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Short communication

Diversity and distribution of co-infecting Botryosphaeriaceae from *Eucalyptus grandis* and *Syzygium cordatum* in South Africa

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ABSTRACT

Species in the fungal family Botryosphaeriaceae are latent pathogens on woody trees. These fungi often have a wide host range, which can include native and introduced hosts in an area. Multi-locus DNA sequence identification on a recent collection of Botryosphaeriaceae from *Eucalyptus grandis* and *Syzygium cordatum* trees in South Africa revealed cross-infectivity of several species, novel host associations and new country reports. *Neofusicoccum eucalyptorum*, *Neofusicoccum kwambonambiense, Neofusicoccum parvum, Neofusicoccum australe* and *Lasiodiplodia pseudotheobromae* were identified from both tree species, with *L. pseudotheobromae* and *N. eucalyptorum* isolated for the first time from *S. cordatum*, similar to *N. kwambonambiense* from *Eucalyptus*. This also represents the first report of *L. pseudotheobromae* from South Africa. Botryosphaeriaceae species on *Eucalyptus* species and *S. cordatum* are fairly well known from South Africa. However, this study revealed new associations, indicating that conclusions should not be generalized and that more intensive sampling from different areas and over time is likely to reveal distinct species and host association patterns.

1. Introduction

Species of the Botryosphaeriaceae associated with *Eucalyptus* (Myrtales, Myrtaceae) trees in plantations have been well studied and are known to occur as endophytes and, in some cases, opportunistic latent pathogens (Burgess et al., 2005; Mohali et al., 2007; Slippers et al., 2004c, 2009; Smith et al., 1994). Countries where Botryosphaeriaceae have been characterised on *Eucalyptus* include Congo (Roux et al., 2000), Uganda (Nakabonge, 2002), Chile (Ahumada, 2003), Australia (Slippers et al., 2004c), South Africa (Burgess et al., 2005; Burgess et al., 2006a; Slippers et al., 2004c, 2009), Ethiopia (Gezahgne et al., 2004), Venezuela (Mohali et al., 2006), Colombia (Rodas et al., 2009), Uruguay (Pérez et al., 2008, 2009) and China (Chen et al., 2011). The Botryosphaeriaceae that occur on these *Eucalyptus* trees in different parts of the world vary considerably. For example, in Venezuela the dominant Botryosphaeriaceae include *Botryosphaeria mamane, Neofusicoccum andium*, *Neofusicoccum parvum, N. pseudofusicoccum*, *N. stromaticum, Lasiodiplodia theobromae*, *L. crassispora* and *Lasiodiplodia pseudotheobromae* (Mohali et al., 2006, 2007). This is different from species combinations present in western Australia that include *Fusicoccum ramsorum, N. parvum, Neofusicoccum australe, N. macroclavatum, P. adansoniae, P. ardesiarum, P. kimberleyense,* and *L. theobromae* (Burgess et al., 2005, 2006a; Pavlic et al., 2008). In South Africa, *N. parvum*, *N. australe*, *Neofusicoccum eucalyptorum* and *N. eucalypticola* are dominant species (Slippers et al., 2004b, 2004c, 2009). These varying species compositions are indicative of a rich diversity of Botryosphaeriaceae on this tree in different parts of the world that potentially arise from local populations.

Many Botryosphaeriaceae that occur on *Eucalyptus* also occur on other hosts. For example, *N. parvum,* which is one of the dominant species on *Eucalyptus* in many parts of the world, has also been found on *Populus nigra* (black poplar) in New Zealand (Slippers et al., 2004a), *Actinidia deliciosa* (kiwifruit) in New Zealand (Slippers et al., 2004a), *Malus sylvestris* (wild apple) in New Zealand (Zhou and Stanosz, 2001), *Ribes* sp. (currents) in Australia (Slippers et al., 2004a), *Tibouchina* sp. in Australia (Slippers et al., 2004c), and *Heteropyxis natalensis* (lavender tree) (Smith et al., 2001), *Terminalia catappa* (Begoude et al., 2010) and *Syzygium cordatum* (Pavlic et al., 2007) in South Africa. *N. australe* has been previously isolated from *Acacia* sp. in Australia (Slippers et al., 2004c), *Wollemia nobilis* in Australia (Slippers et al., 2005), and *Widdringtonia nodiflora* (mountain cypress) in South Africa (Slippers et al., 2005). *L. pseudotheobromae* was found on a *Rosa* sp. (rose) in Netherlands (Alves et al., 2008), a *Coffea* sp. (coffee) in Zaire (Alves et al., 2008), *Citrus aurantium* (sour orange) in Suriname (Alves et al., 2008), *Gmelina arborea* (Beechwood) in Costa Rica (Alves et al., 2008), *Acacia mangium* (black wattle) in Costa Rica (Alves et al., 2008) and *Terminalia catappa* (Bengal almond) in Cameroon (Begoude et al., 2010). In Uruguay the Botryosphaeriaceae on a non-native *Eucalyptus* sp. and various species of native Myrtaceae (Pérez et al., 2008) included *N. parvum*/*N. ribis* and *B. dothidea* on all the Myrtaceae, while *N. eucalyptorum* was found exclusively on *Eucalyptus*. However, after further sampling, *N. eucalyptorum* was also identified on other Myrtaceae such as *Blepharocalyx salicifolius*,

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Myrrhinium atropurpureum var. *octandrum* and *Myrceugenia glaucescens* (Pérez et al., 2009). Clearly the Botryosphaeriaceae often have the ability to infect a great diversity of hosts, with few consistent patterns of host association.

In South Africa Botryosphaeriaceae has also been studied on native Myrtaceae, in particular *S. cordatum*, have been well characterised (Burgess and Wingfield, 2004; Pavlic et al., 2004, 2007, 2008, 2009; Slippers et al., 2004c; Smith et al., 1994). Species that have been described from this tree include *N. ribis, N. kwambonambiense, N. umdonicola, N. cordaticola, N. australe, N. mangiferae, N. parvum, N. luteum, B. dothidea, L. theobromae* and *L. gonubiense* (Pavlic et al., 2007, 2008, 2009). These species have been collected from various locations in South Africa across the natural range of *S. cordatum* and were mostly isolated as endophytes or associated with disease symptoms such as die-back (Pavlic et al., 2004, 2007, 2009). Pathogenicity tests have shown that *N. ribis* and *L. theobromae* are the most pathogenic species on *S. cordatum* (Pavlic et al., 2007). Only *N. parvum* and *N. australe* are known to co-infect *Eucalyptus* spp. and *S. cordatum* in South Africa (Pavlic et al., 2007).

Previous studies identifying the Botryosphaeriaceae on *Eucalyptus* spp. and *S. cordatum* took a broad approach where trees were sampled over a broad geographic area. Furthermore, the samples were collected from both disease symptoms and healthy twigs and leaves. In the present study, a more dense sampling approach was used to directly compare the Botryosphaeriaceae species assemblages from a *Eucalyptus grandis* and a *S. cordatum* tree collected at the same time and from the same geographical location. A multi-gene sequencing approach was followed to identify the various isolates because the ITS region (Internal Transcribed Spacer) alone, which is commonly used for fungal species identification, is insufficient to resolve certain species complexes in the Botryosphaeriaceae, such as the *N. parvum/N. ribis* complex and *N. luteum/N. australe* complex (Pavlic et al., 2008; Slippers et al., 2004c). Additional DNA sequences of the elongation factor 1-alpha (EF-1α) and the RNA polymerase II subunit (RPB2) genes were thus used.

2. Materials and methods

2.1. Sampling site and fungal isolations

Sampling was performed on the eastern coast (Mtubatuba, KwaZulu-Natal) of South Africa, where *Eucalyptus* are grown as non-natives in plantations surrounded by patches of natural vegetation that include *S. cordatum* trees. This particular site (E 32′9′54′, S28′ 29′ 53.0, 33 m above sea level) was chosen because a comprehensive survey of the Botryosphaeriaceae on *S. cordatum* throughout South Africa (Pavlic et al., 2009) had previously shown that trees from this region had a high diversity of Botryosphaeriaceae species. An *E. grandis* and a *S. cordatum* tree were sampled in April 2009, following a relatively high density sampling approach where asymptomatic plant tissues (leaves, increment cores of wood, twigs and petioles were taken from both trees, with the exception that for the *S. cordatum* tree, no petioles were sampled because the leaves are sessile. Four leaves per branch, four branches, and one increment core from one tree per species were sampled. The plant tissues were placed in paper bags and transferred to the laboratory for further processing.

To remove fungal propagules and epiphytic fungi on the plant tissue surfaces (leaves, twigs, petioles and increment) all the substrate tissue samples used in this study were surface disinfested for 5 min using 5% hydrogen peroxide after samples were washed with sterile water. The surface disinfested tissue sections from the trees were placed on full strength malt extract (MEA) agar (20 g malt extract, 20 g agar; Biolab, Midrand, SA) with four to six sub-sections from the same sample placed approximately 4 cm apart. The plates were incubated at 25 °C for approximately 10 days. Growth of endophytic fungi from the plant tissue was checked daily to isolate slow growing fungi before they were overgrown by those that are faster growing. Plant tissues that did not show any initial fungal growth were monitored for a month.

Cultures morphologically resembling the Botryosphaeriaceae (grey to dark in colour with fluffy mycelium, or with black pigment visible from the reverse side of the Petri dish) were sub-cultured to obtain pure cultures by transferring single hyphae onto new MEA agar plates using a sterile needle. Purified cultures were incubated for two weeks under near-UV light and grouped in morphotypes according to colony shape, colour, texture, mycelium type, medium discolouration and colony density. ITS data were generated for these isolates and groupings based on the ITS sequence data were verified with culture morphology (texture, margin, colour, discolouration of the medium) to ensure that the subsets of all groups observed with colony morphology and DNA sequences were included in additional multi-locus sequencing. All cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

2.2. DNA sequence analysis

Mycelium was scraped from the surface of cultures using a sterile scalpel, transferred to 2 ml Eppendorf tubes, freeze-dried and ground to a fine powder using sterile 2 mm metal beads on a Mixer Mill type MM 301 Retsch^{R} tissue lyser (Retsch, Germany) for 3 min at a frequency of 30 cycles. Total fungal genomic DNA was extracted following a method described by Moller et al. (1992). DNA pellets were re-suspended in 50 μl sterile SABAX water (Adcock Ingrams, Bryanston, South Africa). DNA concentrations were determined using the ND-1000 spectrophotometer V3.7.1 (Thermo Fisher Scientific, USA). The DNA was diluted to 50 ng/μl for use in subsequent polymerase chain reactions (PCR's).

The full length ITS region was amplified for initial identifications, including parts of the ribosomal small ribosomal subunit (SSU) and ribosomal large subunit (LSU), using the forward V9G primer (Hoog and Ende, 1998) and reverse LR5 (Vilgalys and Hester, 1990) primer. The PCR reaction consisted of a 25 μl final volume and included 0.5 μl DNA template (50 ng/μl), 1 μl of each primer (10 mM), 2.5 μl (10 mM) dNTP's, 2.5 μl of 10× PCR buffer with $MgCl_2$, 1unit *taq* polymerase (Roche Molecular Biochemicals, Almeda, California) and 17 μl sterile distilled water (SABAX water; Adcock Ingrams, Bryanston). The PCR conditions were as follows: 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s (denaturation), 55 °C for 45 s (annealing), 72 °C for 1 min (elongation) and 72 °C for 4 min (final elongation). The PCR products were then visualized in 2 % agarose gels using Gel Red (Biotium, Haward, California, USA) in 1 × TAE buffer (Tris base 0.4 M, acetic acid 1%, EDTA 0.5 M, pH 8.0). A subset of isolates representing the various species identified based on the ribosomal data (Table 1), were chosen for subsequent multi-gene sequencing. The EF-1α gene region was amplified using the primer pair EF1F and EF2R (Jacobs et al., 2004) and the RBP2 region using the primers RPB2Bot6F and RPB2Bot6R (Sakalidis, 2004). The EF-1α PCR protocol and program parameters were the same as that used for the ITS amplifications, while the protocol for amplifying RBP2 was that of Pavlic et al. (2008). Amplified products were visualized on a 2% agarose gel using Gel red (Biotium, Haward, California, USA) in 1x TAE buffer (Tris base 0.4 M, acetic acid 1%, EDTA 0.5 M, pH 8.0).

Amplified DNA products from the three gene regions were purified using the Sephadex^{R} G-50 columns (Sigma-Aldrich). Sequencing was performed in both directions using the same forward and reverse primers used in the PCR reaction except for the ITS where the internal primer set V9G (Hoog and Ende, 1998) and ITS4 (White et al., 1990) was used. Each sequencing PCR reaction contained 2.5 μl purified DNA, 2.1 μl reaction buffer, 0.5 μl ready reaction buffer (BigDye), 1.5 μl primer (10 mM) and 5.4 μl sterile distilled water (SABAX water; Adcock Ingrams, Bryanston). The sequencing PCR conditions were as follows: 96 °C for 10 s followed by 25 cycles of 53 °C for 5 s, and 60 °C for 4 min.

Consensus sequences from the forward and reverse sequences were built using the CLC Bio Workbench version 5 (CLC bio, Aarhus, Denmark) and sequence inconsistencies were checked manually. ITS sequences generated and the additional genes sequenced were added to the datasets

Table 1
Isolates representing Botryosphaeriaceae used in the phylogenetic study.

Isolate name	Identifcation	Host	Country	Reference	ITS	EF	RBP2
CMW37407	*Lasiodiplodia pseudotheobromae*	*Syzygium cordatum*	South Africa	Gryzenhout, M.	JQ744583	JQ744604	
CMW37408	"	*S. cordatum*	"	"	JQ744584	JQ744605	
CMW37387	*Neofusicoccum eucalyptorum*	*Eucalyptus grandis*	"	"	JQ744579	JQ744600	
CMW37388	"	*E. grandis*	"	"	JQ744580	JQ744601	
CMW37386	"	*E. grandis*	"	"	JQ744581	JQ744602	
CMW 37385	"	*E. grandis*	"	"	JQ744603	JQ744582	
CMW37396	*Neofusicoccum australe*	*E. grandis*	"	"	JQ744576	JQ744597	
CMW37395	"	*E. grandis*	"	"	JQ744577	JQ744598	
CMW37394	"	*E. grandis*	"	"	JQ744578	JQ744599	
CMW37406	*Neofusicoccum parvum*	*S. cordatum*	"	"	JQ744564	JQ744585	JQ744609
CMW37400	*Neofusicoccum kwambonambiense*	*S. cordatum*	"	"	JQ744582	JQ744603	JQ744606
CMW37399	"	*E. grandis*	"	"	JQ744566	JQ744587	JQ744614
CMW37401	"	*S. cordatum*	"	"	JQ744567	JQ744588	JQ744611
CMW37402	"	*S. cordatum*	"	"	JQ744568	JQ744589	JQ744612
CMW37389	"	*E. grandis*	"	"	JQ744569	JQ744590	
CMW37398	"	*E. grandis*	"	"	JQ744570	JQ744591	JQ744606
CMW37391	"	*E. grandis*	"	"	JQ744571	JQ744592	JQ744615
CMW37405	"	*S. cordatum*	"	"	JQ744572	JQ744593	JQ744610
CMW37397	"	*E. grandis*	"	"	JQ744573	JQ744594	JQ744607
CMW37395	"	*E. grandis*	"	"	JQ744574	JQ744598	JQ744613
CMW37404	"	*S. cordatum*	"	"	JQ744575	JQ744596	JQ744608

Abbreviations of isolates and culture collection: CBS, Centraalbureau voor Schimmelcultures Utrecht, Netherlands; CMW, Forestry and Agricultural Biotechnology Institution, University of Pretoria.

(Table 1) that included sequences of species of Botryosphaeriaceae found on *S. cordatum* and *E. grandis* in South Africa and other species known for these genera (Alves et al., 2008; Begoude et al., 2010; Burgess et al., 2006a; Damm et al., 2007; Pavlic et al., 2004, 2007, 2008, 2009; Slippers et al., 2004b, 2004c). These sequences were aligned with the online programme MAFFT version 6 (Katoh et al., 2002) and alignments were verified manually.

A most parsimonious phylogenetic tree (MP) was inferred in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0 (Swofford 2001) for the three gene regions (ITS, EF-1α and RBP2) separately and for the combined ITS and EF-1α sequences. The RBP2 sequences could not be used in the combined dataset because they were only generated for species in the *Neofusicoccum* species complex. Heuristic searches were completed using random stepwise addition with 100 replicates, and the tree bisection and reconstruction (TBR) algorithm as branch swapping algorithm. Gaps were treated as 5th character and nucleotides were defined as unordered and unweighted. A 1000 replicate bootstrap analysis (Felsenstein, 1985) was executed to assess the confidence levels of the branch nodes in the phylogenetic tree. A 1000 replicate partition homogeneity test was applied to the ITS and EF-1α sequence data sets to determine the congruency between the ITS and EF-1α sequence data after the exclusion of uninformative sites (Farris et al., 1995). Maximum likelihood (ML) phylogenetic analyses were performed on the DNA sequence data for the gene regions separately and combined to confirm the groupings obtained with the MP. The online program ATCG phyML 3.0 (http://atgc.lirmm.fr/phyml/) was used. Likelihood substitution models were determined by JModelTest: phylogenetic model averaging version 0.1.1 (2008) using the Akaike information criterion (AIC). The invariable sites were assumed to have a gamma distribution. Confidence levels of the branches were estimated using bootstrap analysis (1000 replicates).

3. Results

3.1. DNA sequence analysis

The ITS dataset consisted of 135 taxa, including 68 reference sequences and 67 generated sequences, thus representing all known genera in the Botryosphaeriaceae. The EF-1α dataset comprised of 103 taxa, including 36 reference sequences representing all genera of Botryosphaeriaceae and 67 sequences from this study. Sequences representing all known genera in the Botryosphaeriaceae were included in the dataset, but not necessarily representing all species. Reference sequences of all species belonging to *Neofusicoccum* and *Lasiodiplodia* were selected for the combined datasets of the ITS and EF-1α because all the sequences generated in this study grouped into those clades. The combined ITS/EF-1α datasets consisted of 63 taxa, including 40 reference sequences and 23 sequences generated from this study. The RBP2 datasets contained only 47 taxa, consisting of 32 reference sequences and 15 sequences from this study, and represented only sequences of *Neofusicoccum* species, with the aim to distinguish isolates belonging to the *N. parvum*/ *N. ribis* species complex (Pavlic et al., 2008).

The results of the PHT test (ITS/EF-1α) revealed that the datasets were incongruent (P-value = 0.001). This was because some isolates in the *N. parvum*/*N. ribis* complex grouped together in the EF-1α tree, but formed distinct groups in the ITS tree (Pavlic et al., 2008). The ITS and EF-1α datasets were, however, combined, keeping the incongruence in the specific group in mind, in order to increase the number of informative sites. Some species could still not be distinguished with confidence in phylogenetic sub-clades based on the ITS and EF-1α data, but these isolates were distinguished based on the RBP2 genes (Pavlic et al., 2008) (Table 2).

The 436 trees generated from the combined ITS and EF-1α datasets differed with respect to the grouping between clades, but were consistent with respect to isolates comprising terminal clades (data not shown). There was strong bootstrap support for clades representing known species in both the parsimony and likelihood analyses (data not shown). The ML tree was chosen for presentation (Fig. 1A) and showed that isolates sequenced in this study grouped with isolates of *N. parvum, N. eucalyptorum, N. australe, N. kwambonambiense* and *L. pseudotheobromae. Neofusicoccum parvum* and *N. kwambonambiense* could not be resolved based on the EF-1α data alone, but were separated in a combined dataset of EF-1α and ITS. The RBP2 sequences further confirmed the identity of these isolates as *N. parvum* and *N. kwambonambiense* (Fig. 1B).

Most *Neofusicoccum* species were present in *E. grandis* and *S. cordatum*. The exception was *N. australe* that was isolated only from *E. grandis* leaves and twigs in this study. The distribution of species of Botryosphaeriaceae within the trees was variable. *N. eucalyptorum* was isolated from *E. grandis* leaves, petioles and twigs, as well as a single leaf of *S. cordatum. N. kwambonambiense* was isolated from the leaves and twigs of *E. grandis* and also the leaves of *S. cordatum. Neofusicoccum parvum* was isolated from trunk increment cores of both tree species,

Table 2
Statistics related to phylogenetic analyses.

	ITS	EF-1α	RBP2	Combined (ITS and EF-1α)
Amplified region size (bp)	700	600	700	1300
No. of characters	500	244	613	744
Parsimony informative	100	132	182	230
Parsimony uninformative	376 constant, 24 variable	97 constant, 15 variable	421 constant, 10 variable	472 constant, 41 variable
No. of trees retained	6	99	2	436
g1	−0.65	−0.7	−0.7	−0.5
Consistency index (CI)	0.8	0.7	1	0.7
Retention index (RI)	0.9	0.9	1	0.9
Substitution model (AIC)	GTR + G	HKY + G	GTR	GTR + G
Gamma shape	0.2	0.4	0.6	0.2

and the petioles of the *E. grandis* tree. The *Lasiodiplodia* clade included only *L. pseudotheobromae* that was found in *S. cordatum* trunk increments.

4. Discussion

This study reports a number of species of the tree pathogen and endophyte family, the Botryosphaeriaceae, for the first time from South Africa. Five Botryosphaeriaceae species were identified from various plant tissue types and occurring on a native *S. cordatum* and a non-native *E. grandis* tree at a particular moment in time. These include *N. eucalyptorum, N. kwambonambiense, N. australe, N. parvum,* and *L. pseudotheobromae*. Three of these species, including *N. eucalyptorum, N. kwambonambiense* and *N. parvum*, were isolated from both trees, while *N. australe* and *L. theobromae* were found only on *E. grandis* or *S. cordatum*, respectively. *N. eucalyptorum* and *L. pseudotheobromae* were found on *S. cordatum*, and *N. kwambonambiense* on *Eucalyptus*, for the first time in South Africa. This is interesting given that relatively wide and thorough surveys of the Botryosphaeriaceae have been conducted on these trees in the past.

The most abundant species of the Botryosphaeriaceae in the two trees sampled in this study was *N. eucalyptorum*, which represented 38% of the total isolates. This fungus was first described by Smith et al. (2001) from cankers on the main stems of *E. grandis* and *E. nitens* in South Africa. In the present study, it was also isolated from leaves, twigs and wood increments of *E. grandis*, and from a leaf of *S. cordatum*. This is the first time that it has been found on the latter host. This fungus is, however, known from other members of the Myrtaceae, such as *Blepharocalyx salicifolius, Myreceugenia glaucescens* and *Myrrhinium atropurpureum* var. *octandrum* in Uruguay (Pérez et al., 2008). A previous study (Slippers et al., 2004c) suggested that *N. eucalyptorum* is native on *Eucalyptus* in Australia based on its abundance, distribution and its association with *Eucalyptus* spp. on that continent (Slippers et al., 2004c). This fungus clearly has the ability to infect various members in the Myrtaceae and can move between native and non-native hosts of these families in countries where it is introduced.

N. kwambonambiense was the second most abundant species, representing 33% of the total isolates in this study. This species was isolated from leaves of *S. cordatum* and the leaves and twigs of *E. grandis*. *N. kwambonambiense* has previously been reported from asymptomatic branches and leaves, dying branches and pulp of the ripe fruit of *S. cordatum* (Pavlic et al., 2009), and it is known only from *S. cordatum* and from a site close to the sampling location used in the present study. However, studies conducted by Sakalidis et al. (2011) also identified *N. kwambonambiense* on *E. dunni* in eastern Australia and *Corymbia torelliana* in northern Australia (Sakalidis et al., 2011). Therefore, the origin, host and geographical ranges of *N. kwambonambiense* remain unknown, but appear to be wide based on the limited reports represented by our study and those of Pavlic et al. (2009a) and Sakalidis et al. (2011). Pathogenicity tests undertaken on both *Eucalyptus* spp. and *S. cordatum* by Pavlic et al. (2009) suggested that *N. kwambonambiense* is more pathogenic than isolates of *N. ribis* and *N. parvum,* and it was more aggressive on *Eucalyptus* spp. than native *S. cordatum.*

N. australe was isolated from the leaves and twigs of *Eucalyptus* and represented 7% of the Botryosphaeriaceae isolates. Pavlic et al. (2007) found low levels of *N. australe* from *S. cordatum*. Those isolates were shown to be pathogenic in greenhouse trials on *S. cordatum* and an *E. grandis x camaldulensis* clone (Pavlic et al., 2007). *Neofusicoccum australe* is a recently described species from diseased stems of native *Acacia* spp. in Australia (Slippers et al., 2004b; Slippers et al., 2004c) and has since been found on *Eucalyptus* spp. in western Australia and South Africa (Slippers et al., 2004c). This species also appears to have a wide host range because it is known from a number of unrelated hosts, also including *W. nobilis* and *W. nodiflora* (Slippers et al., 2005).

N. parvum represented 12% of the isolates and was predominantly isolated from the leaves, twigs and petioles of *Eucalyptus*. Only a single isolate came from a *S. cordatum* leaf. In South Africa this fungus is also known from various hosts, such as a non-native *Tibouchina* sp. (Heath et al., 2011), and native *Heteropyxis natalenis* (Smith et al., 2001) and *S. cordatum* (Pavlic et al., 2007). Studies have shown that *N. parvum* is broadly distributed around the world on numerous hosts. Relevant to this study, it has the potential to cause serious diseases of *Eucalyptus* (Ahumada, 2003; Crous et al., 1989; Slippers et al., 2004b, 2004c).

L. pseudotheobromae represented 11% of the Botryosphaeriaceae isolates and it was isolated from *S. cordatum* bark and trunk increments. This is the first report of *L. pseudotheobromae* from South Africa and from *S. cordatum*. *L. pseudotheobromae* has been previously reported on *Eucalyptus* in Eastern Australia and Venezuela (Alves et al., 2008; Mohali et al., 2005), indicating that this species also has the ability to infect *Eucalyptus* spp. *Lasiodiplodia pseudotheobromae* has recently been separated as a cryptic species from its sister species *L. theobromae* (Alves et al., 2008), a species known from many parts of the world on a wide diversity of hosts.

The large number and diversity of Botryosphaeriaceae encountered in this study on both *Eucalyptus* and *S. cordatum* was not surprising. Previously *N. parvum*, *N. australe* and *L. theobromae* were shown to co-infect both hosts (Pavlic et al., 2007), and results of this study showed that *N. eucalyptorum* and *N. kwambonambiense* can also infect both tree species. It thus appears to be a general characteristic of Botryosphaeriaceae in South Africa to be able to infect different hosts in the Myrtaceae, irrespective of their origin. There thus appears to be little limitation for invasive alien fungi in this group to move from non-native to native hosts, or for endemic fungi in this group to infect non-native hosts. These findings re-affirm previous concerns that such movement of Botryosphaeriaceae could be common and should be considered in quarantine and disease management programs (Burgess and Wingfield, 2002; Burgess et al., 2006b; Slippers and Wingfield, 2007; Wingfield et al., 2001, 2011).

The sampling strategy used in this study was limited with only a single tree of each species being sampled, albeit relatively intensively. Our results are thus not indicative of the full range of infection, geographical distribution, host range and tissue specificity of Botryosphaeriaceae on *E. grandis* and *S. cordatum* in South Africa, or even in the KwaMbonambi/Mtubatuba area. They rather represent a snapshot of these species in a single tree at a particular time. Despite this limited extent, this study

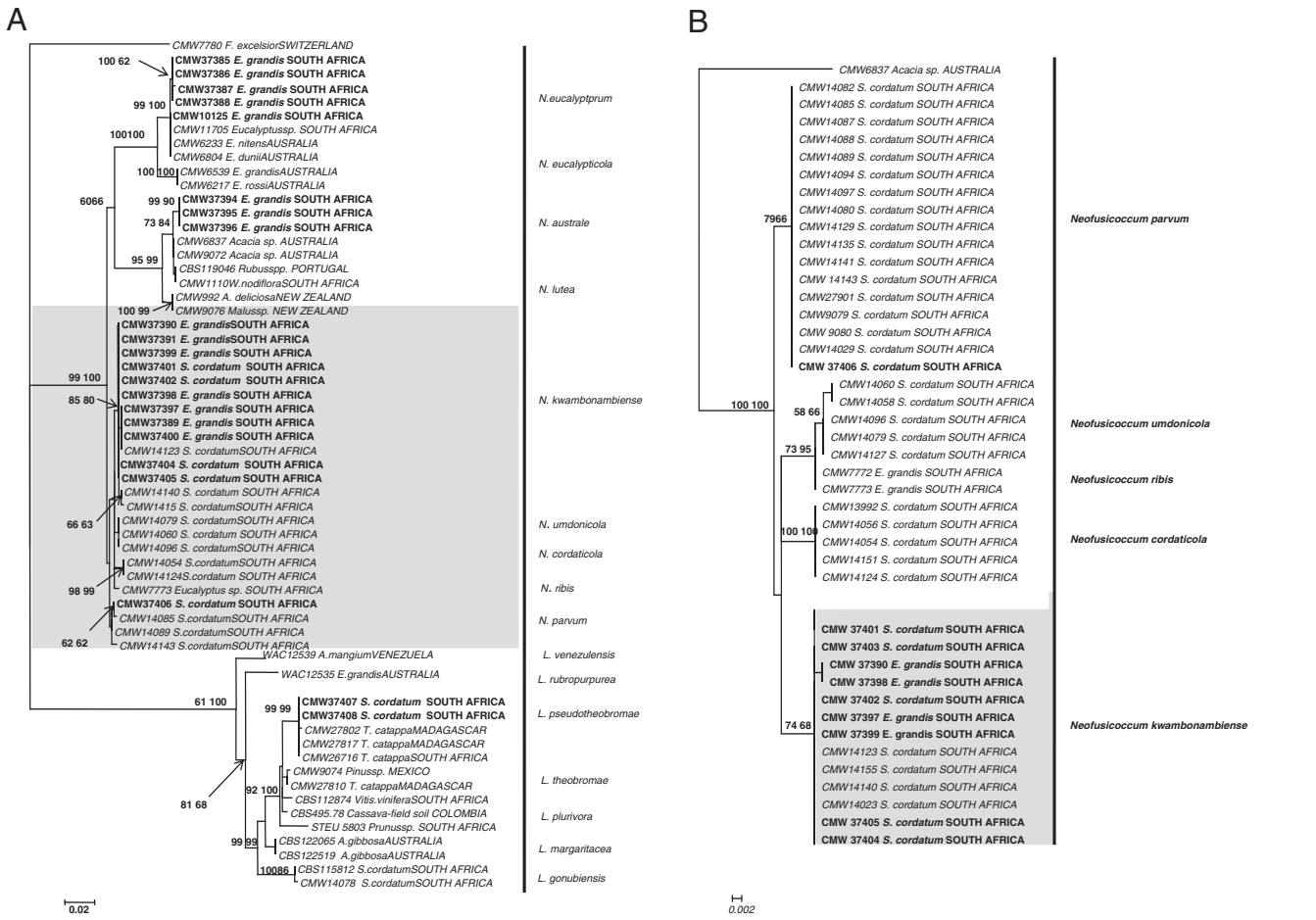

Fig. 1. A. Phylogram produced with the TBR algorithm of a heuristic search on a combined dataset of the ITS and the EF1α. Group frequencies and bootstrap values are indicated (maximum likelihood bootstrap followed by maximum parsimony). B. Phylogram depicting the relationship amongst the *Neofussicoccum parvum*, *Neofussicoccum kwambonambiense, Neofusicoccum umdonicola* and *Neofusicoccum cordaticola* isolates based on maximum likelihood and maximum parsimony analyses of the RBP2 gene.

yielded new reports from hosts well studied in the past. This suggests that continued monitoring, using powerful new identification tools and contemporary taxonomic frameworks will be necessary to fully appreciate the full geographical and host ranges of these species. This is especially so because it is clear from the limited studies on the host and geographical ranges of these fungi that huge gaps exist in our knowledge.

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