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 mahajangana*, *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.

Keywords: *Botryosphaeriales*; *Anacardiaceae*; host range; endophytes; marula; mango

1. 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 and Wingfield 2007). Many species in the family have a wide range of plant hosts, including commercial fruit crops (Chen et al. 2014; Slippers et al. 2005; van Niekerk et al. 2004), forest trees (Burgess et al. 2006b; Slippers et al. 2009), and plants in native woody ecosystems (Jami et al. 2014; Mehl et al. 2011; Pavlic et al. 2007). These fungi occur in healthy plant tissues as latent pathogens and persist endophytically until stress occurs, after which disease symptoms can manifest (Slippers and 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 (Jami et al. 2014; Slippers and Wingfield 2007), 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 and Kotzé 1994) causing disease when fruits ripen or are harvested (Menge and 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 and 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 (Crous et al. 2000; Doidge 1950; Farr and 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*.

2. Materials and Methods

2.1. 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 an 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 ºC. 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.

2.2. 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 µl TE buffer, was used for the 2012

isolates.

2.3. 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 1α (*tef1*), and β-tubulin-2 (*tub2*) loci. Primer sets ITS1 and ITS4 (White et al. 1990), EF1-728F and EF1-986R (Carbone and Kohn 1999) and EF1F and EF2R (Jacobs et al. 2004), and Bt-2a and Bt-2b (Glass and 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 × FastStart PCR buffer (with 3 mM added $MgCl_2$) (Roche Molecular Biochemicals, Almeda, California), 0.2 µM of each primer, 2.5 µM of each dNTP, and 0.5 U FastStart *Taq* Polymerase (Roche). The second mix consisted of 1 × KAPA Taq Buffer A (KAPA Biosystems, Cape Town, South Africa), 0.4 µM of each primer, 2.5 mM of each dNTP, and 1 U KAPA *Taq* Polymerase (KAPA Biosystems). The third mix consisted of 1 × 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 µl 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 ºC for 2 min followed by 40 cycles of 95 ºC for 20 s, 55 ºC for 30 s and 72 ºC for 1 min 30 s, followed by a final extension step of 72 ºC for 7 min. PCR products were visualized using 1.5 % agarose-ethidium bromide gels run in 1 × TAE buffer and product sizes estimated using a Lambda DNA/*Eco*RI + *Hind*III 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/phylows/study/TB2:S19055?x-access-

code=e33df5ddbac9fc1a2b78b8a336f67c5c&format=html). 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.

2.4. 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 and 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 (Katoh and 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 and

Müller 2010).

2.5. 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 × PCR buffer (with 3 mM added $MgCl_2$), 1.25 µM of each dNTP, 0.1 µM of each primer, and 0.2 U FastStart *Taq* Polymerase. The second mix consisted of 1 × MyTaq Reaction Buffer, 0.2 µM 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 µl of this mix was combined with 10 µl of a suspension of LIZ-labelled Genescan 500 size standard (Applied Biosystems, Life Technologies) mixed with formamide (14 µl LIZ ml^{-1} formamide). The products were then run on an Applied Biosystems 3500 Genetic Analyzer. Alleles were scored using GeneMapper® Software (Applied Biosystems).

2.6. 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 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 and 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 and 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 and 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 (Nm) based on rare alleles present (Barton and 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 and Gaggiotti 2008). For the second method, ten iterations were done with burnin set at 300 000 followed by 900 000 runs.

3. Results

3.1. 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).

3.2. 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 194,910 most parsimonious trees (TL = 270, CI = 0.73, RI = 0.953, RC = 0.696). The model selected for ML analysis was TPM1uf (γ = 0.295). The *tef1* dataset consisted of 309 characters (191 parsimony informative, 107 constant, 11 parsimony uninformative), and yielded 738,939 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, γ = 0.789). The combined analysis consisted of 849 characters (344 parsimony informative, 481 constant, 24 parsimony uninformative), and yielded 1,034,389 most parsimonious trees (TL = 756, CI = 0.672, RI = 0.943, RC = 0.633). The model TVM (γ = 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 1 188 characters (20 parsimony informative, 1 151 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, γ = 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 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 iraniensis* (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.

3.3. 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 timepoints (two, four, six, and eight weeks) when isolations were made. Of the eight SSR loci tested, two (*BotF15* and *BotF37*) 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 and 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 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 $\pm$ 0.006, source: *M. indica*: 0.617 $\pm$ 0.007) than the other way round (source: *S. birrea* = 0.126 $\pm$ 0.006, source: *M. indica*: 0.383 $\pm$ 0.007) (Table S3).

4. 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 fabicerciana*, *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 vitifusiforme*) have been isolated previously from other hosts in the country, including both native (Jami et al. 2012, 2014; Mehl et al. 2011; Pavlic et al. 2007) and non-native (Begoude et al. 2010; Damm et al. 2007; Mehl et al. 2014; Pavlic et al. 2007; van Niekerk et al. 2004) trees. This is, however, the first time that any of them have been recorded from *S. birrea*.

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. crassispora* (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 *Ps. 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. crassispora* 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; Gladieux et al. 2015; Liebhold et al. 2012; 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 (Kwa-Zulu 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 sub-clades within the species hinting at potential cryptic relatives have been noted in several studies (Abdollahzadeh et al. 2013; Baskarathevan et al. 2012; 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 multilocus 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 (Bebber et al. 2014; Liebhold et al. 2012) 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 and 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 (Begoude et al. 2012; Jami et al. 2014; Sakalidis et al. 2011). 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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Table S1 – Summary of species collected and counts of associated isolates per site.

Table S2 – Allele sizes (bp) and frequencies at six loci in the *N. parvum* isolates collected in this study, after clone-correction. Frequencies are given for the subsets of isolates from each host collected (*S. birrea* and *M. indica*) as well as for the total combined dataset.

Table S3 – Per population migration rates as calculated by BIMr. Included are migration rates for *N. parvum* from *S. birrea* onto either host and from *M. indica* onto either host, with associated standard errors.

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.

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.

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).

Fig S1 – Maximum likelihood (ML) tree resulting from analysis on the ITS dataset for the first group. The tree is rooted to two isolates of *Melanops tulasnei* (CBS116805, CBS116806). 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.

Fig S2 – Maximum likelihood (ML) tree resulting from analysis on the *tef1* dataset for the first group. The tree is rooted to two isolates of *Melanops tulasnei* (CBS116805, CBS116806). 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.

Table 1

Species	Culture number	Other numbers	Host	Location	Collector(s)	ITS	*tef1*	*tub2*
Botryosphaeria fabicerciana	**CMW 27094**	CBS 127193	*Eucalyptus* sp.	FuJian Province, China	M. J. Wingfield	*HQ332197*	*HQ332213*	
B. fabicerciana	CMW 27121	CBS 127194	*E. grandis* hybrid	FuJian Province, China	M. J. Wingfield	*HQ332198*	*HQ332214*	
B. fabicerciana	CMW 25215		*Mangifera indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997394	KU997130	KU997568
B. fabicerciana		MAN2132	*M. indica*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997460	KU997237	KU997579
B. fabicerciana		MAN25238	*M. indica*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997558	KU997319	
B. fabicerciana		MAR28238	*Sclerocarya birrea* subsp. *caffra*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997549	KU997310	KU997616

Diplodia allocellula	**CMW 36468**	CBS 130408	*Acacia karroo*	Pretoria, S. Africa	F. Jami & M. Gryzenhout	*JQ239397*	*JQ239384*
D. allocellula	CMW 36469	CBS 130409	*Ac. karroo*	Pretoria, S. Africa	F. Jami & M. Gryzenhout	*JQ239398*	*JQ239385*
D. allocellula	CMW 24131		*S. birrea* subsp. *caffra*	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997376	KU997114
Lasiodiplodia crassispora	CMW 13488		*Eucalyptus urophylla*	Venezuela	S. Mohali	*DQ103552*	*DQ103559*
L. crassispora	CMW 14688	WAC12534	*Santalum album*	Ord River, Kununurra, W.A.	T. Burgess	*DQ103551*	*DQ103558*
L. crassispora	**CMW 14691**	WAC12533	*San. album*	Ord River, Kununurra, W.A.	T. Burgess	*DQ103550*	*DQ103557*
L. crassispora	CMW 24111		*S. birrea* subsp. *caffra*	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997362	KU997103
L. gonubiensis	CMW 14077	CBS 115812	*Syzygium cordatum*	Gonubie, Eastern Cape, S. Africa	D. Pavlic	*AY639595*	*DQ103566*
L. gonubiensis	**CMW 14078**	CBS 116355	*Syz. cordatum*	Gonubie, Eastern Cape,	D. Pavlic	*AY639594*	*DQ103567*
L. gonubiensis	CMW 24123		*S. birrea* subsp. *caffra*	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997370	KU997109
L. gonubiensis	CMW 24127		*S. birrea*	Zululand,	B. Hinze	KU997374	KU997113

			subsp. *caffra*	KwaZulu-Natal, S. Africa			
L. iraniensis	**CBS 124710**	IRAN1520C	*Salvadora persica*	Hormozgan, Iran	J. Abdollahzadeh /A. Javadi	*GU945348*	*GU945336*
L. iraniensis	CBS 124711	IRAN1502C	*Juglans* sp.	Golestan, Iran	A. Javadi	*GU945347*	*GU945335*
L. iraniensis	CMW 25232		*S. birrea* subsp. *caffra*	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997384	KU997119
L. mahajangana	CMW 27801	CBS 124925	*Terminalia catappa*	Mahajanga, Madagascar	J. Roux	*FJ900595*	*FJ900641*
L. mahajangana	CMW 27818	CBS 124926	*Ter. catappa*	Mahajanga, Madagascar	J. Roux	*FJ900596*	*FJ900642*
L. mahajangana	**CMW 27820**	CBS 124927	*Ter. catappa*	Mahajanga, Madagascar	J. Roux	*FJ900597*	*FJ900643*
L. mahajangana	CMW 25199		*M. indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997387	KU997121	KU997563
L. mahajangana	CMW 25202		*M. indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997388	KU997122	KU997564
L. mahajangana		MAR1212	*S. birrea* subsp.	Hoedspruit-Klaserie Road,	J. Roux	KU997455	KU997231	

			caffra	Limpopo Province, S. Africa			
L. pseudotheobromae	CBS 447.62		*Citrus aurantium*	Suriname	C. Smulders	*EF622081*	*EF622060*
L. pseudotheobromae	**CBS 116459**	KAS2	*Gmelina arborea*	San Carlos, Costa Rica	J. Carranza-Velásquez	*EF622077*	*EF622057*
L. pseudotheobromae	CMW 25203		*M. indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997389	KU997123
L. pseudotheobromae	CMW 28517		*M. indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997123	KU997226	KU997576
L. pseudotheobromae		MAR25328	*S. birrea* subsp. *caffra*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997547	KU997307
L. theobromae	CMW 10130		*Vitex donniana*	Uganda	J. Roux	*AY236951*	*AY236900*
L. theobromae	**CBS 164.96**		Fruit on coral reef coast	Papua New Guinea	A. Aptroot	*AY640255*	*AY640258*
L. theobromae	CMW 24125		*S. birrea* subsp.	Zululand, KwaZulu-Natal, S.	B. Hinze	KU997372	KU997111

			caffra	Africa				
L. theobromae	CMW 25212		*M. indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997392	KU997128	KU997566
Melanops tulasnei	CBS 116805		*Quercus robur*	Bavaria, Munich, Germany	P. A. Saccardo	*FJ824769*	*FJ824774*	
M. tulasnei	CBS 116806		*Q. robur*	Bavaria, Munich, Germany	P. A. Saccardo	*FJ824770*	*FJ824775*	
Neofusicoccum australe	CBS 112872	STE-U4425	*Vitis vinifera*	Stellenbosch, Western Cape, S. Africa	F. Halleen	*AY343388*	*AY343347*	
N. australe	CBS 112877	STE-U4415	*V. vinifera*	Stellenbosch, Western Cape, S. Africa	F. Halleen	*AY343385*	*AY343346*	
N. australe	CMW 25211		*M. indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997391	KU997127	
N. kwambonambiense	**CMW 14023**	CBS 123639	*Syzygium cordatum*	Kwambonambi, S. Africa	D. Pavlic	*EU821900*	*EU821870*	*EU821840*
N. kwambonambiense	CMW 14123	CBS 123643	*Syz. cordatum*	Kwambonambi, S. Africa	D. Pavlic	*EU821924*	*EU821894*	*EU821864*
N. kwambonambiense	CMW 25198		*M. indica*	Hans Merensky Estate, Limpopo	B. Hinze	KU997386	KU997120	KU997562

				Province, S. Africa				
N. kwambonambiense	CMW 28412		*M. indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997418	KU997143	KU997572
N. kwambonambiense		MAN210316	*M. indica*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997532	KU997290	KU997605
N. mediterraneum	CBS 121558	PD311	*Olea europaea*	Lepre, Scorrano, Italy	C. Lazzizera	*GU799463*	*GU799462*	
N. mediterraneum	**CBS 121718**	CPC13137, PD312	*Eucalyptus* sp.	Rhodes, Greece	P. Crous, M. J. Wingfield, A. Phillips	*GU251176*	*GU251308*	
N. mediterraneum	CMW 24080		*S. birrea* subsp. *caffra*	Kruger National Park, Mpumalanga Province, S. Africa	B. Hinze	KU997339	KU997094	
N. mediterraneum	CMW 24083		*S. birrea* subsp. *caffra*	Kruger National Park, Mpumalanga Province, S. Africa	B. Hinze	KU997341	KU997095	
N. mediterraneum	CMW 24122		*S. birrea* subsp. *caffra*	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997369	KU997108	
N. mediterraneum	MAN21312		*M. indica*	Hoedspruit-	J. Roux	KU997470	KU997247	KU997588

				Klaserie Road, Limpopo Province, S. Africa				
N. parvum	**CMW 9081**	ICMP8003, ATCC58191	*Populus nigra*	TePuke/BP, New Zealand	G. Samuels	*AY236943*	*AY236888*	*AY236917*
N. parvum	CBS 110301	CAP074	*V. vinifera*	Palmella, Portugal	A. Phillips	*AY259098*	*AY573221*	*EU673095*
N. parvum	CMW 28377		*M. indica*	Hans Merensky Estate, Limpopo Province, S. Africa	B. Hinze	KU997395	KU997131	KU997569
N. parvum		MAR11328	*S. birrea* subsp. *caffra*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997539	KU997293	KU997607
N. parvum		MAR21022	*S. birrea* subsp. *caffra*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997456	KU997232	KU997577
N. parvum		MAR2134	*S. birrea* subsp. *caffra*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997474	KU997252	KU997593
N. umdonicola	**CMW 14058**	CBS 123645	*Syz. cordatum*	Kosi Bay, S. Africa	D. Pavlic	*EU821904*	*EU821874*	*EU821844*

N. umdonicola	CMW 14106	CBS 123644	*Syz. cordatum*	Sodwana Bay, S. Africa	D. Pavlic	*EU821905*	*EU821875*	*EU821839*
N. umdonicola		MAN210236	*M. indica*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997531	KU997289	KU997604
N. vitifusiforme	CBS 110880	STE-U5050	*V. vinifera*	Stellenbosch, Western Cape, S. Africa	J. van Niekerk	*AY343382*	*AY343344*	
N. vitifusiforme	**CBS 110887**	STE-U5252	*V. vinifera*	Stellenbosch, Western Cape, S. Africa	J. van Niekerk	*AY343383*	*AY343343*	
N. vitifusiforme	CMW 24068		*S. birrea* subsp. *caffra*	Kruger National Park, Mpumalanga Province, S. Africa	B. Hinze	KU997329	KU997092	
N. vitifusiforme	CMW 24077		*S. birrea* subsp. *caffra*	Kruger National Park, Mpumalanga Province, S. Africa	B. Hinze	KU997336	KU997093	
N. vitifusiforme	CMW 24112		*S. birrea* subsp. *caffra*	Zululand, KwaZulu-Natal, S. Africa	B. Hinze	KU997363	KU997104	
N. vitifusiforme	CMW 24117		*S. birrea* subsp.	Zululand, KwaZulu-Natal, S.	B. Hinze	KU997367	KU997107	

			caffra	Africa			
Pseudofusicoccum olivaceum	**CMW 20881**	CBS 124939	*Pterocarpus angolensis*	Mawewe Nature Reserve, S. Africa	J. Mehl & J. Roux	*FJ888459*	*FJ888437*
Ps. olivaceum	CMW 22637	CBS 124940	*Pt. angolensis*	Pretoriuskop, Kruger National Park, S. Africa	J. Roux	*FJ888462*	*FJ888438*
Ps. olivaceum		MAN22138	*M. indica*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997555	KU997316
Ps. olivaceum		MAN22312	*M. indica*	Hoedspruit-Klaserie Road, Limpopo Province, S. Africa	J. Roux	KU997461	KU997238

Table 2

Locus	*S. birrea*	*M. indica*	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*			
Observed	0.493, P = 0.419	0.330, P = 0.070	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

*Genotypic diversities were computed prior to clone-correction.

Table 3

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

Table S1

Year	Host	Site	Species	Isolates
2006	*S. birrea*	Hans Merensky Estate	*Lasiodiplodia theobromae*	1
		Kruger National Park	*N. mediterraneum*	8
			N. vitifusiforme	30
		Lakelands	*Diplodia allocellula*	2
			L. crassispora	1
			L. gonubiensis	3
			L. iraniensis	8
			L. theobromae	1
			N. mediterraneum	1
			N. vitifusiforme	7
	M. indica	Hans Merensky Estate	*Botryosphaeria fabicerciana*	2
			L. mahajangana	1
			L. pseudotheobromae	2
			L. theobromae	2
			N. australe	1
			N. kwambonambiense	6
			N. parvum	114
			N. umdonicola	13
2012	*S. birrea*	Hoedspruit	*B. fabicerciana*	1
			L. mahajangana	1
			L. pseudotheobromae	1
			N. parvum	29
			N. umdonicola	2
	M. indica	Hoedspruit	*B. fabicerciana*	3
			N. kwambonambiense	8
			N. mediterraneum	1
			N. parvum	137
			N. umdonicola	12
			Pseudofusicoccum olivaceum	2

Table S2

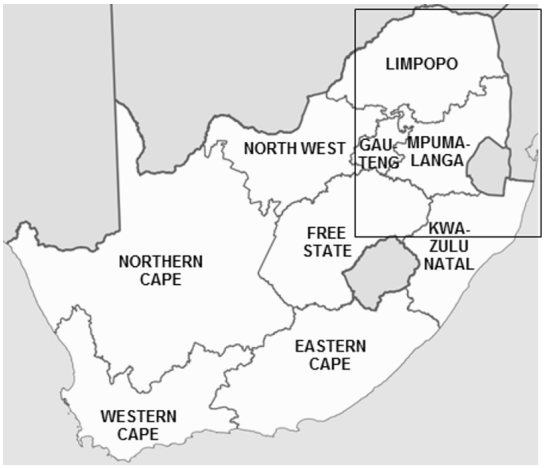
Locus	Allele	*S. birrea*	*M. indica*	Combined
BotF11	420	0.050	0.026	0.034
	429	0.700[+]	0.923[+]	0.847[+]
	433	0.250	0.051	0.119
	Null	0.259	0.235	0.244
*BotF17**	1	0.125	0.306[+]	0.247[+]
	2	0	0.082	0.055
	3	0.292	0.061	0.137
	4	0.167	0.184	0.178
	5	0	0.102	0.068
	6	0.333[+]	0.184	0.233
	7	0.083	0.061	0.068
	8	0	0.020	0.014
	Null	0.111	0.039	0.064
BotF18	234	0.560[+]	0.370[+]	0.437[+]
	244	0.320	0.326	0.324
	250	0.120	0.304	0.239
	Null	0.074	0.098	0.090
BotF21	211	1[+]	0.818[+]	0.879[+]
	221	0	0.182	0.121
	Null	0.185	0.137	0.154
BotF23	424	0.520[+]	0.784[+]	0.697[+]
	427	0.480	0.216	0.303
	Null	0.074	0	0.026
*BotF35**	1	0.182	0.071	0.120
	2	0	0.143	0.080
	3	0.091	0.071	0.080
	4	0.545[+]	0.214	0.360[+]
	5	0	0.071	0.040
	6	0.091	0.357[+]	0.240
	7	0.091	0.071	0.080
	Null	0.593	0.725	0.679

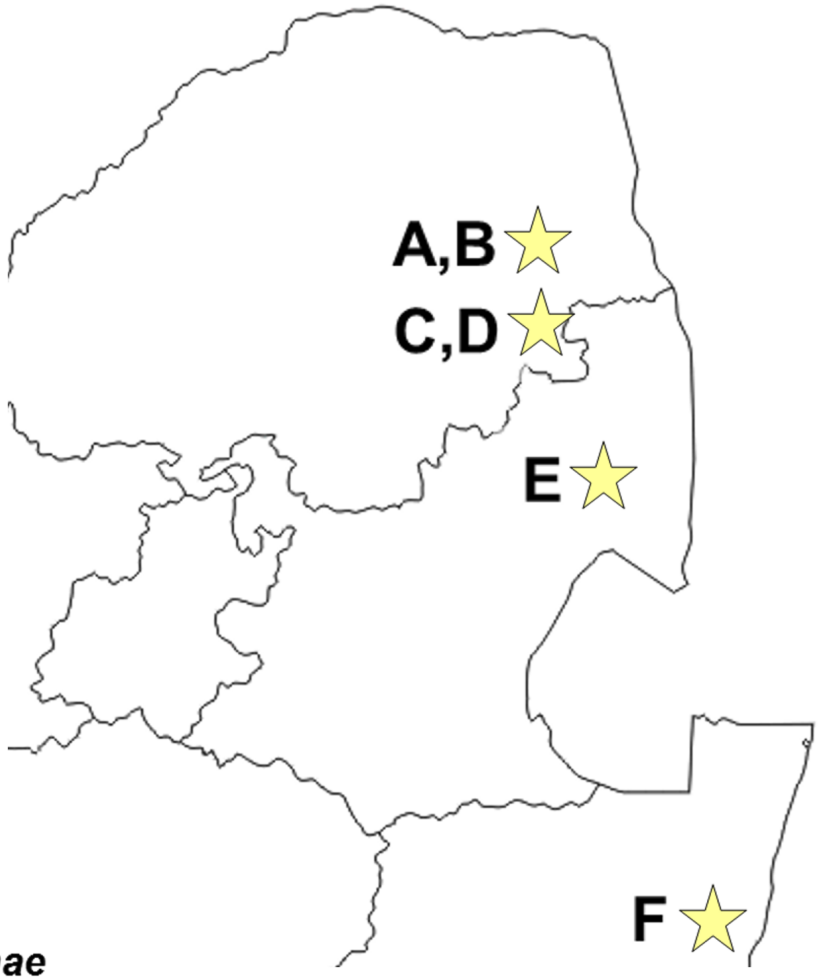
[+]Dominant allele at each locus

*Alleles at these loci were sequenced and the different genotypes observed are denoted by numbers 1-8.

Table S3

Iteration	**Source population → destination population (± standard error)**			
	S. birrea → S. birrea	*S. birrea → M. indica*	*M. indica → M. indica*	*M. indica → S. birrea*
1	0.884 ± 0.004	0.117 ± 0.004	0.375 ± 0.005	0.625 ± 0.005
2	0.884 ± 0.004	0.117 ± 0.004	0.375 ± 0.005	0.625 ± 0.005
3	0.883 ± 0.004	0.117 ± 0.004	0.374 ± 0.005	0.626 ± 0.005
4	0.883 ± 0.004	0.117 ± 0.004	0.374 ± 0.005	0.626 ± 0.005
5	0.883 ± 0.004	0.117 ± 0.004	0.374 ± 0.005	0.626 ± 0.005
6	0.884 ± 0.004	0.116 ± 0.004	0.379 ± 0.008	0.621 ± 0.008
7	0.883 ± 0.004	0.117 ± 0.004	0.374 ± 0.005	0.627 ± 0.005
8	0.884 ± 0.004	0.116 ± 0.004	0.378 ± 0.007	0.622 ± 0.007
9	0.793 ± 0.022	0.207 ± 0.022	0.453 ± 0.020	0.547 ± 0.020
10	0.883 ± 0.004	0.117 ± 0.004	0.375 ± 0.005	0.625 ± 0.005
Average	0.875 ± 0.006	0.126 ± 0.006	0.383 ± 0.007	0.617 ± 0.007

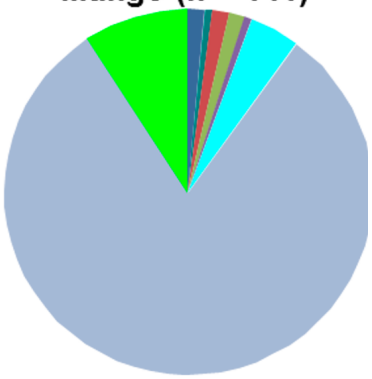




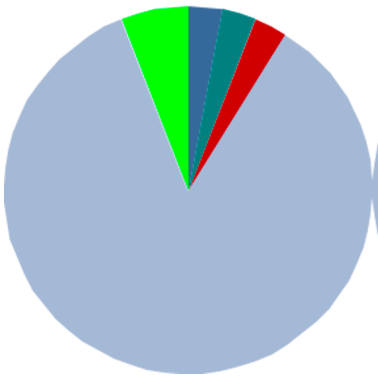
- ***Botryosphaeria fabicerciana***
- ***Diplodia allocellula***
- ***Lasiodiplodia crassispora***
- ***Lasiodiplodia gonubiensis***
- ***Lasiodiplodia iraniensis***
- ***Lasiodiplodia mahajangana***
- ***Lasiodiplodia pseudotheobromae***
- ***Lasiodiplodia theobromae***
- ***Neofusicoccum australe***
- ***Neofusicoccum kwambonambiense***
- ***Neofusicoccum mediterraneum***
- ***Neofusicoccum parvum***
- ***Neofusicoccum umdonicola***
- ***Neofusicoccum vitifusiforme***
- ***Pseudofusicoccum olivaceum***

A. Hans Merensky Estate, Marula (n = 1)

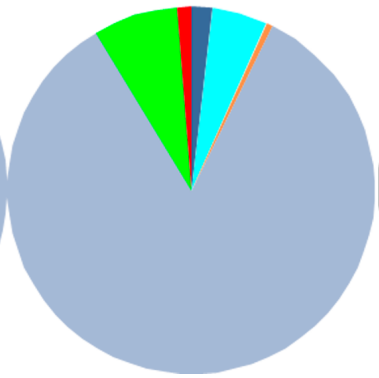
B. Hans Merensky Estate, Mango (n = 141)



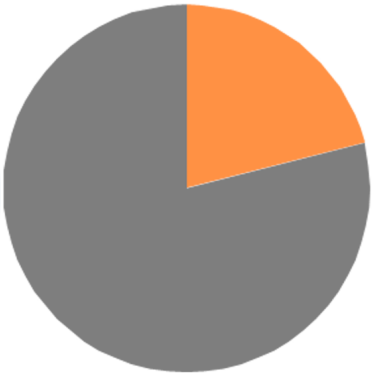
C. Hoedspruit, Marula (n = 34)



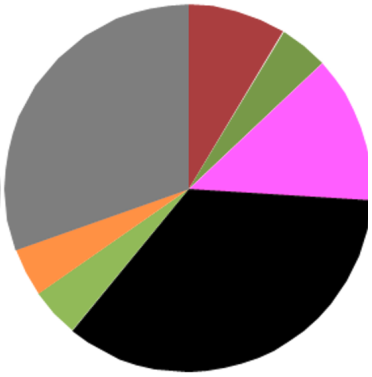
D. Hoedspruit, Mango (n = 163)

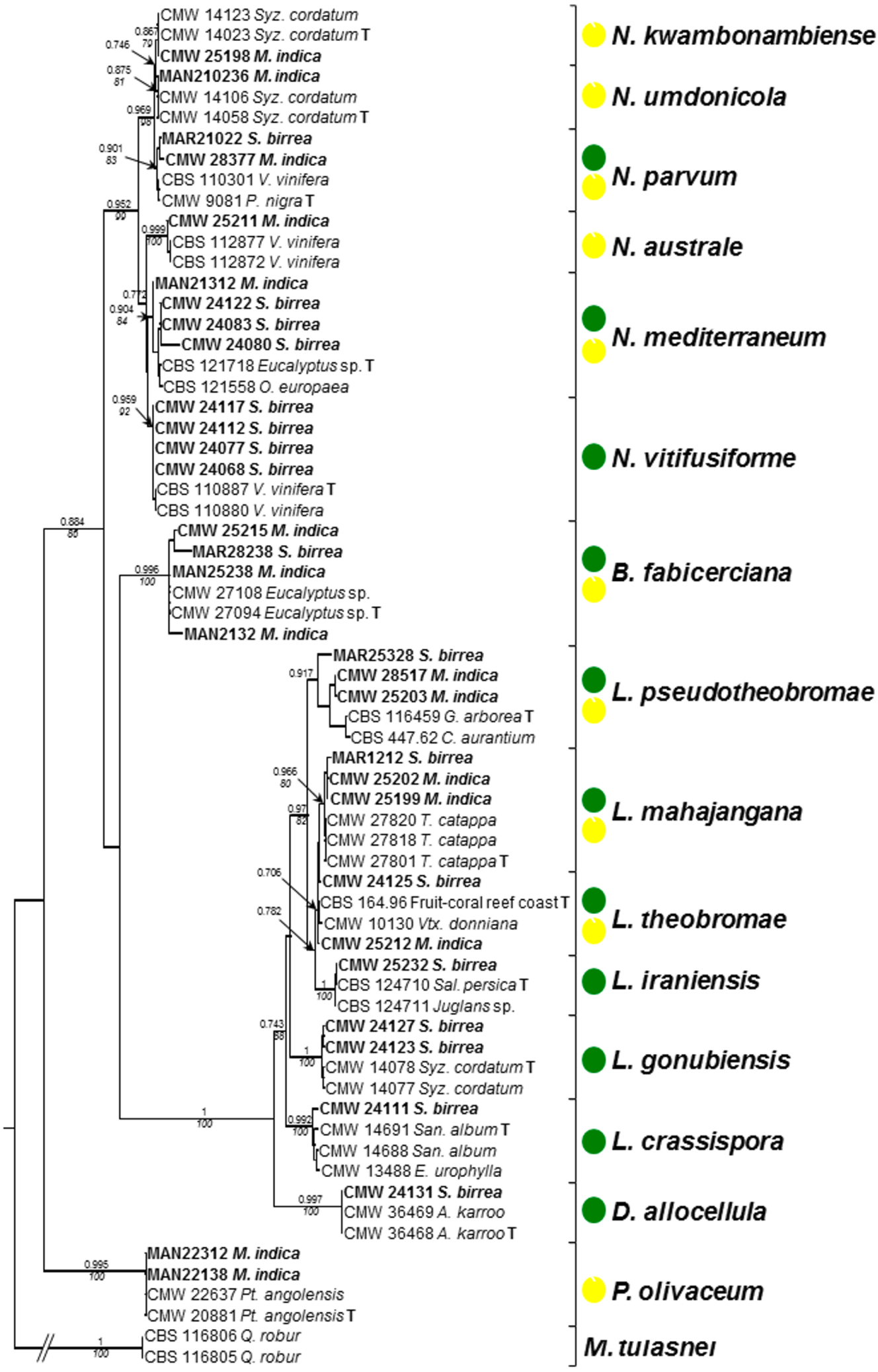


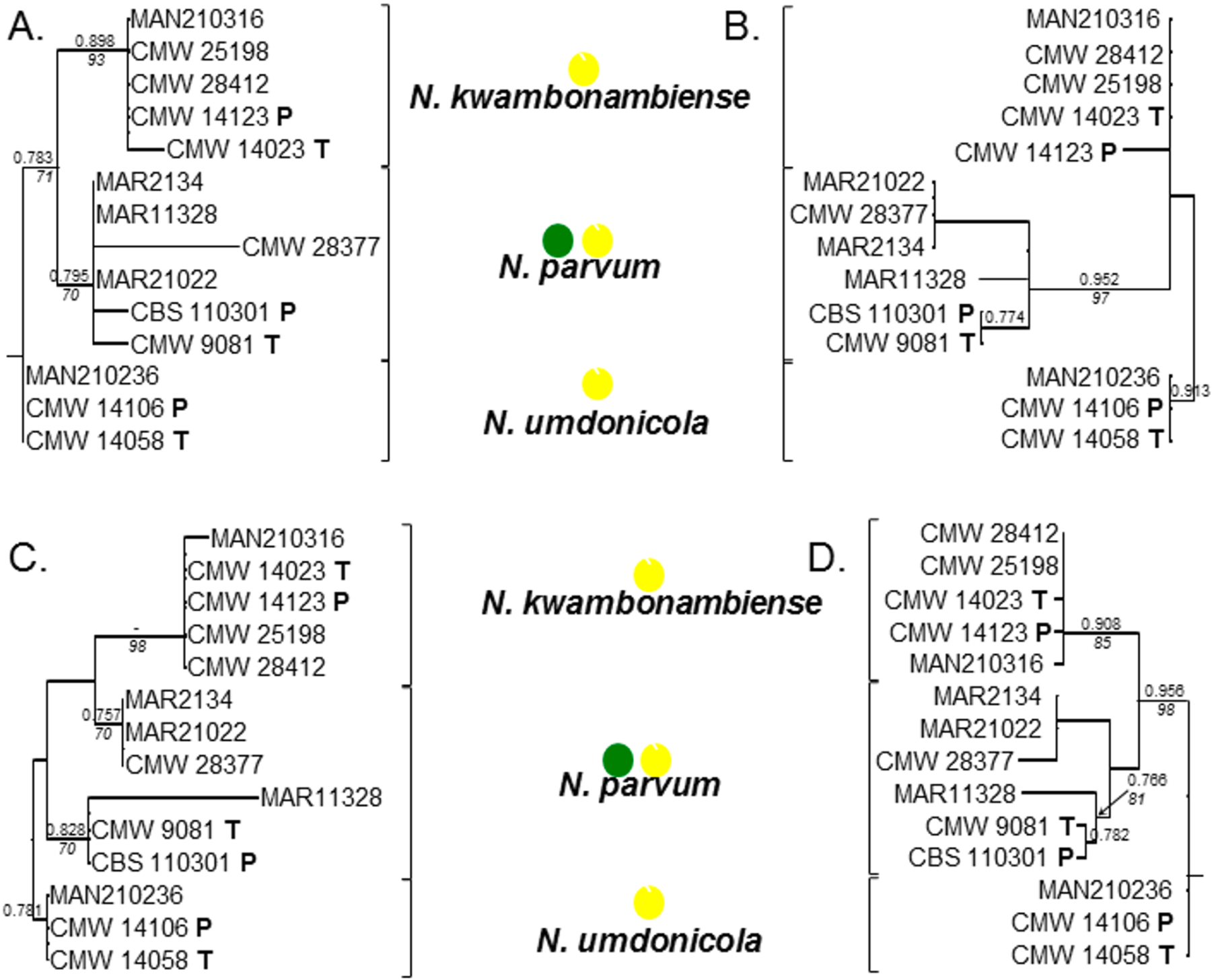
E. Kruger National Park, Marula (n = 38)

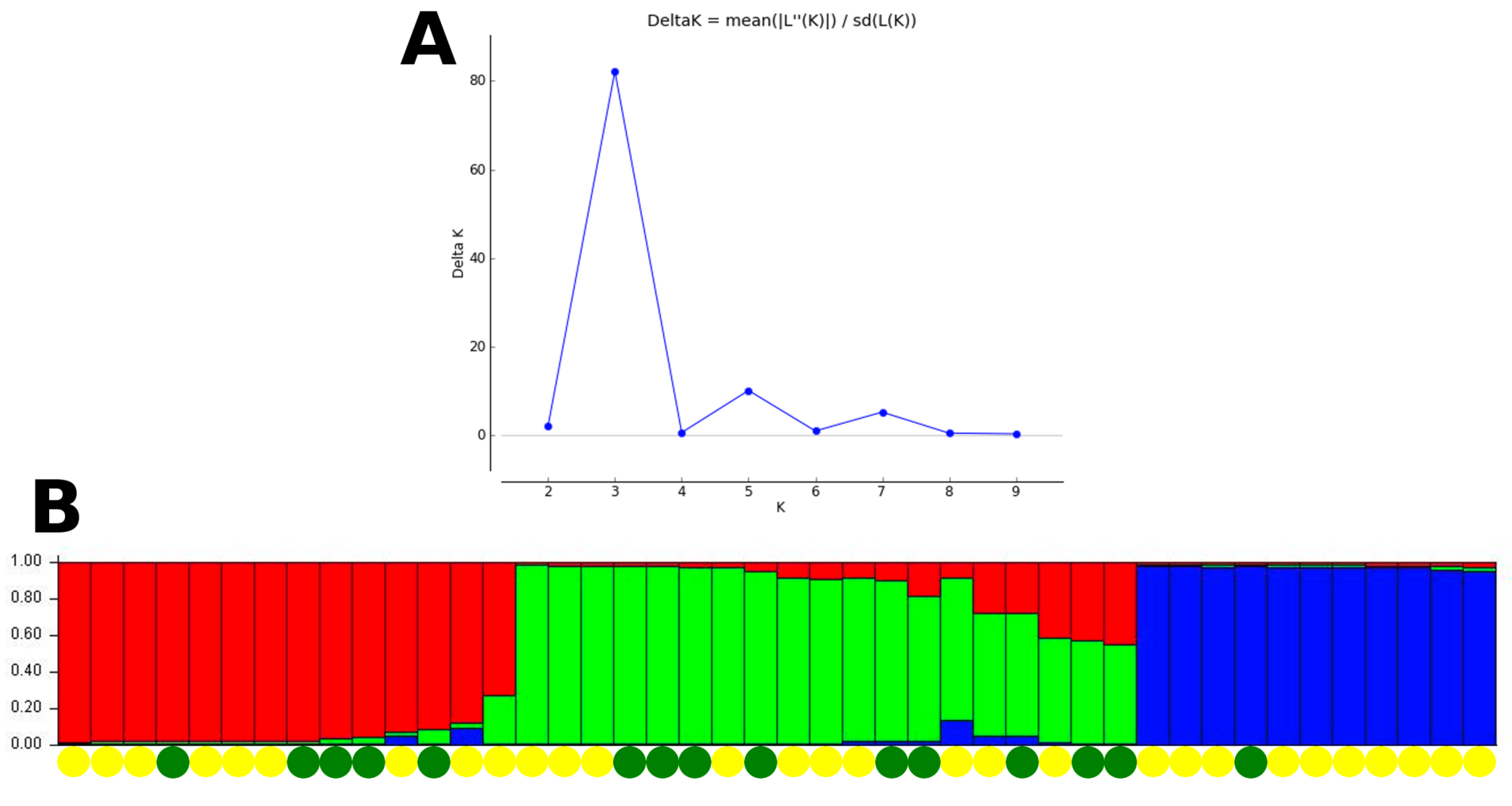


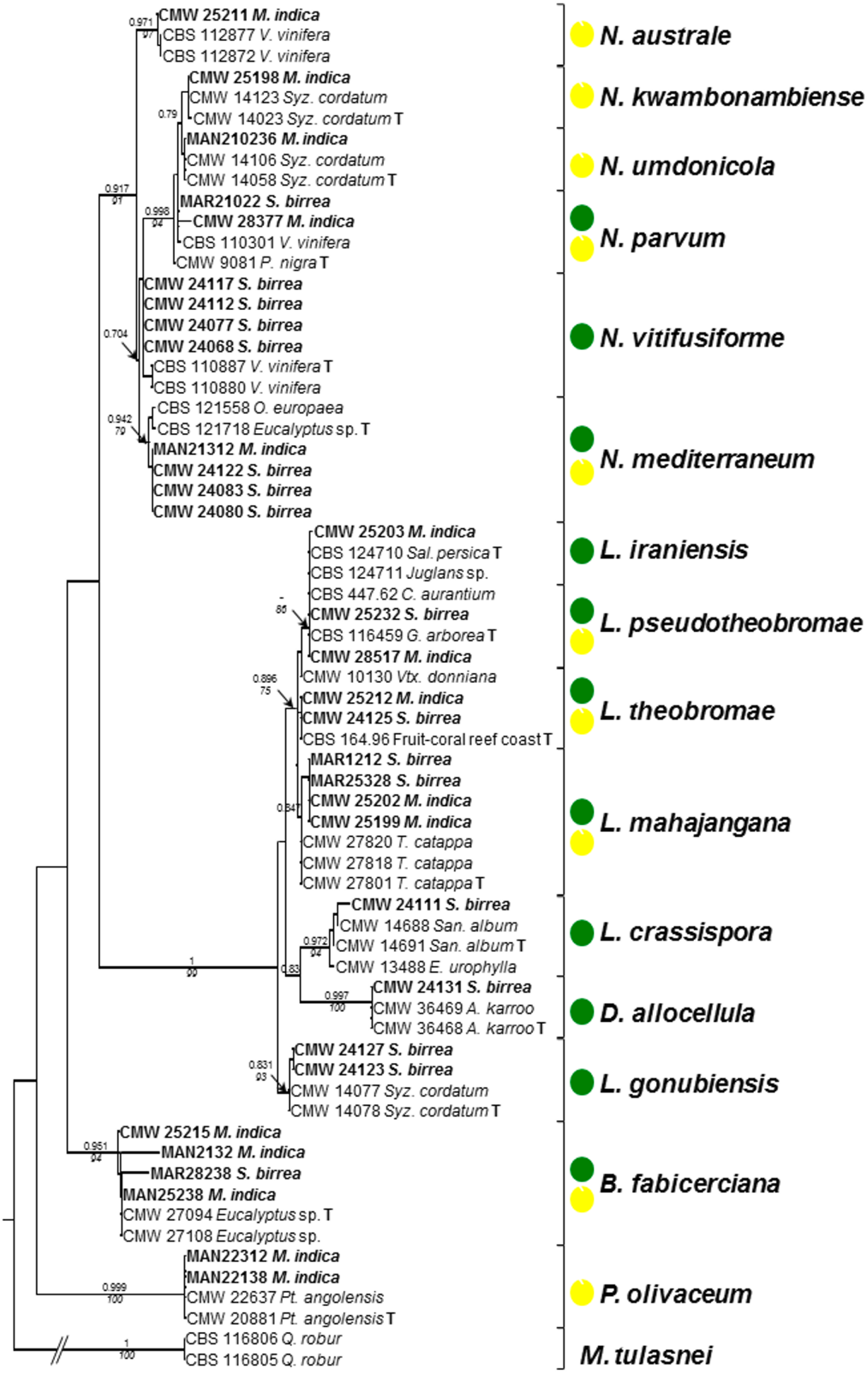
F. Lakelands, Mfolozi Village, Marula (n = 23)




CMW 14123 Syz. cordatum
CMW 14023 Syz. cordatum T
CMW 25198 M. indica
MAN210236 M. indica
CMW 14106 Syz. cordatum
CMW 14058 Syz. cordatum T
MAR21022 S. birrea
CMW 28377 M. indica
CBS 110301 V. vinifera
CMW 9081 P. nigra T
CMW 25211 M. indica
CBS 112877 V. vinifera
CBS 112872 V. vinifera
MAN21312 M. indica
CMW 24122 S. birrea
CMW 24083 S. birrea
CMW 24080 S. birrea
CBS 121718 Eucalyptus sp. T
CBS 121558 O. europaea
CMW 24117 S. birrea
CMW 24112 S. birrea
CMW 24077 S. birrea
CMW 24068 S. birrea
CBS 110887 V. vinifera T
CBS 110880 V. vinifera
CMW 25215 M. indica
MAR28238 S. birrea
MAN25238 M. indica
CMW 27108 Eucalyptus sp.
CMW 27094 Eucalyptus sp. T
MAN2132 M. indica
MAR25328 S. birrea
CMW 28517 M. indica
CMW 25203 M. indica
CBS 116459 G. arborea T
CBS 447.62 C. aurantium
MAR1212 S. birrea
CMW 25202 M. indica
CMW 25199 M. indica
CMW 27820 T. catappa
CMW 27818 T. catappa
CMW 27801 T. catappa T
CMW 24125 S. birrea
CBS 164.96 Fruit-coral reef coast T
CMW 10130 Vtx. donniana
CMW 25212 M. indica
CMW 25232 S. birrea
CBS 124710 Sal. persica T
CBS 124711 Juglans sp.
CMW 24127 S. birrea
CMW 24123 S. birrea
CMW 14078 Syz. cordatum T
CMW 14077 Syz. cordatum
CMW 24111 S. birrea
CMW 14691 San. album T
CMW 14688 San. album
CMW 13488 E. urophylla
CMW 24131 S. birrea
CMW 36469 A. karroo
CMW 36468 A. karroo T
MAN22312 M. indica
MAN22138 M. indica
CMW 22637 Pt. angolensis
CMW 20881 Pt. angolensis T
CBS 116806 Q. robur
CBS 116805 Q. robur
N. kwambonambiense
N. umdonicola
N. parvum
N. australe
N. mediterraneum
N. vitifusiforme
B. fabicerciana
L. pseudotheobromae
L. mahajangana
L. theobromae
L. iraniensis
L. gonubiensis
L. crassispora
D. allocellula
P. olivaceum
M. tulasnei


A.
MAN210316
CMW 25198
CMW 28412
CMW 14123 P
CMW 14023 T
MAR2134
MAR11328
CMW 28377
MAR21022
CBS 110301 P
CMW 9081 T
MAN210236
CMW 14106 P
CMW 14058 T
0.898
93
0.783
71
0.795
70
N. kwambonambiense
N. parvum
N. umdonicola
B.
MAN210316
CMW 28412
CMW 25198
CMW 14023 T
CMW 14123 P
MAR21022
CMW 28377
MAR2134
MAR11328
CBS 110301 P
CMW 9081 T
MAN210236
CMW 14106 P
CMW 14058 T
0.952
97
0.774
0.913
C.
MAN210316
CMW 14023 T
CMW 14123 P
CMW 25198
CMW 28412
MAR2134
MAR21022
CMW 28377
MAR11328
CMW 9081 T
CBS 110301 P
MAN210236
CMW 14106 P
CMW 14058 T
-
98
0.757
70
0.828
70
0.781
N. kwambonambiense
N. parvum
N. umdonicola
D.
CMW 28412
CMW 25198
CMW 14023 T
CMW 14123 P
MAN210316
MAR2134
MAR21022
CMW 28377
MAR11328
CMW 9081 T
CBS 110301 P
MAN210236
CMW 14106 P
CMW 14058 T
0.908
85
0.956
98
0.766
81
0.782


A
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
80
60
40
20
0
2
3
4
5
6
7
8
9
K
B
1.00
0.80
0.60
0.40
0.20
0.00


CMW 25211 M. indica
CBS 112877 V. vinifera
CBS 112872 V. vinifera
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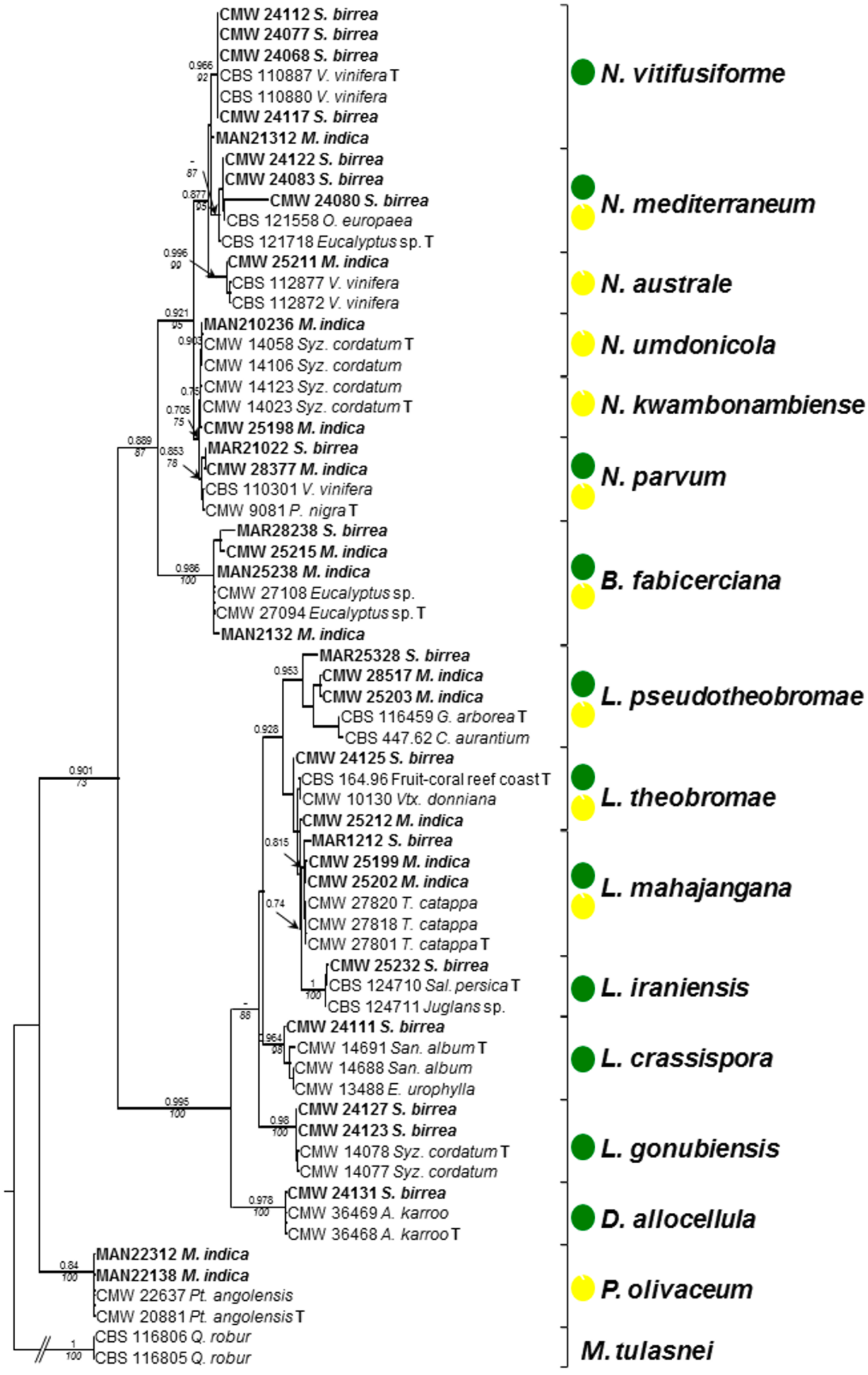
CMW 14123 Syz. cordatum
CMW 14023 Syz. cordatum T
MAN210236 M. indica
CMW 14106 Syz. cordatum
CMW 14058 Syz. cordatum T
MAR21022 S. birrea
CMW 28377 M. indica
CBS 110301 V. vinifera
CMW 9081 P. nigra T
CMW 24117 S. birrea
CMW 24112 S. birrea
CMW 24077 S. birrea
CMW 24068 S. birrea
CBS 110887 V. vinifera T
CBS 110880 V. vinifera
CBS 121558 O. europaea
CBS 121718 Eucalyptus sp. T
MAN21312 M. indica
CMW 24122 S. birrea
CMW 24083 S. birrea
CMW 24080 S. birrea
CMW 25203 M. indica
CBS 124710 Sal. persica T
CBS 124711 Juglans sp.
CBS 447.62 C. aurantium
CMW 25232 S. birrea
CBS 116459 G. arborea T
CMW 28517 M. indica
CMW 10130 Vtx. donniana
CMW 25212 M. indica
CMW 24125 S. birrea
CBS 164.96 Fruit-coral reef coast T
MAR1212 S. birrea
MAR25328 S. birrea
CMW 25202 M. indica
CMW 25199 M. indica
CMW 27820 T. catappa
CMW 27818 T. catappa
CMW 27801 T. catappa T
CMW 24111 S. birrea
CMW 14688 San. album
CMW 14691 San. album T
CMW 13488 E. urophylla
CMW 24131 S. birrea
CMW 36469 A. karroo
CMW 36468 A. karroo T
CMW 24127 S. birrea
CMW 24123 S. birrea
CMW 14077 Syz. cordatum
CMW 14078 Syz. cordatum T
CMW 25215 M. indica
MAN2132 M. indica
MAR28238 S. birrea
MAN25238 M. indica
CMW 27094 Eucalyptus sp. T
CMW 27108 Eucalyptus sp.
MAN22312 M. indica
MAN22138 M. indica
CMW 22637 Pt. angolensis
CMW 20881 Pt. angolensis T
CBS 116806 Q. robur
CBS 116805 Q. robur
N. australe
N. kwambonambiense
N. umdonicola
N. parvum
N. vitifusiforme
N. mediterraneum
L. iraniensis
L. pseudotheobromae
L. theobromae
L. mahajangana
L. crassispora
D. allocellula
L. gonubiensis
B. fabicerciana
P. olivaceum
M. tulasnei


CMW 24112 S. birrea
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CMW 27801 T. catappa T
CMW 25232 S. birrea
CBS 124710 Sal. persica T
CBS 124711 Juglans sp.
CMW 24111 S. birrea
CMW 14691 San. album T
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CMW 13488 E. urophylla
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CMW 24123 S. birrea
CMW 14078 Syz. cordatum T
CMW 14077 Syz. cordatum
CMW 24131 S. birrea
CMW 36469 A. karroo
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MAN22312 M. indica
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N. mediterraneum
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L. theobromae
L. mahajangana
L. iraniensis
L. crassispora
L. gonubiensis
D. allocellula
P. olivaceum
M. tulasnei