingfield MJ, Roux J, 2010. Botryosphaeriaceae associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. *Mycological Progress* 9: 101–123.
- Berraf-Tebbal A, Guerreiro MA, Phillips AJL, 2015. Phylogeny of *Neofusicoccum* species associated with grapevine trunk disease in Algeria, with description of *Neofusicoccum algeriense* sp. nov. *Phytopathologia Mediterranea* 71: 201–214.
- Burgess TI, Barber PA, Mohali S, Pegg G, de Beer ZW, Wingfield MJ, 2006a. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. *Mycologia* 98: 423–435.

- Burgess TI, Sakalidis ML, Hardy GESJ, 2006b. Gene flow of the canker pathogen *Botryosphaeria australis* between *Eucalyptus globulus* plantations and native eucalypt forests in Western Australia. *Austral Ecology* 31: 559–566.
- Carbone I, Kohn LM, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91: 553–556.
- Carlucci A, Raimondo ML, Cibelli F, Phillips AJL, Lops F, 2013. *Pleurostomophora richardsiae*, *Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in southern Italy. *Phytopathologia Mediterranea* 52: 517–527.
- Chen S, Morgan DP, Hasey JK, Anderson K, Michailides TJ, 2014. Phylogeny, morphology, distribution, and pathogenicity of *Botryosphaeriaceae* and *Diaportheaceae* from English walnut in California. *Plant Disease* 98: 636–652.
- Chen S, Pavlic D, Roux J, Slippers B, Xie Y, Wingfield MJ, Zhou X, 2011. Characterization of *Botryosphaeriaceae* from plantation-grown *Eucalyptus* species in South China. *Plant Pathology* 60: 739–751.
- Crous PW, Phillips AJL, Baxter AP, 2000. *Phytopathogenic Fungi from South Africa*. Department of Plant Pathology Press, University of Stellenbosch.
- Damm U, Crous PW, Fourie PH, 2007. *Botryosphaeriaceae* as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. *Mycologia* 99: 664–680.
- Darrida D, Taboada GL, Doallo R, Posada D, 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.
- de Queiroz K, 1999. The general lineage concept of species and the defining properties of the species category. In: Wilson R (ed.), *Species: new interdisciplinary essays*. MIT Press, London, pp. 49–89.
- Desprez-Loustau M-L, Robin C, Buée M, Courtecuisse R, Garbaye J, Suffert F, Satche I, Rizzo DM, 2007. The fungal dimension of biological invasions. *Trends in Ecology and Evolution* 22: 472–480.
- Doidge EM, 1950. The South African fungi and lichens to the end of 1945. *Bothalia* 5: 1–1094.
- Earl DA, vonHoldt BM, 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359–361.
- Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611–2620.
- Excoffier L, Smouse PE, Quattro JM, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.
- Farr DF, Rossman AY, 2016. *Fungal Databases, Systematic Mycology and Microbiology Laboratory*, ARS, USDA, From. <http://nt.ars-grin.gov/fungal-databases/> (retrieved 16/3/2016).
- Faubet P, Gaggiotti OE, 2008. A new Bayesian method to identify the environmental factors that influence recent migration. *Genetics* 178: 1491–1504.
- Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ, 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484: 186–194.
- Gladieux P, Feurtey A, Hood ME, Snirc A, Clavel J, Dutech C, Roy M, Giraud T, 2015. The population biology of fungal invasions. *Molecular Ecology* 24: 1969–1986.
- Glass NL, Donaldson GC, 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323–1330.
- Gramaje D, Agustí-Brisach C, Perez-Sierra A, Moralejo E, Olmo D, Mostert L, Damm U, Armengol J, 2012. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). *Persoonia* 28: 1–13.
- Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O, 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321.
- Halkett F, Simon JC, Balloux F, 2005. Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology & Evolution* 20: 194–201.
- Heath RN, Roux J, Slippers B, Drenth A, Pennycook SR, Wingfield BD, Wingfield MJ, 2011. Occurrence and pathogenicity of *Neofusicoccum parvum* and *N. mangiferae* on ornamental *Tibouchina* species. *Forest Pathology* 41: 48–51.
- Hubisz MJ, Falush D, Stephens M, Pritchard JK, 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9: 1322–1332.
- Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD, 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research* 108: 411–418.
- Jami F, Slippers B, Wingfield MJ, Gryzenhout M, 2014. *Botryosphaeriaceae* species overlap on four unrelated, native South African hosts. *Fungal Biology* 118: 168–179.
- Jami F, Slippers B, Wingfield MJ, Gryzenhout M, 2012. Five new species of the *Botryosphaeriaceae* from *Acacia karroo* in South Africa. *Cryptogamie Mycologie* 33: 245–266.
- Johnson GI, Kotze JM, 1994. Stem-end rot. In: Ploetz RC, Zentmyer GA, Nishijima WT, Rohrbach KG, Ohr HD (eds), *Compendium of tropical fruit diseases*, 1st edn. APS Press, St. Paul, Minnesota, pp. 81–83.
- Katoh K, Toh H, 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298.
- Liebold AM, Brockerhoff EG, Garrett LJ, Parke JL, Britton KO, 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. *Frontiers in Ecology and the Environment* 10: 135–143.
- Marques MW, Lima NB, de Moraes Jr MA, Michereff SJ, Phillips AJL, Câmara MP, 2013. *Botryosphaeria*, *Neofusicoccum*, *Neoscytalidium* and *Pseudofusicoccum* species associated with mango in Brazil. *Fungal Diversity* 61: 195–208.
- Mehl JWM, Slippers B, Roux J, Wingfield MJ, 2011. *Botryosphaeriaceae* associated with *Pterocarpus angolensis* (kiaat) in South Africa. *Mycologia* 103: 534–553.
- Mehl JWM, Slippers B, Roux J, Wingfield MJ, 2013. Cankers and other diseases caused by the *Botryosphaeriaceae*. In: Gonthier P, Nicolotti G (eds), *Infectious Forest Diseases*. CAB International, Oxon, UK, pp. 298–317.
- Mehl JWM, Slippers B, Roux J, Wingfield MJ, 2014. *Botryosphaeriaceae* associated with die-back of *Schizolobium parahyba* trees in South Africa and Ecuador. *Forest Pathology* 44: 396–408.
- Meirmans PG, Van Tienderen PH, 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4: 792–794.
- Menge JA, Ploetz RC, 2003. Diseases of avocado. In: Ploetz RC (ed.), *Diseases of Tropical Fruit Crops*. CAB International, Oxon, UK, pp. 35–71.
- Pavlic D, Slippers B, Coutinho TA, Gryzenhout M, Wingfield MJ, 2004. *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology* 50: 313–322.
- Pavlic D, Slippers B, Coutinho TA, Wingfield MJ, 2007. *Botryosphaeriaceae* occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. *Plant Pathology* 56: 624–636.
- Pavlic D, Slippers B, Coutinho TA, Wingfield MJ, 2009. Molecular and phenotypic characterisation of three phylogenetic species

- discovered within the *Neofusicoccum parvum*/*N. ribis* complex. *Mycologia* **101**: 636–647.
- Pavlic-Zupanc D, Wingfield MJ, Boissin E, Slippers B, 2015. The distribution of genetic diversity in the *Neofusicoccum parvum*/*N. ribis* complex suggests structure correlated with level of disturbance. *Fungal Ecology* **13**: 93–102.
- Peters CR, 1988. Notes on the distribution and relative abundance of *Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* **25**: 403–410.
- Phillips AJL, Alves A, 2009. Taxonomy, phylogeny, and epitypification of *Melanops tulasnei*, the type species of *Melanops*. *Fungal Diversity* **38**: 155–166.
- Ploetz RC, 2003. Diseases of mango. In: Ploetz RC (ed.), *Diseases of Tropical Fruit Crops*. CAB International, Oxon, UK, pp. 327–363.
- Rousset F, 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* **8**: 103–106.
- Sakalidis ML, Hardy GESJ, Burgess TI, 2011. Class III endophytes, clandestine movement amongst hosts and habitats and their potential for disease; a focus on *Neofusicoccum australe*. *Australasian Plant Pathology* **40**: 510–521.
- Sakalidis ML, Slippers B, Wingfield BD, Hardy GESJ, Burgess TI, 2013. The challenge of understanding the origin, pathways and extent of fungal invasions: global populations of the *Neofusicoccum parvum*–*N. ribis* species complex. *Diversity and Distributions* **19**: 873–883.
- Shackleton SE, Shackleton CM, Cunningham T, Lombard C, Sullivan CA, Netshiluvhi TR, 2002. Knowledge on *Sclerocarya birrea* subsp. *caffra* with emphasis on its importance as a non-timber forest product in South and southern Africa: a summary. Part 1: taxonomy, ecology and role in rural livelihoods. *Southern African Forestry Journal* **194**: 27–41.
- Slippers B, Burgess TI, Pavlic D, Ahumada R, Maleme H, Mohali S, Rodas CA, Wingfield MJ, 2009. A diverse assemblage of *Botryosphaeriaceae* infect *Eucalyptus* in native and non-native environments. *Southern Forests* **71**: 101–110.
- Slippers B, Burgess TI, Wingfield BD, Crous PW, Coutinho TA, Wingfield MJ, 2004. Development of simple sequence repeat markers for *Botryosphaeria* spp. with *Fusicoccum* anamorphs. *Molecular Ecology Notes* **4**: 675–677.
- Slippers B, Johnson GI, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ, 2005. Phylogenetic and morphological re-evaluation of the *Botryosphaeria* species causing diseases of *Mangifera indica*. *Mycologia* **97**: 99–110.
- Slippers B, Wingfield MJ, 2007. *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**: 90–106.
- Smith H, Wingfield MJ, Crous PW, Coutinho TA, 1996. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86–88.
- Snyman JC, 1998. Origin and history of the mango. In: de Villiers EA (ed.), *The Cultivation of Mangoes*. Institute for Tropical and Subtropical Crops, Agricultural Research Council, Nelspruit, pp. 1–3.
- Stanosz GR, Smith DR, Leisso R, 2007. *Diplodia* shoot blight and asymptomatic persistence of *Diplodia pinea* on or in stems of jack pine nursery seedlings. *Forest Pathology* **37**: 145–154.
- Stöver BC, Müller KF, 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**: 7.
- Swart WJ, Wingfield MJ, Knox-Davies PS, 1987. Conidial dispersal of *Sphaeropsis sapinea* in three climatic regions of South Africa. *Plant Disease* **71**: 1038–1040.
- Swofford DL, 2003. PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods)*, version 4. Sinauer Associates, Sunderland, MA.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731–2739.
- Trakunyingcharoen T, Cheewangkoon R, To-anun C, Crous PW, van Niekerk JM, Lombard L, 2014. *Botryosphaeriaceae* associated with diseases of mango (*Mangifera indica*). *Australasian Plant Pathology* **43**: 425–438.
- Úrbez-Torres JR, Peduto F, Gubler WD, 2010. First report of grapevine cankers caused by *Lasiodiplodia crassispora* and *Neofusicoccum mediterraneum* in California. *Plant Disease* **94**: 785.
- van Niekerk JM, Bester W, Halleen F, Crous PW, Fourie PH, 2010. First report of *Lasiodiplodia crassispora* as a pathogen of grapevine trunks in South Africa. *Plant Disease* **94**: 1063.
- van Niekerk JM, Crous PW, Groenewald JE, Fourie PH, Halleen F, 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia* **96**: 781–798.
- van Wyk M, van der Merwe NA, Roux J, Wingfield BD, Kamgan GN, Wingfield MJ, 2006. Population genetic analyses suggest that the *Eucalyptus* fungal pathogen *Ceratocystis fimbriata* has been introduced into South Africa. *South African Journal of Science* **102**: 259–263.
- White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*. Academic Press, San Diego, pp. 315–322.
- Wingfield MJ, Brockerhoff EG, Wingfield BD, Slippers B, 2015. Planted forest health: the need for a global strategy. *Science* **349**: 832–836.
- Wright LP, Davis AJ, Wingfield BD, Crous PW, Breneman T, Wingfield MJ, 2010. Population structure of *Cylindrocleftidium parasiticum* infecting peanuts (*Arachis hypogaea*) in Georgia, USA. *European Journal of Plant Pathology* **127**: 199–206.
- Wright S, 1978. *Evolution and the Genetics of Populations: a treatise in four volumes*. In: *Variability within and among Natural Populations*, vol. 4. University of Chicago Press, Chicago.