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Botryosphaeriaceae on native and exotic *Myrtaceae* trees in southern and eastern Africa

by

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DECLARATION

I, Ntaoleng Angel Maduke declare that the dissertation, which I hereby submit for the degree *Magister Scientiae* at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Preface

Members of the *Botryosphaeriaceae* represent a diverse family of fungi with over 180 species. They have been reported from a wide range of hosts occurring as endophytes, saprophytes or pathogens. These fungi are commonly referred to as latent opportunistic pathogens as they cause disease when the host is under abiotic or biotic stress. Symptoms associated with infection include tip die-back, stem and branch cankers, fruit rots, leaf spots and in severe cases mortality of trees. However, they are best known as canker and die-back pathogens on woody plants including species such as *Eucalyptus* (*Myrtaceae*). This dissertation focusses on the *Botryosphaeriaceae* on native and introduced *Myrtaceae* in southern and eastern Africa and aimed to increase current scientific knowledge of these fungi in the region.

The literature review of this dissertation provides background to the research chapter by discussing the historic taxonomic changes in the *Botryosphaeriaceae,* and the current methods used for identification and classification of the species in this family. Furthermore, it summarizes the knowledge pertaining to *Botryosphaeriaceae* species on *Eucalyptus* and native *Myrtaceae* trees, particularly in eastern and southern Africa. Importantly, it considers fungal host jumps/shifts and their future impact in relation to *Eucalyptus* plantation forestry in Africa. Consideration is also given to the biology, ecology and possible future impacts of the *Botryosphaeriaceae.*

Eucalyptus is the largest genus in the family *Myrtaceae*. Most species in this genus originates from Australia and were introduced to other countries as non-natives. In most parts of the world, *Eucalyptus* plantations are established in close proximity to native trees making it possible for pathogens to move from the native to the non-native trees and *vice versa*. This movement could result in considerable damage to the *Eucalyptus* plantations and the native trees.

Chapter two of this dissertation focuses on identifying the *Botryosphaeriaceae* species occurring on both native and the non-native *Myrtaceae* trees in the Limpopo Province of South Africa and selected countries in southern and eastern Africa. Furthermore, it aimed at understanding the implications of the host jumps/shifts between the native and the non-native trees and their importance in relation to plantation forestry in eastern and southern Africa. This was achieved by sampling trees from South Africa, Malawi, Mozambique, Tanzania and Zimbabwe. Species resembling *Botryosphaeriaceae* were identified to species level using multi-gene DNA sequence data. To study the implications of the host jumps/shifts on *Eucalyptus* plantations, isolates obtained from native *Myrtaceae* trees were tested for pathogenicity under glasshouse conditions.

The work presented in this dissertation provides a foundation of knowledge regarding the occurance and distribution of *Botryosphaeriaceae* species on several native *Myrtaceae* and *Eucalyptus* trees in eastern and southern Africa. This information is especially important in light of the fact that the areas from which isolates were obtained had not been considered previously. Furthermore, it suggests future directions to better understand the biology and taxonomy, movement, role and influence of *Botryosphaeriaceae* species on both native and non-native *Myrtaceae* trees.

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CHAPTER 1

Literature review

Botryosphaeriaceae on *Myrtaceae*: impact and future threats

1.0 Introduction

The *Botryosphaeriaceae* Theissen & Sydow (Botryosphaeriales, Dothideomycetes, Ascomycota) is a diverse family of fungi with a cosmopolitan distribution. Most species in this family occur on a wide range of hosts, while some seem host-specific (Crous *et al.* 2006, 2017; Slippers and Wingfield 2007; Slippers *et al*. 2009, 2017; Mehl *et al.* 2013, 2017; Marsberg *et al.* 2017; Yang *et al*. 2017). The ecology of *Botryosphaeriaceae* species and their hosts encompasses many different life styles, ranging from saprophytes on dead plant material, symptomless endophytes or pathogenic species (Slippers and Wingfield 2007; Jami *et al*. 2014; Slippers *et al*. 2014; Crous *et al.* 2017). They are often referred to as opportunistic pathogens as they remain endophytic in infected plants, only causing disease when the host is under stress, such as that caused by hail, drought and damage by pests and pathogens (Slippers and Wingfield 2007). Symptoms associated with infection by species of *Botryosphaeriaceae* include tip die-back, stem and branch cankers, fruit rots, leaf spots and in severe cases mortality of trees (Slippers and Wingfield 2007; Mehl *et al*. 2013). It has been predicted that the impact of these fungi may increase as the global climate changes and where conditions stressful to plants arise (Slippers and Wingfield 2007).

The taxonomy of species and genera in the *Botryosphaeriaceae* has undergone significant changes over the past century and continues to change with evolving DNA-based techniques. Initially, species in the *Botryosphaeriaceae* were described based on morphology, especially that of the asexual states, because the sexual states are rarely encountered in nature; and based on host associations (Denman *et al*. 2000; Slippers *et al.* 2004; Crous *et al.* 2006; Phillips *et al.* 2013). However, the confusion caused by the overlap of morphological characteristics between the species and the ability of some species in the family to occur on wide host range resulted in considerable taxonomic confusion. In recent years, the advent of molecular techniques has contributed considerably to resolving the taxonomy of the *Botryosphaeriaceae* (Slippers *et al.* 2004; Crous *et al.* 2006; Phillips *et al.* 2013; Yang *et al*. 2017). The numbers of species identified have increased significantly, and there are over 10 000 gene sequences lodged in GenBank for the order Botryosphaeriales (Slippers *et al.* 2017). It is currently accepted that the *Botryosphaeriaceae* resides in the order Botryosphaeriales and it comprises 23 phylogenetically described genera with over 180 species that are known from culture and sequence data (Dissanayake *et al*. 2016; Linaldeddu *et al*. 2016; Crous *et al.* 2017; Slippers *et al.* 2017; Yang *et al.* 2017).

Diseases caused by fungi in the *Botryosphaeriaceae* affects native and exotic trees world-wide, and have been recorded on more than 100 plant species (Mehl *et al*. 2013). Several species of *Botryosphaeriaceae* have been associated with plantation-grown *Eucalyptus* L'Héritier de Brutelle species globally. One of the first diseases of plantation grown eucalypt species described in South Africa was Botryosphaeria stem canker (Smith *et al*. 1994). Infection results in stem and branch cankers, tip die-back, wood stain and in some cases death of trees (Slippers and Winfield 2007; Mehl *et al.* 2013, 2017). Prior to 1995, there were 27 species of *Botryosphaeriaceae* in the genera *Botryosphaeria* Cesati & De Notaris, *Diplodia* Fries, *Dothiorella* Liu and *Lasiodiplodia* Ellis reported on *Eucalyptus* (Sankaran *et al*. 1995). Currently there are 20 species reported on *Eucalyptus* (Slippers *et al.* 2009; Pavlic-Zupanc *et al.* 2017). *Neofusicoccum* Crous, followed by *Botryosphaeria* and *Lasiodiplodia*, are the most commonly reported genera on *Eucalyptus* in both native and non-native environments (Slippers *et al.* 2009; Pérez *et al.* 2010; Pavlic-Zupanc *et al.* 2017), while other genera are rarely found.

The *Myrtaceae* De Jussieu is a family of shrubs and trees that comprises over 130 genera and 5600 species, predominately distributed in the Southern Hemisphere (Johnson and Briggs 1981; Govaerts *et al.* 2008). *Eucalyptus* is the largest genus in the family, with over 700 species. Most *Eucalyptus* species originate from Australia, with a few originating from the South East Asian islands (Iglesias-Trabado and Wilstermann 2008). They were introduced to the rest of the world in the nineteenth century (Doughty 2000), and they are currently planted globally as non-natives because of their fast growth and ability to withstand harsh environments (Turnbull 2000). Currently more than 20 million hectares of eucalypt species are planted as non-natives in more than 100 countries (FAO 2010). In South Africa, the eucalypt industry comprises about 568 000 ha and forms a major part of the forestry industry, providing the country with pulp, fuel wood, timber and essential oils (FAO 2010). Several other members of the *Myrtaceae* are also economically important crops. These include, for example, *Psidium guajava* (L.) Singh U.R. Pandey, Upadhyay, Tripathi BM and *Syzygium aromaticum* (L.) Merrill & Perry, while others such as *S. cordatum* Linnaeus are ecologically important species and used for medicinal purposes, fruit and as ornamentals (de Wet *et al*. 2010).

Pathogens affecting *Eucalyptus* species in their exotic environments could be introduced from natural vegetation surrounding the plantations. In most parts of the world, eucalypt plantations are established in close proximity to native trees making it possible for pathogens to move from the native to the non-native trees and *vice versa*. Native trees could serve as an important source of pathogens affecting *Eucalyptus*, as it has been found with the pathogens *Austropuccinia psidii* (G. Winter) Beenken (Castro *et al.* 1983; Coutinho *et al.* 1998; Tommerup *et al*. 2003; Simpson *et al*. 2006; Pérez *et al*. 2011; Carnegie *et al*. 2016) and *Chrysoporthe austroafricana* Gryzenhout & Wingfield (Heath *et al*. 2006; Maússe-Sitoe *et al.* 2016). The rust fungus *A. psidii* that occurs on a wide variety of native *Myrtaceae* trees in South America has become one of the main pathogens on non-native *Eucalyptus* planted in the same areas (Coutinho *et al*. 1998; Pérez *et al*. 2011; Carnegie *et al*. 2016). In recent years, several studies have investigated possible host jumps/shifts of *Botryosphaeriaceae* species between native and non-native *Myrtaceae* (Pavlic *et al.* 2007; Pérez *et al*. 2008; Slippers *et al*. 2009; Pérez *et al.* 2010). It is of great concern that the exchange between the exotic and the native trees could result in huge economic loss and ecological disturbance.

This literature review focusses on the *Botryosphaeriaceae* associated with plants in the *Myrtaceae*. Firstly, it provides a summary of the major changes in the taxonomy of the *Botryosphaeriaceae* and the species concepts that are currently used in the identification of *Botryosphaeriaceae* species. Thereafter, it focuses on the ecology and the diseases caused by *Botryosphaeriaceae* species. Lastly, it considers the apparent host range expansions and host shifts/jumps between native and non-native *Myrtaceae* trees, and the implications of these events.

2.0 The *Botryosphaeriaceae*

The ascomycete family *Botryosphaeriaceae* (Botryosphaeriales, Dothideomycetes) was first introduced in 1918 by Theissen and Sydow as a sub-family in the *Pseudosphaeriaceae* to accommodated the genus *Botryosphaeria*, which was described in 1863 by Cesati and De Notaris (Cesati and De Notaris 1863; Theissen and Sydow 1918). The genus *Botryosphaeria* is considered the type genus of the family and *B. dothidea* as the type species (Slippers *et al*. 2004). Cesati and De Notaris described *B. dothidea* based on the morphological characteristics of the conidia (asexual morph). Petrak (1923), however, rejected the classification of Theissen and Sydow (1918) and placed *Botryosphaeria* in the order Sphaeriales. A few years later Miller (1928) placed *Botryosphaeria* species in the order Dothideales because they lacked true perithecial walls. Most recently, Schoch *et al*. (2006) re-classified the genus *Botryosphaeria* in the family

Botryosphaeriaceae and in the order Botryosphaeriales which currently includes 10 families (Yang *et al.* 2017). It is currently accepted that the *Botryosphaeriaceae* comprises of 23 genera with 180 species that are known from culture and sequence data (Phillips *et al.* 2013; Dissanayake *et al*. 2016; Slippers *et al.* 2017). A summary of the major taxonomic changes that have occurred in the *Botryosphaeriaceae* family is presented in Table 1. More detailed descriptions of the history of the taxonomic changes in the family can also be found in the publications of Crous *et al.* 2006; Liu *et al*. 2012; Phillips *et al.* 2013; Slippers *et al.* 2013, 2017; Dissanayake *et al*. 2016; Yang *et al*. 2017; and only a brief summary of the main issues are, therefore, discussed in the next sections.

3.0. Species concepts in the *Botryosphaeriaceae*

The taxonomy of species and genera in the *Botryosphaeriaceae* have undergone significant changes over the past century. Initially species and genera were identified solely based on morphology and host association. Later, the advent of DNA techniques led to many cryptic species being described in the *Botryosphaeriaceae*. Today, the most widely accepted method of species delineation and classification includes the combination of morphological, ecological and phylogenetic species concepts. It is predicted that whole genome sequencing will provide greater insights into the biology, phylogeny and the diversity of the group (Lopes *et al*. 2017).

3.1. Morphological species concept

Species in the *Botryosphaeriaceae* were initially characterized based on the morphology of their typically hyaline and aseptate ascospores (Barr 1987, 1989; Phillips *et al*. 2008). However, this tool of characterization became inadequate as species with dark and septate ascospores were reported. The phylogenetic and taxonomic status of genera in the *Botryosphaeriaceae* was verified by Phillips *et al*. (2008), who recognized six genera with pigmented ascospores, four of which were septate. For example, ascospores of species such as *Diplodia corticola* A.J.L. Phillips, Alves & Luque, *N. eucalytporum* Crous, Slippers & A.J.L. Phillips, and *N. protearum* Crous, Slippers & A.J.L. Phillips become pigmented and septate with age, and the walls typically appear rough due to melanin deposition on the inner surfaces. In subsequent years, Liu *et al*. (2012) defined the characteristics of ascospores in the family as hyaline and aseptate, but pigmented and 1-septate with age. However, this characterization for the family was rejected as ascospores of some species become pigmented and septate at an earlier stage of development. For example, ascospores of

Dothiorella iberica A.J.L Phillips, Luque & A. Alves, *Do. Sarmentorum* (Fries) A.J.L. Phillips, Alves & Luque, *Spencermartinsia viticola* A.J.L. Phillips & Luque, *Sphaeropsis eucalypticola* (Doilom, J.K. Liu & K.D. Hyde) A.J.L. Phillips, and *S. visci* (Albertini & Schweinitz) Saccardo become pigmented at an earlier stage of development, and so the definition of characteristics of genera in the *Botryosphaeriaceae* by Liu *et al*. (2012) was also rejected. Furthermore, the ascospores represent the sexual state of the fungi, which are rarely found in nature, therefore making descriptions based on ascospores alone unreliable (Slippers *et al*. 2013).

The asexual state is most common among species of *Botryosphaeriaceae* and it was initially considered to represent more robust morphological diversity to distinguish between different genera and species (Shoemaker 1964; Laundon 1973). The conidia of the *Botryosphaeriaceae* are of two basic types namely: fusicoccum-like conidia that are narrow and thin walled, and diplodia-like conidia with wide and thick walls (Denman *et al*. 2000; Zhou and Stanosz 2001). The fusicoccum-like conidia are typically hyaline and non-septate but can develop septa and become pale brown with age. The diplodia-like conidia can be septate or non-septate, hyaline or brown. The most informative features of the asexual states include pigmentation, wall thickness, septation of conidia, and presence or absence of paraphyses (Phillips *et al*. 2013). Similar to the sexual state, it is now recognized that conidial morphology alone should be used with caution as the morphological changes that occur with age are difficult to interpret and have caused confusion in the identification of species in the *Botryosphaeriaceae*.

3.2 Ecological species concept

Host association has in the past been used as one of the characteristics for classification to identify *Botryosphaeriaceae* species. Many asexual (‘anamorph’) and sexual (‘teleomorph’) species in this family have been associated with, and described from the host on which they occur. For example, *N. protearum* was isolated from *Protea* species in South Africa (Denman *et al*. 2000, 2003), *N. eucalyptorum* (Crous, H. Sm. ter. & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, and *N. eucalypticola* (Slippers, Crous & M.J. Wingf) Crous, Slippers & A.J.L. Phillips from *Eucalyptus* species in South Africa and Australia, respectively (Smith 2001; Slippers *et al.* 2004), and *Diplodia tsugae* (A. Funk) A.J.L. Phillips & Alves from *Tsuga* species in Canada (British Columbia) (Funk 1964). However, it was later shown that host association cannot delimit most species of

Botryosphaeriaceae because most species are not host specific. For example, *N. parvum* has been reported on a range of hosts including *S. cordatum*, *Eucalyptus*, *Acacia* Martius and at least 90 other host genera (Sakalidis *et al.* 2013). The morphological and ecological species concepts are suggested to have underestimated the true diversity of species in the *Botryosphaeriaceae* (Slippers and Wingfield 2007; Phillips *et al*. 2013; Slippers *et al.* 2017).

3.3 Phylogenetic species concept

The use of DNA sequence data to deleniate species of fungi emerged in 1996 when Berbee used the 18S rRNA gene (Small Subunit) to study the phylogenetic relationship amongst the genera and orders of the Luculoascomycetes. However, phylogenetic analyses of this gene region alone could not determine with confidence whether species of the *Botryosphaeria-Guignardia* clade corresponded to the Dothideales or the Pleosporales. In subsequent years, the Internal Transcribed Spacer (ITS) region, including the 5.8S gene of the ribosomal RNA gene was predominantly used to identify the species in the *Botryosphaeriaceae.* The ITS is the standard barcoding gene (Chase and Fay 2009; Schoch *et al*. 2012) and is still the most widely used locus in taxonomic studies of *Botryosphaeriaceae*. However, the ITS tends to underestimate the true diversity of species in the *Botryosphaeriaceae* as it cannot always distinguish closely related and cryptic species (Burgess *et al*. 2001; de Wet *et al*. 2003; Slippers *et al*. 2004; Pavlic *et al.* 2009; Sakalidis *et al*. 2011; Cruywagen *et al*. 2017, Slippers *et al*. 2017).

Gene phylogenies using multiple, unlinked, gene regions are the currently accepted method of species delineation in the *Botryosphaeriaceae* (Pavlic *et al.* 2009; Phillips *et al*. 2013; Slippers *et al*. 2013; Yang *et al*. 2017). The most widely used loci include the ITS, β-tubulin (TUB), Translation Elongation Factor-1α (TEF-1α), Large Sub-unit (LSU) and RNA polymerase II (RPB2). Studies have revealed that the LSU and the ITS loci are suitable to consider taxonomic sub-divisions only at the generic level but not the species level, and that it should be combined with other loci to give more resolution (White *et al*. 1990; Crous *et al*. 2006; Yang *et al.* 2017).

The TUB gene has been used commonly to identify *Botryosphaeriaceae* species but has been described as lacking phylogenetic signal (Slippers *et al*. 2017). The RPB2 is the most trusted locus as it provides good resolution in distinguishing cryptic species (Pavlic *et al*. 2009; Sakalidis *et al*.

2011; Yang *et al.* 2017). However, there are incomplete databases for this gene region since most of the work has been done on the genera *Neofusicoccum* and *Lasiodiplodia* and not much work on the other genera in the family (Pavlic *et al*. 2009; Cryuwagen *et al*. 2017). Translation Elongation Factor-1α is considered as one of the most difficult gene regions to amplify and lacks phylogenetic signal (Slippers *et al*. 2017; Cryuwagen *et al*. 2017). However, TEF-1α is still the most widely used locus in comparison to the other gene regions used in delineating species of *Botryosphaeriaceae.* The use of multiple loci has shown that every locus discussed above is problematic in some way. Consequently, to obtain a sufficient and more informative phylogenetic signal, combination of four or more datasets is necessary in some genera of *Botryosphaeriaceae*.

Multiple phylogenies have the added advantage of allowing for the recognition of hybrid species within the *Botryosphaeriaceae*. Five hybrids were recently identified in the genus *Lasiodiplodia* based on multigene phylogenies (Cryuwagen *et al*. 2017; Rodríguez-Gálvez *et al*. 2017). In those studies, they defined a hybrid as an individual that groups with different species in single-gene genealogies. For example, in the paper by Rodríguez-Gálvez (2017), one *Lasiodiplodia* isolate grouped between *L. citricola* Abdollahzadeh, Javadi & A.J.L. Phillips and *L. parva* A.J.L. Phillips, Alves & Crous in multigene phylogeny, while in single gene genealogies it grouped with one or the other, suggesting possible hybridization. This has added emphasis to the importance of using multiple gene regions as hybrids cannot be recognized where only a single locus is used. So far, hybrids have only been described in the genus *Lasiodiplodia*. Hybridization in *Lasiodiplodia* is not surprising as it is one of the most commonly isolated genera in the *Botryosphaeriaceae*. It has a wide host range and a cosmopolitan distribution, which could possibly result in sexual reproduction between two different species. The general concern is that the hybrids can expand their host range, and evolve more rapidly than the parent species, possibly causing more damage (Brasier 2001).

3.4 Whole genome sequencing

Whole genome sequencing is a powerful tool for research that refers to a process of determining the complete DNA sequence of an organism's genome at a single time. This method is beginning to gain attention and it is set to shed light on the diversity, taxonomy, classification and biology of the *Botryosphaeriaceae* and other species. To date, five *Botryosphaeriaceae* genomes have been sequenced namely those of *B. dothidea, Diplodia sapinea* (Fries) Fuckel, *D. scrobiculata* J. de

Wet, Slippers & M.J. Wingfield, *D. seriata* De Notaris, *Macrophomina phaseolin*a (Tassi) Goidànich, and *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (Islam *et al.* 2012; Bihon *et al*. 2014; Wingfield *et al.* 2015; Marsberg *et al*. 2017). The genome sequences have created a platform to answer some of the most intriguing questions regarding the biology and endophytic nature of species in the *Botryosphaeriaceae*.

Genome sequences have also for example, revealed the reproductive strategy of *B. dothidea*, *D. sapinea* and *N. parvum*. *Diplodia sapinea* has a heterothallic reproductive system while *B. dothidea* and *N. parvum* appears to be homothallic (Bihon *et al.* 2014; Lopes *et al*. 2017; Marsberg *et al*. 2017). Why *D. sapinea* has a heterothallic mating system that differes from *B. dothidea* and *N. parvum*, and how the reproductive strategy influences the diversity and distribution of the species in this family is still unknown. Further research into the genomes of other species in the family is needed to fully understand the biology of this species and how they differ from each other.

3.5 Detection of *Botryosphaeriaceae* species using metabarcoding

Metabarcoding is the study of genetic material recovered directly from environmental samples. The metabarcoding method can be used to detect endophytes in plant tissues, including those in the *Botryosphaeriaceae*. Metabarcoding has certain advantages such as identification of a true diversity of *Botryosphaeriaceae* in the plant material sampled, identification of the slow-growing *Botryosphaeriaceae* species that can be over grown by fast growing species (Slippers *et al*. 2017), and therefore missed wth other identification methods. Metabarcding can also be used to characterize the full community of endophytes in a tree (Kemler *et al.* 2013). For example, Kemler *et al*. (2013) showed that *Botryosphaeriaceae* species make up a dominant 30% of all the identified taxonomic species in a single *Eucalyptus* tree. This study further proves that high levels of different endopyhytic *Botryosphaeriaceae* species can occur on a single tree at the same time. Lastly, metabarcoding can be used to understand host association and phylogeography of *Botryosphaeriaceae* species. Although this method is currently rather costly and the species identified are not culturable, it remains effective to characterize the general community of endophytes in woody plants.

4.0 *Botryosphaeriaceae* – ecology

The *Botryosphaeriaceae* comprise an ecologically and evolutionary diverse group of fungi commonly isolated from woody hosts. They are associated with a wide range of hosts including ornamental, forestry and agricultural trees (Slippers and Wingfield 2007; Mehl *et al*. 2013). The species of *Botryosphaeriaceae* are able to establish different types of relationships with their hosts. They can either be saprophytes feeding on dead material, endophytes in a healthy host or pathogens causing diseases. These fungi are also commonly known as opportunistic pathogens as they cause disease when the host plant is subjected to biotic or abiotic stress. Symptoms associated with infection by species of *Botryosphaeriaceae* include tip die-back, stem and branch cankers, fruit rots, leaf spots and in severe cases mortality of trees (Slippers and Wingfield 2007; Mehl *et al*. 2013).

4.1 *Botryosphaeriaceae* as saprophytes

Saprophytes are defined as organisms that feed on dead organic matter. *Botryosphaeriaceae* species have often been regarded as saprophytes or secondary pathogens that overwinter as fruiting bodies on dead tissues and move to healthy tissues. There have been uncertainties regarding the saprophytic life cycle of these fungi as they are known to be saprophytes while some species have been shown to be pathogens or endophytes (Milholland 1991). Fisher *et al*. (1993), suggested that *B. dothidea* occurring on leaf spots and on dead *Eucalyptus* leaves might be derived from earlier latent endophytic infections of healthy leaves rather than from saprophytic infections. It is also believed that the saprophytic cycle in these fungi depends on the relationship between the host and the *Botryosphaeriaceae* species. For example, *B. dothidea* is an important pathogen of *Pistachio vera* Linnaeus (Ma *et al*. 2001), however this species was found causing grape cluster rot in Taiwan (Kuo *et al*. 1989). Furthermore, *B. dothidea* was found causing excoriose in Portuguese grapevines while some of the strains were found pathogenic causing stem dieback (Phillips 1998). Grossenbacher and Duggar (1911) distinguished between the saprophytic and the pathogenic form of *B. dothidea* (reported as *Botryosphaeria ribis*) causing cane blight. They found that the pathogenic *B. dothidea* (as *B. ribis f. chromogena*) formed purplish pink colour on starch paste similar to the one observed on the diseased host while the saprophytic form, *B. dothidea* (as *B. ribis f. achromogena*), developed no colour. Philllips (2002) showed that *B. dothidea* represents a species complex and that the weakly or saprophytic species were more predominant than those that are pathogenic. Subsequent studies also reported *Botryosphaeriaceae* species as saprophytes. For example, Thomidis *et al*. (2011) found the overwintered pycnidia of *N. parvum* from plant debris of peach trees. Similarly, the pycnidia and pseudothecia of *B. dothidea* were found overwintering in dead kiwi orchards in China (Zhou *et al*. 2015). Currently, species of *Botryosphaeriaceae* are isolated from dead, or rotting wood in attempts to isolate saprophytic species. It is currently feared that the saprophytic cycle might serve as primary inoculum for healthy trees as *Botryosphaeriaceae* species can colonize dead wood and can move from dead to healthy wood.

4.2 *Botryosphaeriaceae* as pathogens

Botryosphaeriaceae species have been reported as pathogens of more than 100 host plants and are associated with various symptoms on different hosts (Mehl *et al.* 2013). The symptoms observed on plants infected by species of the *Botryosphaeriaceae* include die-back of branches, stem and twig cankers, sooty cankers, blue staining, post-harvest diseases, crown thinning, abortion of seed capsules, leaf spots, root cankers, damping off of seedlings, kino exudation and in severe cases plant mortality (Punithalingham and Holliday 1973; Slippers and Wingfield 2007; Mehl *et al.* 2013; Slippers *et al*. 2017).

Diseases caused by species in the *Botryosphaeriaceae* are usually expressed when the tree is exposed to stressful conditions (Smith *et al*. 1994; Paoletti *et al.* 2001; Stanosz *et al*. 2001). Stresses include abiotic factors such as drought, hail damage, attack from insect pests, or pathogen other than the *Botryosphaeriaceae* (Slippers and Wingfield 2007; Mehl *et al*. 2013; Slippers *et al*. 2017). The most commonly reported stress factor is water stress, with several studies illustrating the effect of water stress on the pathogenicity of *Botryosphaeriaceae* species (Ma *et al.* 2001; Desprez-Loustau *et al*. 2006; Slippers and Wingfield 2007; Piškur *et al*. 2010; Qiu *et al.* 2014). *Botryosphaeria dothidea*, for example, was found to be more aggressive on *Pistachio vera* and *Ostrya carpinifolia* Scop. under reduced water content than when exposed to humid conditions (Ma *et al*. 2001; Piškur *et al*. 2010). Furthermore, this species was found to be the causal agent of die-back disease of *O. carpinifolia* in Slovenia and Italy under extreme weather conditions (Piškur *et al*. 2010).

Diplodia sapinea is a widely distributed pathogen that is mostly associated with pine trees. It is regarded as the most important pathogen of pine plantations (Waterman 1939) and is usually associated with stress. Drought and hailstorms contribute largely to diseases caused by this fungus. The diseases caused by species of *D. sapinea* on *Pinus* include stem cankers, root diseases, seedling blight, blue staining and tip dieback (Buchanan 1967). The damage caused by *D. sapinea* in South Africa in 1990 was calculated to be between 11% and 28% volume loss, while the potential loss of production was up to 55% after hail damage and die-back (Zwolinski *et al*. 1990). The damage caused by *D. sapinea* is one of the main factors restricting the planting of *Pinus radiata* to only certain parts of the country (Swart and Wingfield 1991). This pathogen has been reported as an economically important pathogen in many other countries such as the USA where it caused major losses of seedlings under stress (Stanosz *et al*. 2001), and it was also reported as the pathogen responsible for the die-back of *Pinus nigra* in the Netherlands (Kam and Dam 1987) and in Nothern and Central Italy (Maresi *et al*. 2002). In Zwaziland, more than 50% of the *P. taeda* Linnaeus trees died due to diseases caused by *D. sapinea*. The diseases occurred more severely on sites which were stressed and the most common stress factor was drought.

Prior to 1995, there were 27 species of *Botryosphaeriaceae* reported on *Eucalyptu*s, residing in the genera *Botryosphaeria*, *Diplodia*, *Dothiorella* and *Lasiodiplodia* (Sankaran *et al*. 1995). Twenty three species were reported on *Eucalyptus* by Slippers *et al*. 2009. The number of species reduced due to reanalysis of the original specimens using DNA sequencing data. Currently, there are 30 species of *Botryosphaeriaceae* reported on *Eucalyptus* in the world (Table 3). On *Eucalyptus* trees, *Botryosphaeriaceae* species cause a wide range of symptoms such as tip dieback, stem cankers, kino exudations and leaf spots (Barnard *et al*. 1987; Smith *et al*. 1994; Slippers and Wingfield 2007). In most cases, the diseases observed on *Eucalyptus* were associated with environmental stress such as hail or drought (Wene and Schoeneweiss 1980; Smith *et al*. 1994; Roux *et al*. 2005; Slippers and Wingfield 2007). For example, in South Africa, die-back and stem cankers were observed on *Eucalyptus* species where the diseases caused by *B. dothidea* were associated with extreme environmental conditions such as drought, frost or hail (Smith *et al*. 1994). In India, *L. theobromae* (reported as *Botryodiplodia theobromae*) was reported as the causal agent of stem canker disease on *Eucalyptus* species planted under dry climates. *Eucalyptus camaldulensis*

Dehnhardt was found to be a more susceptible host to the disease compared to *E. tereticornis* Smith. This disease resulted in 20% mortality of trees and is the reason why *E. camaldulensis* is no longer planted in India, even though its growth is more superior to that of *E. tereticornis* (Sharma *et al*. 1984).

Interestingly, the most pathogenic species of the *Botryosphaeriaceae* are those with a wide host and geographic range, such as *L. theobromae, N. parvum* and *D. sapinea* (Slippers and Wingfield 2007). This could be due to their frequent contact with hosts that have not co-evolved resistance to them (Parker and Gilbert 2004; Slippers *et al*. 2005). However, not all species with a wide host range cause disease on all the hosts that they infect, in all areas. For example, *B. dothidea* is a very serious pathogen of fruit and nut trees in the USA but is found rarely on these hosts in South Africa and other regions (Michailides 1991; Slippers *et al.* 2007; Chen *et al*. 2014).

4.3 *Botryosphaeriaceae* as plant endophytes

Endophytes are defined as microbes that can live in a host without causing any apparent symptoms. Most of the species in the *Botryosphaeriaceae* display the typical characteristics of endophytes such as a wide host range, cosmopolitan distribution and high levels of diversity (Denman *et al*. 2000; Slippers and Wingfield 2007). The first evidence of endophytism in the *Botryosphaeriaceae* was discovered by Petrini and Fisher in 1988 when they isolated *D. sapinea* (reported as *D. pinea*) from healthy stems of Scots pine (*Pinus sylvestris* Linnaeus). Later, more evidence of endophytism emerged as Fisher *et al*. (1993) isolated *B. dothidea* from healthy leaves, twig bark and xylem of *Eucalyptus nitens*. Since their establishment as endophytes in the 1990's, more studies have shown that the majority of the genera in the *Botryosphaeriaceae* have been recorded as endophytes (Crous *et al*. 2006). These include *Botryosphaeria*, *Diplodia*, *Dothiorella* Sacc., *Lasiodiplodia* and *Neofusicoccum*. Studies in forestry plant communities have shown that woody plants have high levels of endophytic *Botryosphaeriaceae* species (Burgess *et al*. 2005; Pavlic *et al*. 2008; Jami *et al*. 2012; Osorio *et al*. 2017). For example, Pavlic *et al*. (2007) found eight endophytic species of *Botryosphaeriaceae* on native *S. cordatum* trees in South Africa. Furthermore, in subsequent studies, *Botryosphaeriaceae* were isolated from various plant tissues on a single *S. cordatum* tree (Pillay *et al*. 2013). This emphasizes their ability to colonize the entire tree and implies that other species in the *Myrtaceae* might also have high levels of endophytic *Botryosphaeriaceae* species.

5.0 Host associations

Botryosphaeriaceae species have a wide host range and occur on both gymnopserms and angiosperms (Slippers and Wingfield 2007). However, their occurrence has mostly been recorded on angiosperms and less commonly on gymnosperms (de Wet *et al.* 2008). Currently, 18 genera in the family have been reported on angiosperms, while some species in five of the genera (*Diplodia, Dothiorella, Lasiodiplodia, Macrophomina* Petrak and *Neofusicoccum*) have been reported from both host groups (Dissanayake *et al.* 2016; Mehl *et al.* 2017; Slippers *et al.* 2017; Yang *et al.* 2017). Of 180 species of *Botryosphaeriaceae*, only three species, *Diplodia cupressi, D. tsugae* and *Dothiorella sempervirentis* have been reported to occur exclusively on gymnosperms. The latter observations support the suggestion that the ancestors of the *Botryosphaeriaceae* evolved on angiosperms and later moved to gymnosperms (de Wet *et al.* 2008).

Many *Botryosphaeriaceae* species are generalists, however some species are considered host specific. For example, the genus *Alanphillipsia* P.W. Crous & M.J. Wingf., which was described in 2013, has been exclusively isolated from *Aloe* Burman species in South Africa (Crous *et al*. 2013, 2014). Other species that have shown host specificity, include *N. eucalypticola* found exclusively on *Eucalyptus* species, while *N. vitifusiforme* on *Vitis vinifera* Linnaeus (van Niekerk *et al*. 2004) was thought to be host specific, but was recently identified from *Eucalyptus* species (Deidda *et al.* 2016). *Neofusuicoccum protearum* was thought to be host specific on *Protea* Linnaeus species, however, it was recently isolated from *Santalum acuminatum* de Candolle (Taylor *et al.* 2009). In this paper, we distinguish the species movement to other hosts in to host jumps, hosts shifts and host range expansions.

5.1 Host jumps

Host jumps occur when a pathogen moves between distant, or taxonomically unrelated family hosts (Stukenbrock and McDonald 2008). Host jumps are generally attributed to evolution, hybridization or a change in the genetic make-up of species over time (Burdon *et al*. 2006). Several examples have been recorded of fungi in the *Botryosphaeriaceae* that have undergone host jumps.

Jami *et al*. (2014), for example, conducted a study where they found *Botryosphaeriaceae* species overlapping on four unrelated native South African tree families. She isolated seven species of *Botyrosphaeriaceae* from four tree families growing in close proximity, of which five occurred only on a single host, but *Spencermartinsia viticola* was found on three of the species sampled. This shows the ability of *Botryosphaeriaceae* to jump hosts and it confirms that many of the species are not host specific and can occur on a number of hosts.

5.2 Host shifts

Host shifts are defined as the movement of a pathogen between phylogenetically related family hosts (Stukenbrock and McDonald 2008). Numerous examples exist for host shifts in the *Botryosphaeriaceae*. *Botryosphaeria dothidea, N. eucalyptorum,* and the members of the *N. parvum*/ *N. ribis* complex were found co-occuring on non-native *Eucalytpus* and native *Myrtaceae* (*Myrceugenia glaucescens* D. Legrand & Kausel, *Myrciaria tenella* Berg, *Myrcianthes pungens* Legrand, *Blepharocalyx saclifolius* Berg) in Uruguay at the same point in time (Pérez *et al*. 2010). Furthermore, three species of *Botryosphaeriaceae* (*N. eucalyptorum, N. kwambonambiense, N. parvum*) were identified on both non-native *Eucalyptus* and native *S. cordatum* in South Africa in the same geographical region and at a particular moment in time (Pillay *et al*. 2013). A recent study by Mehl *et al.* (2017) also serves as an example of such a movement where *N. parvum* was found infecting both native (*Sclerocarya birrea* Hochst) and non-native (*Mangifera indica* Linnaeus) *Anacardiaceae* in South Africa. This is a clear indication that there is biotic exchange between the non-native and native *Myrtaceae* and that this shifting of *Botryosphaeriaceae* species from one host to another should be fully monitored as they pose a threat to commercial crops and native plants.

5.3 Host range expansions

Host range expansion occurs when a species colonizes a new host species while remaining pathogenic to the ancestral host (Lê Van *et al.* 2012). Host shifts and host range expansions are often used interchangeably, however host shifts are known to lead to host expansions. Host expansion causes loss of host specialisation and leads to species that are generalists (Agosta *et al*. 2010). Factors determining the success of host range expansion are the phylogenetic relationship of the hosts and the geographic proximity between the original host and the new host (Diegisser *et* *al*. 2009). Non-native *Eucalyptus* trees are usually planted in close proximity to native *Myrtaceae* trees providing opportunities for some fungal species to expand to new hosts. For example, *Chrysoporthe austroafricana*, *Austropuccina psidii* and a number of *Botryosphaeriaceae* species such as *L. theobromae, L. pseudotheobromae, N. eucalyptorum* and *N. parvum* have been shown to have expanded from native trees to the non-native trees or *vice versa* (Pérez *et al*. 2010). However, host range expansions can also occur on phylogenetically distant hosts. For example, *Diplodia sapinea,* which was previously known to occur exclusively on the *Pinaceae* (gymnosperm) family, was recently found on a phylogenetically distant host, *Fagus sylvatica* Linnaeus (angiosperm) (Zlatković *et al.* 2017). This fungus seems to have expanded its host range while still pathogenic on the *Pinus* trees.

6.0 *Botryosphaeriaceae* on *Myrtaceae*

The *Botryosphaeriaceae* on trees in the *Myrtaceae* have received a considerable amount of attention over the past decade. This has been driven by the economic importance of *Eucalyptus* species in many countries globally. In many countries where eucalypts are grown commercially, they are planted in close proximity to native *Myrtaceae*, and in some cases other non-native *Myrtaceous* crops. This has provided ample opportunity for host shifts/jumps and expansions.

The movement of *Botryosphaeriaceae* species from non-native to native *Myrtaceae* or *vice versa* has been well studied in countries such as Australia, Uruguay and South Africa. Species that have been isolated from native *Mytraceae* in Uruguay include *B. dothidea, D. pseudoseriata* C.A. Pérez, Blanchette, Slippers & M.J. Wingfield*, D. seriata, Do. iberica, L. pseudotheobromae* A.J.L. Phillips,. Alves & Crous, *N. eucalyptorum* and *N. parvum* (Pérez *et al*. 2008, 2010). From these species, *B. dothidea, N. eucalyptorum* and *N. parvum* were found occurring on both *Eucalyptus* and the native *Myrtaceae* hosts, while *L. psedudotheobromae* and *D. pseudoseriata* were isolated exclusively from native *Myrtaceae*. Interestingly, *N. eucalyptorum* which was thought to be host specific to *Eucalyptus* species was isolated from the native *Myrtaceae* trees. This suggests that *N. eucalyptorum* might have shifted and expanded from the *Eucalyptus* species to the native *Myrtaceae*. In Western Australia, Burgess *et al*. (2006) clearly demonstrated that there is no restriction in the movement of *N. australe* from native to non-native *Eucalyptus* species. The lack of host specificity of some of the *Botryosphaeriaceae* species such as *N. parvum* means they can move from the native and the introduced trees, therefore, causing infections. Once species are introduced into an unfamiliar environment, they can jump/shift and expand to new hosts and cause economic and/or ecological damages. The host shift in the *Botryosphaeriaceae* has become an interesting topic to researchers due to the ecological and economic impact that these jumps and shifts can cause on native and non-native trees.

6.1 *Botryosphaeriaceae* on *Myrtaceae* in Africa

In Africa, *Botryosphaeriaceae* species have been reported in more than 35 countries on various hosts. Of the 54 countries found in the African continent, 9 countries (Ethiopia, Kenya, Ghana, Mozambique, Republic of Congo, South Africa, Uganda, Zambia, Zimbabwe) have reported *Botryosphaeriaceae* on *Eucalyptus* (*Myrtaceae*) species (Roux *et al*. 2000, 2001, 2005; Gezahgne *et al*. 2004; Chungu *et al.* 2010; Jimu *et al*. 2015; Maússe-Sitoe *et al*. 2016) (Table 3) and one country has reported it on other native *Myrtaceae*. In South Africa, studies have focused on native *Myrtaceae* trees such as *S. cordatum* and *Heteropyxidaceae* (Smith 2001; Pavlic *et al*. 2007; Mehl *et al*. 2017). Other countries in Africa have focused solely on species that cause diseases on economically and ecologically important trees. This is alarming as the ability of *Botryosphaeriaceae* species to move from native to non-native *Myrtaceae* and *vice versa* could result in considerable ecological and economical damage to native *Myrtaceae* or *Eucalyptus*.

6.2 *Botryosphaeriaceae* on native *Myrtaceae* in Africa

There are four genera in the *Myrtaceae* that are native to South Africa including *Syzygium, Eugenia* Linnaeus, *Meterosideros* Gaertner, and *Heteropyxis* Harvey (http://posa.sanbi.org/searchspp.php). In this country, studies of *Botryosphaeriaceae* on native *Myrtaceae* have been conducted only on *Heteropyxis natalensis* Harvey (Smith 2001) and *S. cordatum* (Pavlic *et al.* 2007). There are a total of twenty *Botryosphaeriaceae* species currently known from native *Myrtaceae* in South Africa (Table 2). This is of concern given that the biotic exchange between the native and the introduced *Myrtaceae* could result in economic loss and ecological disturbance. It is also known that fungal movement is more likely to be successful when the native trees and the non-natives are planted in close proximity to each other and are phylogenetically related, which is the case with *Eucalyptus* and native *Myrtaceae* trees in South Africa. Native trees could thus serve as source of pathogenic fungi that could infect non-native trees and *vice versa*. The biotic exchange from native to non-native *Myrtaceae* trees have been well documented in a number of studies (Wingfield *et al*. 2001; Wingfield 2003; Burgess *et al*. 2006; Pavlic *et al*. 2007; Pérez *et al*. 2010).

There are over 35 species of *Botryosphaeriaceae* reported from native woody tree hosts in South Africa (Pavlic *et al*. 2007; Jami *et al*. 2012; Pillay *et al.* 2013; Mehl *et al.* 2017). Of these only 20 have been reported on native *Myrtaceae* trees in the country (Table 2). Pavlic *et al.* (2007) reported eight species of *Botryosphaeriaceae* on *S. cordatum*, but five years later advanced DNA sequencing techniques revealed additional cryptic species and currently 19 species (Table 2) are known from *S. cordatum* (Pavlic *et al*. 2007, 2009; Pillay *et al*. 2013; Mehl *et al*. 2017). According to past reports, there have been insufficient studies on native *Myrtaceae* in other African countries, other than *Eucalyptus* and *Psidium guajava*.

6.3 *Botryosphaeriaceae* on *Eucalyptus* in Africa

The *Botryosphaeriaceae* are important pathogens of eucalypt species in Africa. *Botryosphaeriaceae* cause a range of disease symptoms on eucalypts on the continent. This includes stem cankers, tip die-back, kino exudations, and mortality of trees (Slippers and Wingfield 2007). In East Africa, most of the work has been focused on isolating *Botryosphaeriaceae* species associated with diseases on *Eucalyptus*, and the causal agents in most cases are found to be *L. theobromae* and *N. parvum* (Roux *et al*. 2000, 2001, 2005; Jimu *et al*. 2015; Mamle and Roux 2015). In South Africa, there are currently twelve *Botryosphaeriaceae* species reported on *Eucalyptus,* either as endophytes or from stem cankers (Table 3) (Roux *et al*. 2000, 2001; Pillay *et al*. 2013; Pavlic-Zupanc *et al.* 2017).

7.0 Conclusions

Over the past decade, the taxonomy of the *Botryosphaeriaceae* has changed greatly due to the advent of DNA techniques to identify these fungi. This has allowed for a much better understanding of species concepts in the genus, which has in turn allowed for a better understanding of the host and geographic ranges of species within the family. Furthermore, whole genome sequencing has made it possible to better understand and study the biology and phylogeography of the *Botryosphaeriaceae*. This technology will allow researchers to answer questions such as the ecological role of the species of *Botryosphaeriaceae* in a broader ecosystem, their reproductive strategy, the colonization of their hosts, and most importantly the changing interaction of *Botryosphaeriaceae* species with their hosts under conditions imposed by climate change. It is expected that the impact of fungi in the *Botryosphaeriaceae* will increase in future. This is due to multiple factors including their endophytic nature, their ability to infect multiple hosts, and their cosmopolitan distribution.

Studies have focused largely on the taxonomy of the *Botryosphaeriaceae* species and the diseases they cause on economically important trees. However, there have been very few studies to understand the host shifts of this species to new hosts. In South Africa, studies have focused on one native *Myrtaceae* tree (*S. cordatum*) while in the rest of Africa no studies have been made on any native *Myrtaceae* trees. Given the ability of the *Botryosphaeriaceae* species to shift hosts, it is important to study species occurring on native *Myrtaceae* trees as they could serve as inoculum for newly introduced trees. The potential impact of the species obtained from native trees on the non-native trees need to be fully monitored and studied. The aims of studies in this thesis are, therefore, to expand on previous surveys done on native and non-native *Myrtaceae* trees in Africa, and specifically to increase knowledge pertaining to the species diversity, host range and ecology of *Botryosphaeriaceae* species on the *Myrtaceae*. The research project for which this review was prepared focusses on both the native and non-native *Myrtaceae* trees to provide further insight into the movement of the *Botryosphaeriaceae* from the native *Myrtaceae* trees to the non-natives. The research should aslo add to the body of knowledge regarding the pathogenicity of *Botryosphaeriaceae* species obtained from native *Myrtaceae* on *Eucalyptus* species.

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Table 1. Timeline of the major taxonomic changes in the *Botryosphaeriaceae*

Year	**Taxonomic changes**	**References**
1863	The genus *Botryosphaeria* was introduced by Cesati and De Notaris	Cesati and De Notaris 1863
1918	The *Botryosphaeriaceae* was introduced as a sub-family in the *Pseudosphaeriaceae*. Subsequently the sub-class the Dothideineae was established to accommodate the order Pseudosphaeriales, family *Botryosphaeriaceae,* and the genus *Botryosphaeria*	Theissen and Sydow 1918
1923	*Botryosphaeria* was placed in the sub-family *Pseudosphaeriaceae, Pleosporaceae* (Sphaeriales)	Petrak 1923
1928	*Botryosphaeriae* species were then placed in the order Dothideales because they lacked true perithecial walls	Miller 1928
1955	Lutrell recongised two major groups, the unitunicate and the bitunicate Ascomycetes. He replaced the name Pseudosphaeriales with Pleosporales, and assigned *Botryosphaeria* species to this order based on a centrum typical of the Pleosporales with pseudothecia and pseudoparaphyses	Luttrell 1955
1975	Von Arx and Müller placed all bitunicate ascomycetes in the single order Dothideales, which comprised two sub-orders and 34 families, including the *Botryosphaeriaceae*	Von Arx and Müller 1975
1979	In 1979 Barr accepted the placement of *Botryosphaeria* species in the Pleosporales due to the centrum development	Barr 1979
1996	Berbee used gene sequences of 18S rRNA gene (SSU) to study phylogenetic relationships of genera and orders of lucoloascomycetes.	Berbee 1996
1998	First ITS DNA sequence data used to reveal the distinction between asexual morphs with hyaline *Fusicoccum*-like conidia and pigmented, *Diplodia*-like conidia within *Botryosphaeria* species examined	Jacobs and Rehner 1998; Denman *et al.* 2000
2006	Based on multigene phylogeny the new order Botryophaeriales was introduced to accommodate the single family *Botryosphaeriaceae*	Schoch *et al.* 2006
2006	Based on 28s rRNA, Crous introduced 10 clades within the *Botryosphaeriaceae* including unresolved clade (*Diplodia/ Lasiodiplodia*, *Tiarosporella*), *Botryosphaeria s.str*, *Macrophomina*,	Crous *et al.* 2006

	Neoscytalidium, *Dotthidotthia*, *Neofusicoccum*, *Pseudofusicoccum*, *Saccharata*, "*Botryosphaeria*" quercuum, and *Guignardia*	
2008	Based on multigene sequence-based phylogeny, Phillips recognized at least six genera among *Botryosphaeriaceae* species with dark ascospores	Phillips *et al.* 2008
2012	The ascospores of *Botryosphaeriaceae* were defined as hyaline and aseptate, but pigmented and septate with age. Liu describe the family *Botryosphaeriaceae* as having 29 genera of which 17 known in culture	Liu *et al*. 2012
2013	Six families were recognized in the Botryosphaeriales based on sequence data of six loci (SSU, LSU, ITS, EF1, BT, mtSSU), including *Botryosphaeriaceae* (17 genera), *Aplosporellaceae*, *Melanopsaceae*, *Phyllostictaceae*, *Planistromellaceae* and *Saccharataceae*	Slippers *et al.* 2013
2013	Comprehensive descriptions of 17 genera and 110 species in the *Botryosphaeriaceae*	Phillips *et al.* 2013
2016 - 2017	*Botryosphaeriaceae* represented by 23 genera and 180 species described based on morphological, phylogenetical and ecological differences	Dissanayake *et al.* 2016; Slippers *et al.* 2017; Yang *et al.* 2017

Table 2. *Botryosphaeriaceae* on native *Myrtaceae* trees in Southern Africa

Host	**Identity**	**Reference**
Heteropyxis natalensis	*Botryosphaeria dothidea*	Smith 2001
Syzygium cordatum	*B. dothidea*	Pavlic *et al*. 2007, 2008, 2009; Pillay *et al*. 2013; Mehl *et al*. 2017
	Lasiodiplodia egyptiacae	
	L. gilanensis	
	L. gonubiensis	
	L. iraniensis	
	L. theobromae	
	L. pseudotheobromae	
	L. rubropurpurea	
	Neofusicoccum australe	
	N. batangarum	
	N. cordaticola	
	N. cryptoaustrale	
	N. eucalyptorum	
	N. kwambonambiense	
	N. luteum	
	N. mangiferae	
	N. parvum	
	N. ribis	
	N. umdonicola	

Table 3. *Botryosphaeriaceae* species reported from *Eucalyptus* species in the world

Distribution	**Identity**	**Host**	**References**
Australia	*B. ramosa* *L. rubropurperea* *N. australe* *N. eucalyptorum* *N. eucalypticola* *N. macroclavatum* *N. occulatum* *N. vitifusiforme*	*E. corticola, E. camaldulensis, E.pauciflora, E. marginata, E. rubida, E. viminalis* *Eucalyptus* spp. *E. gomphocephala, E. marginata, E. globulus, E. diversicola, E. saligna* *E. grandis*	Barber *et al*. 2005; Taylor *et al*. 2009; Summerell *et al*. 2006 Sutton 1980 Burgess *et al*. 2005 Burgess *et al*. 2006 Pavlic *et al*. 2008 Sakalidis *et al*. 2011 Slippers *et al*. 2004
Brazil	*N. iraniensis*	*Eucalyptus* spp.	Machado *et al.* 2014
China	*B. fabicerciana* *L. theobromae* *L. pseudotheobromae* *L. ruboropurpurea* *N. parvum* *N.ribis*	*E. grandis*, *Eucalyptus* spp.	Chen *et al*. 2011
Columbia	*B. dothidea* *N. ribis*	*E. grandis*	Rodas *et al.* 2009
Ethiopia	*N. parvum* *N. ribis*	*Eucalyptus* spp.	Gezahgne *et al.* 2004
Ghana	*Botryosphaeria* sp.	*E. grandis*	Mamle and Roux 2015
Greece	*N. meditterraneum*	*Eucalyptus* spp.	Crous *et al*. 2007
India	*L. theobromae*	*Eucalyptus* spp.	Sharma *et al.* 1984
Italy	*N. australe* *N. luteum* *N. mediterraneum* *N. parvum* *N. vitifusiforme*	*Eucalyptus* spp.	Deidda *et al*. 2016
Kenya	*B. obtusa*	*E. grandis*	Mutitu *et al*. 2008; Roux *et al.* 2005
Mozambique	*N. kwambonambiense* *N. parvum*	*Eucalyptus* spp.	Maússe-Sitoe *et al*. 2016
Portugal	*B. dothidea* *D. corticola* *D. seriata* *N. australe* *N. algeriense* *N. eucalyptorum* *N. kwambonambiense* *N. parvum*	*E. globulus, Eucalyptus* spp.	De la Mora-Castaneda *et al*. 2014; Barradas *et al*. 2016

Republic of Congo	*L. theobromae*	*Eucalyptus* spp.	Roux 2000
South Africa	*B. dothidea* *C. atrovirens* *L. pseudotheobromae* *L. theobromae* *N. australe* *N. eucalypti* *N. eucalypticola* *N. eucalyptorum* *N. kwambonambiense* *N. parvum* *N. ursorum* *N. cryptoaustrale*	*Eucalyptus* spp. *E. grandis* *E. urophylla*	Pavlic *et al*. 2007 Pillay *et al*. 2013 Pillay *et al*. 2013 Burgess *et al*. 2006 Pillay *et al*. 2013 Smith *et al*. 1994, 2001; Pavlic-Zupanc *et al*. 2017
Spain	*N. australe* *N. parvum*	*Eucalyptus* spp. *E. globulus*	Armerngol *et al.* 2008 Iturritxa *et al.* 2011
Thailand	*C. atrovirens* *S. eucalypticola*	*Eucalyptus* spp.	Liu *et al*. 2012
Uganda	*L. theobromae*	*E. grandis*	Roux *et al*. 2001
Uruguay	*B. dothidea* *L. crassispora* *L. theobromae* *N. eucalyptorum* *N. parvum*	*E. urophylla, E. grandis, Eucalyptus* spp.	Pérez *et al*. 2008, 2010
USA	*N. meditterraneum*	*Eucalyptus* spp.	Inderbitzin *et al.* 2010
Venezuela	*B. mamane* *C. atrovirens* *N. andinum* *N. parvum* *N. ribis*	*E. urophylla, Eucalyptus* spp.	Mohali *et al*. 2006
Zambia	*L. theobromae* *N. eucalyptorum* *N. parvum*	*Eucalyptus* spp.	Chungu *et al.* 2010
Zimbabwe	*N. eucalyptorum* *N. parvum*	*Eucalyptus* spp.	Jimu *et al.* 2016

CHAPTER 2

Botryosphaeriaceae on native and non-native *Myrtaceae* in eastern and southern Africa

ABSTRACT

Botryosphaeriaceae species are important latent pathogens causing diseases on many woody plants, usually when they are subjected to stress. Little is, however, known regarding the diversity and occurrence of *Botryosphaeriaceae* on native *Myrtaceae* species in eastern and southern Africa. In this study, we identified endophytic *Botryosphaeriaceae* species on several native *Myrtaceae* and two non-native *Myrtaceae* from different countries in the region. Fourteen species in the *Botryosphaeriaceae* were identified based on analyses of DNA sequence data of the ITS rDNA, β-tubulin, TEF-1α and RPB2 gene regions. The species identified included *Diplodia* sp., *Neofusicoccum cordaticola, N. eucalyptorum, N. kwambonambiense, N. umdonicola, N. parvum,* three novel *Neofusicoccum* species, two potential *Neofusicoccum* hybrids*, Lasiodiplodia gonubiensis, Lasiodiplodia* sp. 1 and one potential *Lasiodiplodia* hybrid. *Neofusicoccum parvum* was the most abundant species collected and was found occurring on both native and non-native *Myrtaceae* in eastern and southern Africa. Pathogenicity trials conducted suggest that *N. parvum* is the most pathogenic species on *Eucalyptus* trees.

1.0 Introduction

Botryosphaeriaceae species are amongst the most commonly isolated fungi associated with trees, both in native and non-native environments (Slippers and Wingfield 2007; Slippers *et al*. 2009; Pérez *et al*. 2010). They have a cosmopolitan distribution and occur on a wide range of hosts (Crous *et al*. 2006; Mehl *et al*. 2013). The association between species in the *Botryosphaeriaceae* and their hosts can be saprophytic, endophytic or pathogenic (Slippers and Wingfield 2007). The pathogenic species are mostly known to cause diseases when the host plant is subjected to stress (Slippers and Wingfield 2007). Symptoms associated with infection by species of *Botryosphaeriaceae* include tip die-back, stem and branch cankers, fruit rots, leaf spots and in severe cases mortality of trees (Slippers and Wingfield 2007; Mehl *et al*. 2013). It has been predicted that the impact of these fungi may increase as the global climate changes and becomes more stressful to plants in some areas (Slippers and Wingfield 2007).

The taxonomy of species and genera in the *Botryosphaeriaceae* has undergone significant changes over the past century. Initially, species were described based only on morphology, especially that of the asexual states because the sexual states are rarely encountered in nature (Denman *et al.* 2000; Slippers *et al*. 2004; Crous *et al*. 2006; Phillips *et al*. 2013). Host associations were also commonly used for species delineation, resulting in many novel species being described based on their occurrence on novel hosts (Slippers and Wingfield 2007; Slippers *et al*. 2009). The overlap of morphological characteristics between the species and their wide host ranges resulted in considerable misidentification of *Botryosphaeriaceae* species.

The advent of molecular techniques has contributed to resolving the taxonomy of the *Botryosphaeriaceae* (Slippers *et al*. 2004; Crous *et al*. 2006; Phillips *et al*. 2013). The most commonly used approach to identify species of *Botryosphaeriaceae* includes analyses of sequences for a combination of two or more loci, such as the Internal Transcribed Spacer (ITS), β-tubulin (BT), Translation Elongation Factor (TEF-1α), and RNA polymerase II subunit (RPB2). The combined analyses provide sufficient phylogenetic support for the differentiation of cryptic species (Slippers *et al.* 2017). Recently, several examples of hybridization have been identified between species in this group and these can only be identified if two or more loci are used for analyses (Cruywagen *et al.* 2017). It is currently accepted that the *Botryosphaeriaceae* resides in the order Botryosphaeriales and it comprises 23 phylogenetically distinct genera with over 180 species that are known from culture and sequence data in Genbank (Dissanayake *et al.* 2016; Linaldeddu *et al*. 2016; Crous *et al.* 2017; Slippers *et al*. 2017; Yang *et al.* 2017).

The *Myrtaceae* (Order Myrtales) is a family of shrubs and trees that comprises about 130 genera and 5600 species, predominately in the southern hemisphere (Johnson and Briggs 1981; Govaerts *et al*. 2008). *Eucalyptus* is the largest genus in the family, with over 700 species. Most *Eucalyptus* species originate from Australia, with a few originating from south east Asian islands. These trees have been planted globally, as non-natives, because of their fast growth and the variety of species available for multiple environmental conditions. It was introduced to east Africa in the 19th century (Evans 1992; Chamshama and Nwonwu 2004) and expanded to the tropics and sub-tropics between the years 1940-1980 (Chamshama and Nwonwu 2004). In South Africa, the first *Eucalyptus* species were established in 1875 after timber demand had exceeded the supply available from indigenous forests, and by the 1940s at least 149 species had been established in the country (Poynton 1977; Albaugh *et al*. 2013). *Eucalyptus* species were recognized for their fast growth and timber quality and today form the basis of commercial plantation forestry in many countries globally (Albaugh *et al*. 2013). Several other members of the *Myrtaceae* are also economically important crops. These include, for example, *Psidium guajava* and *Syzygium aromaticum,* while others such as *S. cordatum* are ecologically important species, and used for medicinal and ornamental purposes.

Botryosphaeriaceae species are amongst the most commonly isolated fungi associated with *Eucalyptus* species, both in native and non-native environments. Prior to 1995 there were 27 species of *Botryosphaeriaceae* reported on *Eucalyptus,* belonging to the genera *Botryosphaeria*, *Diplodia*, *Dothiorella* and *Lasiodiplodia* (Sankaran *et al.* 1995). In 2009, the use of multiple phylogenies confirmed 23 of the 27 species in the *Botryosphaeriaceae* on *Eucalyptus* species (Slippers *et al*. 2009). However, the taxonomy of the *Botryosphaeriaceae* has recently undergone major revisions and some genera that were reported on *Eucalyptus* are now considered separate families in the order *Botryosphaeriales,* such as the *Pseudofusicoccumaceae* (Yang *et al.* 2017). Currently, there are over 30 species of *Botryosphaeriaceae* reported on *Eucalyptus* in the world (Dissanayake *et al.* 2016). The species belong to the genera *Botryosphaeria* (Mohali *et al.* 2007; Taylor *et al*. 2009; Pérez *et al*. 2010; Pavlic-Zupanc *et al*. 2017), *Cophinforma* (Liu *et al*. 2012), *Diplodia* (Barradas *et al*. 2016), *Dothiorella* (Slippers *et al*. 2009), *Lasiodiplodia* (Burgess *et al.*

2006; Abdollahzadeh *et al.* 2010; Pérez *et al*. 2010), *Neofusicoccum* (Denman *et al*. 2000; Smith 2001; Slippers *et al*. 2004; Burgess *et al*. 2006; Crous *et al*. 2007; Pérez *et al*. 2010; Sakalidis *et al.* 2011), *Neoscytalidium* (Sutton and Dyko 1989) and *Sphaeropsis* (Liu *et al.* 2012). *Neofusicoccum* and *Lasiodiplodia* are the most commonly reported on *Eucalyptus* in both native and non-native environments (Crous *et al*. 2007; Slippers *et al*. 2009; Abdollahzadeh *et al*. 2010; Pérez *et al*. 2010), while other genera are rarely found.

Species of *Botryosphaeriaceae* are important pathogens of *Eucalyptus* species in Africa, resulting in tip die-back, stem and branch cankers and xylem discoloration (Smith *et al*. 1994; Roux *et al*. 2000, 2001; Slippers *et al*. 2004, 2009). In South Africa, there are currently twelve *Botryosphaeriaceae* species reported from *Eucalyptus*, either as endophytes or from stem cankers (Pavlic *et al*. 2007; Pillay *et al*. 2013; Pavlic-Zupanc *et al*. 2017). Research on the *Botryosphaeriaceae* associated with *Eucalyptus* species on the rest of the African continent is limited to single reports from the Republic of Congo (Roux *et al*. 2000, 2005), Ethiopia (Gezahgne *et al*. 2004; Roux *et al*. 2005), Uganda (Roux *et al*. 2001; Nakabonge 2005), Zambia (Chungu *et al*. 2010), Mozambique (Maússe-Sitoe *et al*. 2016), Zimbabwe (Jimu *et al*. 2015), and Kenya (Roux *et al*. 2005).

Pathogens affecting *Eucalyptus* species in their non-native environments could be introduced or from natural forest stands surrounding the plantations. Native trees could serve as an important source of *Eucalyptus* pathogens as it has been found with pathogens such as *Austropuccinia psidii* (Coutinho *et al*. 1989; Beenken 2017) and *Chrysoporthe austroafricana* (Heath *et al*. 2006). In recent years, several studies have investigated possible host jumps/shifts of *Botryosphaeriaceae* species between native and non-native *Myrtaceae* (Pavlic *et al*. 2007; Slippers *et al*. 2009; Pérez *et al*. 2008, 2010). In South Africa, thirteen *Botryosphaeriaceae* species have been found on both non-native *Eucalyptus* species and native *S. cordatum*. These include, *Botryosphaeria dothidea*, *Lasiodiplodia gonubiensis*, *L. iraniensis*, *L. pseudotheobromae*, *L. rubropurpurea*, *L. theobromae*, *Neousicoccum australe*, *N. cryptoaustrale*, *N. eucalyptorum*, *N. kwambonambiense*, *N. luteum*, *N. parvum*, *N. ribis* and *N. ursorum* (Pavlic *et al*. 2007; Pillay *et al*. 2013; Pavlic-Zupanc *et al.* 2017). *Neofusicoccum parvum* has also been isolated from another native family in the Myrtales, the *Heteropyxidaceae*, in South Africa (Smith 2001). Interestingly, some species in the *Botryosphaeriaceae* have been isolated only from *S. cordatum* in South Africa but not from

Eucalyptus. Examples include *L. egyptiacae*, *L. gilanensis, N. batangarum, N. cordaticola*, *N. mangiferae* and *N. umdonicola* (Pavlic *et al*. 2007; Mehl *et al*. 2017). In Uruguay*, B. dothidea*, *N. eucalyptorum*, and the members of the *N. parvum*/*N. ribis* complex were found co-occurring on non-native *Eucalyptus* and native *Myrtaceae* (*Blepharocalyx salicifolius*, *Myrceugenia glaucescens, Myrrhinium atropurpureum var*. *octandrum*) (Pérez *et al*. 2010).

While considerable work has been done to identify species of *Botryosphaeriaceae* associated with *Eucalyptus* and *S. cordatum* in South Africa, there have been limited studies focusing on *Botryosphaeriaceae* species on other native *Myrtaceae* trees in Africa. The aim of this study was, therefore, to identify the species of *Botryosphaeriaceae* occurring on both native *Myrtaceae* and non-native *Eucalyptus* species from five countries in eastern and southern Africa using sequence data for four gene regions (BT, ITS, TEF-1α, and RPB2). Furthermore, pathogenicity trials were conducted on *Eucalyptus* trees to understand the possible implications of host shift/jumps to introduced *Eucalyptus* trees.

2.0 Materials and methods

2.1 Isolates

Isolates used in this study were divided into two groups. The first group of isolates was obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (UP), South Africa. They originated from collections made in 2009 in East Africa (Malawi, Mozambique, Tanzania and Zimbabwe) including those from native *S. cordatum* and non-native *Eucalyptus* trees in the sampled regions. A total of 34 isolates were regrown and considered in the study (Table 1).

The second group of isolates were collected from two areas (Soutpansberg and Tzaneen) in the Limpopo Province of South Africa during April and August 2015 (Fig. 1, Table 1). The samples were taken from three sites in each area. In Soutpansberg the sampling sites included Lajuma Nature Reserve near Louis Trichardt and the Fundudzi area near Thohoyandou. In Tzaneen, the samples were taken from Georges Valley, Grootbos and New Agatha (Fig. 1). The samples were collected from two non-native trees (*Eucalyptus* species and *Psidium guajava*) and from six native trees chosen according to their proximity to the *Eucalyptus* plantations. These included *Eugenia* *natalitia*, *E. woodii*, *Syzygium cordatum*, *S. legattii* and *S. gerarrdii* (*Myrtaceae)* and *Heteropyxis natalensis (Heteropyxidaceae)* in the Myrtales. Random sampling was used to collect the samples. At each of the six sites, 10 twigs per tree species (one twig per tree) were collected. For the *Eucalyptus* species 10 additional dead twigs were collected at each site. The collected samples were kept in open paper bags, and isolations were made immediately.

The samples were first surface sterilized with 70 % alcohol for one minute, then rinsed in distilled water for 30 seconds. The twigs were cut in half and for each collected twig four pieces were cut from the centre, placed on 2 % MEA and incubated at 25 °C. Single hyphal-tips from isolates displaying typical cultural morphological characteristics of *Botryosphaeriaceae* (white to grey mycelium with aerial hyphae, rapid growth) were transferred to clean 2 % MEA plates to obtain pure cultures. The pure cultures are kept in the Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria.

2.2 DNA extraction, PCR amplification and Sequencing

DNA was extracted from fungal mycelium of 7-day-old cultures using the DNA extraction protocol described by Möller *et al*. (1992). DNA concentrations were determined using a ND-1000 spectrophotometer V3.7.1 (Thermo Fisher Scientific, USA). The DNA was diluted with Sabax water to 50ng/µl for use in Polymerase Chain Reactions (PCR).

Four gene regions, including the Internal Transcribed Spacer (ITS) regions ITS1 and ITS2, and the intermediate 5.8S gene of the ribosomal RNA (rRNA), β-tubulin (BT), Translation Elongation Factor (TEF-1α), and RNA polymerase II subunit (RPB2) were amplified for DNA sequencing and subsequent phylogenetic analyses. The primers used to amplify the selected gene regions, their sequences and their respective annealing temperatures are presented in Table 2. The ITS gene region of the rRNA operon was amplified for initial screening of all isolates using the primers ITS1/4 (White *et al*. 1990). The other three gene regions were amplified for selected isolates (Table 1) using primers listed in the Table 2.

Genomic DNA was used for amplification of the selected gene regions in Polymerase Chain Reactions (PCR's). The PCR reaction mixes consisted of 1 µl DNA template, 0.5 µl (10Mm) of each forward and reverse primer, 0.5 µl Taq polymerase, 5.0 µl Taq buffer (Bioline GmbH, 21

Germany) and 17.5 µl Sabax water which made up a total volume of 25 µl. The PCR protocol used was as follows: 94 °C for 4 min, 35 cycles of denaturation at 94 °C at 30 s, annealing at 55 °C for 45 s, elongation at 72 °C for 1 min and a final elongation cycle at 72 °C for 7 min. The PCR products were visualized using 1 % agarose gel containing Gel red (Biotium, Hayward, US) in 1×TAE buffer solution. PCR product sizes were estimated using a 100 bp marker under UV light. Eight µl Exosap mixture was used to clean the PCR products before sequencing PCRs. The sequencing PCR conditions were as follows: 96 °C for 10 s followed by 35 cycles of 5 °C for 10 s and 60 °C for 4 min. Sodium acetate mixture containing 2 µl of NaAc, 8 µl of Sabax water and 50 µl of absolute alcohol was used to clean the final products. Sequence reactions were performed with the ABI PRISM ™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems Inc., Foster City, California) in both directions, following the manufacturer's instructions, and run on an ABI PRISM 3100™ automated DNA sequencer.

2.3 Phylogenetic analyses

Sequences were edited using CLC BioWorkbench v.5 and inconsistencies of sequences were checked using Bioedit v7.2.6 software. As an initial screening of all isolates, ITS sequences were included in an ITS dataset that included the types of all known genera in the *Botryosphaeriaceae.* Thereafter, ITS, β-tubulin, TEF-1α and RPB2 sequences of each genus were compiled into a dataset of known and described species in that genus. Sequence alignments were done using online software, MAFFT version 7 (http://timpani.genome.ad.jp/~mafft/server/) (Katoh and Standley 2013). Parsimony analyses were conducted on individual datasets and on combined datasets after partition homogeneity tests were run in PAUP (Phylogenetic Analyses Using Parsimony) v.4.0.b10 (Posada and Buckley 2004) to determine the congruency of the ITS and TEF-1α, BT and TEF-1α, ITS and BT gene region combinations, and between all four gene regions combined. Only individual trees were drawn for the RPB2 gene region because the data base for this gene region is incomplete and comprises mostly species of *Neofusicoccum* and *Lasiodiplodia* (Slippers *et al*. 2017). The gaps were removed before analyses and all characters were unweighted and unordered. Maximum likelihood (ML) trees with a 1000 replicate bootstrap analyses were executed to assess the confidence levels of the branch nodes (Felsenstein 1985). The tree length (TL), consistency index (CI), retention index (RI), and the homoplasy index (HI) was determined for all data sets analysed (Table 3).

2.4 Pathogenicity trials

Nine isolates representing the species of *Botryosphaeriaceae* identified in this study were selected for pathogenicity trials under greenhouse conditions. The selected isolates represented putative novel species or species representing novel host records. The isolates were grown on 2 % MEA at room temperature for seven days prior to inoculation. Two-year-old *Eucalyptus grandis* clone TAG5 trees in pots were left to acclimatize to the glasshouse conditions of ~25 °C for 3-4 weeks with natural day/night conditions of ~14 h daylight and 10 h darkness before inoculations.

Each of the selected nine isolates were inoculated into the stems of 10 trees each. Ten trees were inoculated with sterile 2 % MEA plugs to serve as controls. For inoculations, the bark on the stem was removed with a 5 mm diameter cork borer to expose the cambium. Plugs of equal size from seven-day-old cultures were inserted into wounds with mycelium facing the exposed cambium. The wounds and mycelial plugs were then sealed with Parafilm (Bemis®, USA) to prevent desiccation and contamination. The inoculated trees were randomly arranged in the glasshouse and maintained at a temperature of 25 °C for four weeks under natural day/night conditions and watered once each day. External lesion development on the inoculated stems was measured after four weeks.

Two randomly selected trees inoculated with each of the *Botryosphaeriaceae* isolates, and the control, were selected for re-isolation to confirm pathogenicity re-isolations were made by cutting out a small piece of wood at the edge of the lesion and placing this on 2 % MEA. DNA sequences of the ITS gene region were used to confirm the identity of the fungal isolates obtained. The entire pathogenicity trial was repeated once under the same conditions. The data were analyzed using one-way analyses of variance (ANOVA) using PROC GLM (General Linear Model) in SAS version 8 (Littell 1996). A significance level of $P< 0.05$ for the F-value was used.

3.0 Results

3.1 Isolates

A total of 34 isolates were obtained from the CMW culture collection, of which 27 originated from *S. cordatum* and seven from *Eucalyptus* species in Malawi, Mozambique, Tanzania and Zimbabwe (Table 1). In South Africa, a total of 157 isolates were obtained from the following *Myrtaceae* trees: *Eucalyptus camaldulensis* (11), *E. cloeziana* (17), *E. grandis* (36), *Eugenia natalitia* (5), *H. natalensis* (30), *S. cordatum* (48), *S. gerrardii* (4) and *P. guajava* (6) (Table 1).

3.2 DNA sequencing and phylogenetic analyses

ITS sequences of the 191 isolates were compared with the nucleotide sequence data base of NCBI (http://www.ncbi.nlm.nih.gov) using the blast search function, identifying three possible genera (*Diplodia*, *Lasiodiplodia* and *Neofusicoccum*) amongst the isolates sequenced in this study. The ITS sequences were then compiled into a data set comprising types of all genera in the *Botryosphaeriaceae*, confirming the presence of three genera amongst the isolates from southern and eastern Africa (Figure 2). The identified genera were *Diplodia* (2 isolates), *Lasiodiplodia* (28 isolates) and *Neofusicoccum* (161 isolates).

Representative isolates, of each genus identified based on ITS sequence analyses, were selected for further analyses using sequences of the BT, TEF-1α and RPB2 gene regions. The isolates were selected to represent different geographical origins and hosts. Datasets for each of the gene regions sequenced were analyzed separately and in various combinations to investigate the congruency of the phylogenetic clades obtained. Tree parameters obtained from the maximum likelihood analysis of the ITS region, BT, TEF-1α, RPB2 gene and combined datasets for all regions are represented in Table 3.

Diplodia species were analyzed using ITS, BT and TEF-1α sequences. However, only the results of ITS and TEF-1α regions are shown because the data for the BT gene region was incomplete and there were no reference sequences in GenBank for species that group closely related to the isolates obtained in this study. In the individual analyses of ITS and TEF-1α sequences two *Diplodia* isolates obtained in this study (CMW46906, CMW46899) grouped in a clade with the known species *D. alatafructa, D. insularis* and *D. pseudoseriata* (Figs 3 and 4). African isolates were identical to the type isolate of *D. pseudoseriata* and to an isolate of *D. alatafructa* in the ITS (Fig 3). The two African isolates formed a sub-clade (without bootstrap support) in the clade of the three known *Diplodia* species in the TEF-1α dataset (Fig 4). Furthermore, the reference isolates representing *D. alatafructa* and *D. pseudoseriata* separated from the ex-type isolates of both species in the TEF-1α dataset. In the combined ITS and TEF-1α data set the two African isolates grouped in separate sub-clades within a larger clade with *D. alatafructa*, *D. insularis* and *D.*

pseudoseriata (Fig 5). The two African isolates could be identified only to the genus level and were thus treated as *Diplodia* sp.

Lasiodiplodia species were analyzed using four gene regions including ITS, BT, TEF-1α and RPB2 for individual trees and a combined analysis of all four gene regions (Table 3). Based on analyses of the ITS dataset, *Lasiodiplodia* isolates grouped into three broad phylogenetic clades. Nine of the isolates grouped with *L. gonubiensis*, one (CMW46912) grouped on its own and the other eight isolates grouped within a larger clade in the *L. theobromae* complex which included 18 species (Fig 6). Analyses of the BT dataset confirmed the grouping of the isolates in the *L. theobromae* complex (7) and *L. gonubienses* (9), while isolate CMW46912 also grouped in the *L. gonubienses* clade (Fig 7). This gene region could also not resolve the identities of the other eight isolates (Fig 7), but grouped them closest to *L. iraniensis*, *L. plurivora* and *L. pseudotheobromae*. A combined dataset for the ITS and BT gene regions confirmed the identity of the nine isolates (CMW46921, CMW46916, CMW46908, CMW46915, CMW46914, CMW46913, CMW46930, CMW46927, CMW31829) from Africa as *L. gonubiensis* (Fig 8). The combined dataset separated isolate CMW46912 into a sub-clade from the *L. gonubiensis* clade with a high bootstrap value of 80 %. Seven of the isolates (CMW46893, CMW46891, CMW46943, CMW46861, CMW46860, CMW46859, CMW46870) again grouped together within a larger clade containing multiple species, and were most similar to *L. plurivora*. The bootstrap values were generally low to support the separation of the isolates of other species in the larger *L. theobromae* complex.

Analyses of the TEF-1α gene region of *Lasiodiplodia*, grouped five isolates (CMW46870, CMW46859, CMW46860, CMW46861, CMW46891) selected from a group of seven unresolved isolates identified in the ITS and BT gene regions with *L. gilanensis*, with a strong bootstrap value of 89 % (Fig 9). Isolate CMW46912 again grouped with *L. gonubiensis*, while CMW31829 that in the ITS, BT and combined ITS/BT grouped with *L. gonubiensis*, now grouped with *L. gilanensis*. Isolate CMW31829 could be a hybrid as it grouped in different clades in the different gene trees.

Phylogenetic analyses of the RPB2 gene region suggested that the clade of seven unresolved isolates identified in the individual ITS, BT, TEF-1α trees represent a novel undescribed species, as five representative isolates of that group (CMW46870, CMW46859, CMW46860, CMW46861, CMW46891) formed a separate clade in the RPB2 (Fig 10) and in combined analyses of all four gene regions (Fig 11). Therefore, these isolates were treated as *Lasiodiplodia* sp. (Table 1). In the RPB2 sequence analyses, two isolates (CMW31829 and CMW46912) formed a separate clade closest to the *L. gonubiensis* clade with 100 % bootstrap support. However, in the combined analyses of all four gene region sequences, they grouped closely to *L. gonubiensis* with low bootstrap support (Fig 11). The identity of these two isolates was not resolved and they were treated as *Lasiodiplodia* sp. (Table 1).

Neofusicoccum species were analyzed using ITS, BT, TEF-1α and RPB2 sequences. However, only the results of ITS, BT, TEF-1α and ITS and BT combined regions are shown. The data for the RPB2 gene region were incomplete because there were insufficient reference sequences in GenBank for species that group closely with the isolates obtained in this study. However, this gene region is used in Table 4 to compare species that are undescribed with some of the closely related species available in GenBank. The ITS dataset for the genus *Neofusicoccum* consisted of 60 ingroup taxa and was rooted to *Botryosphaeria dothidea*. Based on the ITS gene region, the *Neofusicoccum* isolates analyzed in this study reside in seven clades identified as *N. cordaticola*, *N. eucalyptorum*, *N. kwambonambiense*, *N. parvum*, *N. ribis/ N. occulatum*, *N. umdonicola* and two possibly novel species, *Neofisicoccum* sp. 1 (CMW46887, CMW46888) and *Neofusicoccum* sp. 2 (CMW31802, CMW31803, CMW31797, CMW31798) (Fig 12). A total of 30 *Neofusicoccum* isolates from eastern and southern Africa, representing each of the phylogenetic groups identified with ITS sequences, were selected for further analysis using the BT gene region.

Analyses of the BT data for the 30 *Neofusicoccum* isolates from this study, grouped them in seven clades representing *N. parvum* (12), *N. batangarum/ N. umdonicola* (3), *N. kwambonambiense* (5), *N. eucalyptorum* (2), a clade close to *N. cordaticola* and two possible novel species, of which one clade (CMW46887, CMW46888) represented the same novel clade as identified in the ITS gene region (Fig 13). Some isolates grouped in clades different to which they occurred in the ITS analyses. Isolate CMW46965 in the BT grouped closer to CMW31796 and not with *N. parvum* as in the ITS, with these two forming a separate sub-clade in the BT. Similarly, isolate CMW31825, which in the ITS grouped with *N. parvum* grouped with *N. cordaticola* in the BT. The shared polymorphisms between this isolate and the parental species are highlighted in yellow in Table 4. Isolate CMW31797 groups together with CMW31802, CMW31803 and CMW31798 in all three gene regions, However, it differs with two SNP's grouping this isolate with *N. umdonicola* while the three other isolates group with *N. cordaticola* as highlighted in pink in Table 4. Isolates CMW31825 and CMW31797 displayed incongruency between the two gene sequences and could be explained by accepting that some of these species are hybrids. Furthermore, the two potential hybrids have gaps in parsimony informative regions in both TEF-1α and RPB2 gene regions that are not seen in other *Neofusicoccum* species. Based on the analyses of SNP's (Table 4) in ITS and BT, there are potentially four new *Neofusicoccum* species (CMW46887, CMW46888, CMW31802, CMW31803, CMW31798 and CMW31796) and two of this species could be hybrids (CMW31797 and CMW31825).

The TEF-1α gene region was problematic because some of the isolates were difficult to amplify. Of the 30 isolates selected for further analysis, only 22 could be amplified. These isolates grouped into five phylogenetic clades including *N. parvum* (11), *N. eucalyptorum* (2), *N. batangarum*, *N. cordaticola* and *N. kwambonambiense* clade (4), and two novel clades for isolates (CMW46888, CMW46887), treated as *Neofusicocum* sp. 1 and isolate CMW31796 treated as *Neofusicoccum* sp. 3 (Fig 14). The novelty of two *Neofusicoccum* species was consistent in all three individual dataset analyses.

3.3 Pathogenicity trials

Lesions were observed around the points of inoculation on *Eucalyptus grandis* (TAG 5) trees approximately three weeks after inoculation with the selected *Botryosphaeriaceae* isolates. The lesion lengths were measured four weeks after inoculation. Variations in the lesion lengths were observed between the species and between isolates of the same species, as well as between the control inoculations and some species (Fig 15). *Neofusicoccum kwambonambiense* (CMW46991, CMW46862), *N. parvum* (CMW46962, CMW46944) and *Neofusicoccum* sp. 3 (CMW31796) produced the largest lesions on average and their lesions differed significantly from those of the controls ($P<0.05$). *Neofusicoccum* sp. 1 produced the smallest lesions of all tested isolates and in some cases no lesions at all, and they did not vary significantly from those of the controls ($P>0.05$). *Lasiodiplodia* sp. 1 (CMW46854, CMW46870) also produced lesions that differed significantly from the controls and were intermediate in size for the isolates tested. Of the six *Botryosphaeriaceae* species selected for pathogenicity testing, four species representing *Lasiodiplodia* sp. 1, *N. kwambonambiense*, *N. parvum* and the *Neofusicoccum* sp. 3 were re-isolated from inoculation sites to confirm the pathogenic status for these isolates. An additional 10 trees were selected to attempt to re-isolate isolates CMW46888 and CMW46887. Despite of these attempts, this species could not be re-isolated from the inoculated trees.

4.0 Discussion

This study presents new knowledge on the host range and distribution of species of *Botryosphaeriaceae* in southern and eastern Africa. Isolates from native and introduced *Myrtaceae* in five countries (Malawi, Mozambique, South Africa, Tanzania, and Zimbabwe) were analyzed, identifying fourteen species in three genera, *Diplodia*, *Lasiodiplodia* and *Neofusicoccum*. Four of these species are apparently new to science, and three of these are potential hybrids.

The genus *Neofusicoccum* is the third largest in the *Botryosphaeriaceae* with 29 species following *Dothiorella* (30 species) and *Lasiodiplodia* (31) (Dissanayake *et al.* 2016). The majority of isolates obtained from *Myrtaceae* in this study represented species of *Neofusicoccum* and they were obtained from both native and non-native trees. *Neofusicoccum parvum* was the most abundant species identified in this study. This is not surprising given the cosmopolitan distribution and the wide host range of this species (Slippers and Wingfield 2007). In African countries, such as Ethiopia, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe, *N. parvum* is known to cause stem canker and tip die-back on *Eucalyptus* species (Smith *et al*. 1994; Gezahgne *et al*. 2004; Slippers *et al.* 2004; Roux *et al*. 2005; Chungu *et al*. 2010; Jimu *et al*. 2015; Maússe-Sitoe *et al.* 2016; Pavlic-Zupanc *et al*. 2017). It is also well-known from *S. cordatum* in South Africa (Pavlic *et al*. 2007). This study is, however, the first to report *N. parvum* on *S. cordatum* in Malawi, Mozambique, Tanzania and Zimbabwe. Furthermore, it reports novel host associations of *N. parvum* in South Africa where it was found on most tree species sampled in this study, including *Eucalyptus* species, *E. natalitia*, *P. guajava* and *S. cordatum*.

The majority of the *Neofusicoccum* isolates, other than *N. parvum*, obtained in this study grouped with *N. batangarum, N. cordaticola, N. kwambonambiense* and *N. umdonicola*. These species are part of a larger group in the *N. parvum/N. ribis* species complex (Pavlic *et al.* 2009). In this study, *N. cordaticola* and *N. umdonicola* were found occurring only on *S. cordatum* and this is the first report of these species from Tanzania and Mozambique. *Neofusicoccum kwambonambiense* was isolated from both *S. cordatum* and *E. grandis* in this study and in all the regions sampled in

Limpopo. This species was first isolated from symptomless and dying branches, leaves, and pulp of ripe fruits of *S. cordatum* in South Africa (Pavlic *et al.* 2009). Since its description, *N. kwambonambiense* has been reported on various hosts in three countries including Brazil (Lopes *et al.* 2017), South Africa (Pavlic *et al.* 2009) and Uruguay (Pérez *et al.* 2010).

Two isolates (CMW46950, CMW46952) representing a larger group of 13 were identified as *N. eucalyptorum*. Twelve of the isolates identified as *N. eucalyptorum* were obtained from *E. grandis* in the Tzaneen area and one isolate was obtained from *E. cloeziana* in Louis Trichardt. *Neofusicoccum eucalyptorum* was first isolated from stem cankers of *E. grandis* and *E. nitens* in South Africa (Smith 2001) and it was thought to be specific to *Eucalyptus* (Slippers *et.al* 2004) as it was found in abundance on this host in Australia. However, in subsequent years this species has been reported on various native *Mytraceae* trees in Uruguay (Pérez *et al.* 2010) and in South Africa (Pillay *et al.* 2013). It has not been reported on any other host outside of the *Myrtaceae*. This suggests that *N. eucalyptorum* is possibly specific to hosts in the the *Myrtaceae* in which case it would have shifted from *Eucalyptus* trees to native *Myrtaceae* trees. Further intensive sampling, and investigation of *N. eucalyptorum* is necessary to fully understand the association of this pathogen on *Myrtaceae* trees and the implications that might arise from this association.

Three novel *Neofusicoccum* species (*Neofusicoccum* sp. 1, *Neofusicoccum* sp. 2 and *Neofusicoccum* sp. 3) were identified in this study. *Neofusicoccum* sp. 1 was represented by two isolates obtained from *E. natalitia* in South Africa. This study is the first to report *Botryosphaeriaceae* species on *E. natalitia.* No morphological descriptions were, however, possible for these fungi as no fruiting structures could be induced, even after multiple attempts and several months. *Neofusicoccum* sp. 2 (four isolates) from *S. cordatum* in Tanzania, and *Neofusicoccum* sp. 3 (one isolate) from *S. cordatum* in Tanzania. This study reports novel host associations on *S. cordatum* in Tanzania.

Two *Lasiodiplodia* species, *Lasiodiplodia* sp. 1 and *L. gonubiensis*, were obtained from *Myrtaceae* in southern and eastern Africa in this study. This is the largest genus in the *Botryosphaeriaceae* family inclucing 31 species (Dissanayake *et al.* 2016). It has a cosmopolitan distribution and has been reported on more than 500 plant hosts (Punithalingam 1976; Farr and Rossman 2017). *Lasiodiplodia gonubiensis* was first described from native *S. cordatum* in the Eastern Cape Province of South Africa (Pavlic *et al*. 2004). In the current study, *L. gonubiensis* was found on native *H. natalensis* from Georges Valley and Lajuma and non-native *Eucalyptus* from Lajuma. This is the first report of this species on these two hosts.

Host range expansions, host shifts and host jumps are well studied in the *Botryosphaeriaceae* family (Pérez *et al*. 2008, 2010; Jami *et al.* 2014; Mehl *et al*. 2017). Species with a cosmopolitan distribution such as *L. theobromae* and *N.parvum* are regarded as generalists as they have undergone host range expansions and are reported on various hosts. While some species such as the ones in the *Alanphillipsia* genus are found exclusively on Aloe (Crous *et al*. 2013, 2014). In this study *Diplodia* sp., *Lasiodiplodia* sp. 1, *L. gonubiensis, N. kwambonambiense* and *N. parvum* were found occurring on both the native and the non-native *Myrtaceae,* while *N. umdonicola* was found exclusively on *S. cordatum* and *N. eucalyptorum* was found only on *Eucalyptus*. It is however not surprising that *N. eucalyptorum* was found exclusively on *Eucalyptus* as it was intitially thought to be specific on this host, but has now shifted to other *Myrtaceae* trees in other countries. *Neofusicoccum umdonicola* has only been reported from Africa and not other countries in the world on *S. cordatum* as it was also found in this study. This could mean that so far, *N. umdonicola* is host specific on *S. cordatum* and that it originates from Africa. Further studies are needed to understand the host specificity of *N. umdonicola*.

Cryptic species are defined as species that have undergone genetic divergence (Sakalidis *et al*. 2011). These species cannot be separated based on morphological or ecological characteristics and the statistical support from phylogenetic analyses such as bootstrap values are generally poor. Furthermore, all genetic loci may have not achieved monophyly. In this study, *Lasiodiplodia* sp. 1 grouped within the *L. theobromae* species complex that includes 17 species. Based on the TEF-1α, this species is identical to *L. gilanensis,* however in BT, RPB2 and in a combined analyses of the four gene regions, it represents a novel species. The low bootstrap value between *L. gilanensis* and the isolates described in this study raises a question as to whether this is a novel cryptic species or whether it represents some of the genetic variation that makes up *L. gilanensis*. However, based on the defintion of cryptic species by Sakalidis *et al*. (2011), we conclude that this species is a cryptic species.

The genus *Diplodia* comprises 26 species known from culture (Dissanayake *et al*. 2016; Slippers *et al.* 2017). The most intensively used gene regions to identify species in this genus are ITS and TEF-1α, while the Large subunit (LSU) and BT are rarely used. Only two isolates (CMW46906, CMW48899) of *Diplodia* were obtained in the current study. They were from non-native *Eucalyptus camaldulensis* and from native *Syzygium gerrardii* in the Limpopo Province. This is the first report of *Diplodia* species from these two hosts. In all analyses (ITS, TEF-1α and combined), the African isolates grouped in a larger clade containing *D. alatafructa*, *D. insularis* and *D. pseudoseriata*. *Diplodia insularis* is phylogenetically related to *D. alatafructa* and *D. insularis*, however this species can be separated from the latter two species by the size and shape of the conidia and by a combined analysis of ITS and TEF-1α (Linaldeddu *et al.* 2016). Based on the phylogenetic analyses of *Diplodia* by Dissanayake *et al*. (2016) and this study, the bootstrap value was below 70 % and did not support the separation of *D. insularis* from *D. alatafructa, D. pseudoseriata* and *Diplodia* sp. 1.

Mehl *et al*. (2011) described *D. alatafructa* based on both morphology and the phylogenetic species concept using ITS and TEF-1α and described this species as being closely related to *D. seriata*. However, subsequent studies showed that *D. alatafructa* is more closely related to *D. pseudoseriata* and *D. insularis* and not *D. seriata*. Phillips *et al.* (2012), suggested that *D. alatafructa* and *D. pseudoseriata* be regarded as synonyms as there is no clear distinction between the two species based on both morphology and DNA sequence data. These species form a complex and cannot be completely separated with ITS, TEF-1α, or in a combined analysis of the both sequence datasets. *Diplodia alatafructa, D. pseudoseriata*, *D. insularis* and *Diplodia* sp. could possibly be a single, genetically diverse, species. Additional isolates and studies are needed to clarify the taxonomy of species in this complex.

A number of isolates in this study could not be conclusively identified in any of the currently known *Botryosphaeriaceae* species. For example, isolate CMW31796 grouped with *N. ribis/ N. occulatum* in the ITS, in a novel clade together with CMW46965 in the BT and in its own, unique clade in the TEF-1α. Similarly, CMW31825 grouped with *N. umdonicola* in the ITS and TEF-1α, but with *N. cordaticola* in the BT. The irregularities within the *Botryosphaeriaceae* could be explained as intraspecific genetic variability or as hybrids (Sakalidis *et al.* 2011; Cruywagen *et al*. 2017). In a study by Sakalidis *et al*. (2011), species such as this were not described or taxonomically classified, however in other studies, such species were described as new species or hybrid species (Cryuwagen *et al*. 2017). Based on the decision tree represented by Cryuwagen *et al*. (2017) on hybridisation, we conclude that CMW31825 and CMW31797 in the *Neofusicoccum* genus are hybrids as they are incongruent in multiple genes in non-sister groups. In *Lasiodiplodia*, isolate CMW31829 is incongruent in one gene region (TEF-1α) and is grouping between two non-sister groups (*Lasiodiplodia* sp. 1, and *L. gonubiensis*). Therefore, we conclude that it also represents a possible hybrid species. To the best of our knowledge, there have not been any reports on hybridisation in the *Neofusicoccum* genus.

The taxonomy of *Botryosphaeriaceae* has been revised several times in the recent past. Yet there are many questions that remain to be addressed. In this study, the isolates of *Diplodia* could not be taxonomically resolved because there are only two complete gene regions (ITS and TEF-1α) in the NCBI database. Similar to *Diplodia*, four gene regions were used to identify species in *Neofusicoccum*, however, only three could be used because the RPB2 gene region database is incomplete. For *Lasiodiplodia*, all four gene regions were successfully used to identify species. However, all four gene regions could not provide sufficient support for the eight isolates belonging to the *L. theobromae* complex. According to Sakalidis *et al*. (2011) and Cruywagen *et al.* (2017), the most informative loci used to delineate species in the *Lasiodiplodia* genus is TEF-1α. However, this gene region was not congruent with three other gene regions used and it is usually the most problematic gene region to amplify. For the identification of new species in the *Botryosphaeriaceae*, at least four gene regions should be used in addition to ITS as a standard method of identification so that problems such as this are not encountered in the future. Additionally, hybrid species cannot be identified with only one or two loci.

Pathogenicity trials showed that all but one of the tested species was able to cause lesions on *Eucalyptus* trees. The most pathogenic species were *N. parvum* and *N. kwambonambiense* followed by *Lasiodiplodia* sp. 1. Although its sister species *L. theobromae* is pathogenic on *Eucalyptus* species, *L. gonubiensis* was found to result in lesions not significantly different from the controls by Pavlic *et al.* (2007). Isolates of species in the *Botryosphaeriaceae* clearly differ in their pathogenicity with variable results emerging from different studies. This could be due to the fact that there is more genetic variation and further cryptic species in the *Botryosphaeriaceae* than previously studied.

This study has expanded information on the host range and distribution of the *Botryosphaeriaeae* in previously unexplored areas of southern and eastern Africa. This information will assist future studies to better understand factors influencing patterns related to the diversity of these important stress associated pathogens. However, the larger part of the African continent, and many other hosts in the Myrtales, remain unexplored. The relevance and threat of these fungi to native hosts also remain unclear and needs further study.

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Table 1. Isolates representing the *Botryosphaeriaceae* species considered in a phylogenetic study and pathogenicity trial

Species	**Isolate ID**[1, 2, 3]	**Host**	**Locality**[4]	**ITS**	**BT**	**TEF-1α**	**RPB2**
*Neofusicoccum arbuti**	CBS 117090	*Arbutus menziesii*	U.S.A.	AY819724	KF531794	KF531791	N/A
N. arbuti	CBS 116131	*A. menziesii*	U.S.A.	AY819720	KF531793	KF531792	N/A
*N. australe**	CMW 6837	*Acacia* sp.	Australia	AY339262	AY339254	AY339270	N/A
N. australe	CMW 37396	*Eucalyptus grandis*	South Africa	JQ744576	AY339255	JQ744597	N/A
*N. batangarum**	CBS 124923	*Terminalia catappa*	Cameroon	FJ900608	FJ900635	FJ900654	N/A
N. batangarum	CBS 124924	*T. catappa*	Cameroon	FJ900607	FJ900634	FJ900653	N/A
*N. cordaticola**	CBS 123635	*Syzigium cordatum*	South Africa	EU821903	EU821843	EU821873	N/A
N. cordaticola	CBS 123634	*S. cordatum*	South Africa	EU821898	EU821838	EU821868	N/A
*N. cryptoaustrale**	CMW 23785	*Eucalyptus* sp.	South Africa	FJ752742	FJ752756	FJ752713	N/A
*N. eucalypticola**	CBS 115679	*E. grandis*	Australia	AY615141	AY615127	AY615133	N/A
N. eucalypticola	CBS 115766	*Eucalyptus rossii*	Australia	AY615143	AY615125	AY615135	N/A
N. eucalyptorum	CMW 46993	*Eucalyptus cloeziana*	Louis Trichardt, SA				
N. eucalyptorum	CMW 46949	*E. grandis*	Georges valley, SA				
N. eucalyptorum	**CMW 46950**	*E. grandis*	Georges valley, SA				
N. eucalyptorum	**CMW 46951**	*E. grandis*	Georges valley, SA				
N. eucalyptorum	**CMW 46952**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46953**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46954**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46955**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46956**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46957**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46958**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46959**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46960**	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46961**	*E. grandis*	Grootbos, SA				
N. parvum	***CMW 46962***	*E. grandis*	Grootbos, SA				
N. eucalyptorum	**CMW 46963**	*E. grandis*	Grootbos, SA				
*N. eucalyptorum**	CMW 10126	*E. grandis*	South Africa	AF283687	AY236921	AY236892	N/A

N. eucalyptorum	CBS 115791	*E. grandis*	South Africa	AF283686	AY236920	AY236891	N/A
N. kwambonambiense	**CMW 46885**	*Eugenia natalitia*	New Agatha, SA				
N. kwambonambiense	**CMW 46871**	*S. cordatum*	Fundudzi, SA				
N. kwambonambiense	**CMW 46872**	*S. cordatum*	Fundudzi, SA				
N. kwambonambiense	**CMW 46873**	*S. cordatum*	Lajuma, SA				
N. kwambonambiense	**CMW 46878**	*S. cordatum*	Lajuma, SA				
N. kwambonambiense	**CMW 46879**	*S. cordatum*	Lajuma, SA				
N. kwambonambiense	**CMW 46880**	*S. cordatum*	Lajuma, SA				
N. kwambonambiense	**CMW 46881**	*S. cordatum*	Lajuma, SA				
N. kwambonambiense	**CMW 46882**	*S. cordatum*	Lajuma, SA				
N. kwambonambiense	**CMW 46883**	*S. cordatum*	Lajuma, SA				
N. kwambonambiense	**CMW 46884**	*S. cordatum*	Lajuma, SA				
*N. kwambonambiense**	CBS 123641	*S. cordatum*	South Africa	EU821919	EU821859	EU821889	N/A
N. kwambonambiense	CBS 123639	*S. cordatum*	South Africa	EU821900	EU821840	EU821870	N/A
*N. luteum**	CBS 110497	*Vitis vinifera*	Portugal	EU673311	EU673092	EU673277	N/A
N. luteum	CBS 110299	*V. vinifera*	Portugal	AY259091	DQ458848	AY573217	N/A
*N. macroclavatum**	WAC 12446	*Eucalyptus globulus*	Australia	DQ093197	DQ093208	DQ093218	N/A
N. macroclavatum	CBS 118223	*E. globulus*	Australia	DQ093196	DQ093206	DQ093217	N/A
*N. mangiferae**	CBS 118532	*Mangifera indica*	Australia	AY615186	AY615173	DQ093220	N/A
N. mangiferae	CBS 118531	*M. indica*	Australia	AY615185	AY615172	DQ093221	N/A
*N. mediterraneum**	CBS 121718	*Eucalyptus* sp.	Greece	GU251176	GU251836	GU251308	N/A
N. mediterraneum	CBS 121558	*Eucalyptus* sp.	U.S.A.	GU799463	GU799461	GU799462	N/A
*N. occulatum**	CBS 128008	*E. grandis*	Australia	EU301030	EU339472	EU339509	N/A
N. occulatum	MUCC 286	*E. pellita*	Australia	EU736947	EU339474	EU339511	N/A
N. parvum	**CMW 46907**	*E. camaldulensis*	Lajuma				
N. parvum	**CMW 46977**	*E. cloeziana*	Louis Trichardt, SA				
N. parvum	**CMW 46978**	*E. cloeziana*	Louis Trichardt, SA				
N. parvum	**CMW 46979**	*E. cloeziana*	Louis Trichardt, SA				
N. parvum	**CMW 46980**	*E. cloeziana*	Louis Trichardt, SA				
N. parvum	**CMW 46981**	*E. cloeziana*	Louis Trichardt, SA				
N. parvum	**CMW 46982**	*E. cloeziana*	LouisTtrichardt, SA				
N. parvum	**CMW 46983**	*E. cloeziana*	Louis Trichardt, SA				

N. parvum	**CMW 46984**	*E. cloeziana*	Louis Trichardt, SA
N. parvum	**CMW 46985**	*E. cloeziana*	Louis Trichardt, SA
N. parvum	**CMW 46986**	*E. cloeziana*	Louis Trichardt, SA
N. parvum	**CMW 46987**	*E. cloeziana*	Louis Trichardt, SA
N. parvum	**CMW 46988**	*E. cloeziana*	Louis Trichardt, SA
N. parvum	**CMW 46989**	*E. cloeziana*	Louis Trichardt, SA
N. parvum	**CMW 46990**	*E. cloeziana*	Louis Trichardt, SA
N. kwambonambiense	***CMW 46991***	*E. cloeziana*	Louis Trichardt, SA
N. parvum	**CMW 46992**	*E. cloeziana*	Louis Trichardt, SA
N. parvum	**CMW 31839**	*E. cloeziana*	Mozambique
N. parvum	**CMW 31840**	*E. cloeziana*	Mozambique
N. parvum	**CMW 31838**	*E. cloeziana*	Mozambique
N. parvum	**CMW 46964**	*E. grandis*	Fundudzi, SA
N. parvum	**CMW 46965**	*E. grandis*	Fundudzi, SA
N. parvum	**CMW 46966**	*E. grandis*	Fundudzi, SA
N. parvum	**CMW 46967**	*E. grandis*	Fundudzi, SA
N. parvum	***CMW 46944***	*E. grandis*	Georges valley, SA
N. parvum	**CMW 46945**	*E. grandis*	Georges valley, SA
N. parvum	**CMW 46946**	*E. grandis*	Georges valley, SA
N. parvum	**CMW 46947**	*E. grandis*	Georges valley, SA
N. parvum	**CMW 46948**	*E. grandis*	Georges valley, SA
N. parvum	**CMW 46968**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46969**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46970**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46971**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46972**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46973**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46974**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46975**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46976**	*E. grandis*	Lajuma, SA
N. parvum	**CMW 46941**	*E. grandis*	New Agatha, SA
N. parvum	**CMW 46942**	*E. grandis*	New Agatha, SA

N. parvum	**CMW 31847**	*E. grandis*	Zimbabwe				
N. parvum	**CMW 31848**	*E. grandis*	Zimbabwe				
N. parvum	**CMW 31849**	*E. grandis*	Zimbabwe				
N. parvum	**CMW 31850**	*E. grandis*	Zimbabwe				
N. parvum	**CMW 31851**	*E. grandis*	Zimbabwe				
N. parvum	**CMW 46885**	*E. natalitia*	Grootbos, SA				
N. parvum	**CMW 46886**	*E. natalitia*	New Agatha, SA				
*N. parvum**	CMW 10122	*E. grandis*	South Africa	AF283681	EU673095	AY236882	N/A
N. parvum	**CMW 46925**	*Heteropyxis natalensis*	Georges valley, SA				
N. parvum	**CMW 46926**	*H. natalensis*	Georges valley, SA				
N. parvum	**CMW 46928**	*H. natalensis*	Georges valley, SA				
N. parvum	**CMW 46929**	*H. natalensis*	Georges valley, SA				
N. parvum	**CMW 46931**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46932**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46933**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46934**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46935**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46936**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46937**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46938**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46939**	*H. natalensis*	New Agatha, SA				
N. parvum	**CMW 46940**	*H. natalensis*	New Agatha, SA				
N. parvum	CMW 9081	*Populus nigra*	New Zealand	AY236943	AY236917	AY236888	N/A
N. parvum	**CMW 46890**	*Psidium guajava*	Fundudzi, SA				
N. parvum	**CMW 46864**	*S. cordatum*	Fundudzi, SA				
N. parvum	**CMW 46867**	*S. cordatum*	Fundudzi, SA				
N. parvum	**CMW 46868**	*S. cordatum*	Fundudzi, SA				
N. parvum	**CMW 46869**	*S. cordatum*	Fundudzi, SA				
N. parvum	**CMW 46838**	*S. cordatum*	Grootbos, SA				
N. parvum	**CMW 46839**	*S. cordatum*	Grootbos, SA				
N. parvum	**CMW 46840**	*S. cordatum*	Grootbos, SA				
N. parvum	**CMW 46841**	*S. cordatum*	Grootbos, SA				

N. parvum	**CMW 46842**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46843**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46844**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46845**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46846**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46847**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46848**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46849**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46850**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46851**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46852**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46853**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46856**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46857**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46858**	*S. cordatum*	Grootbos, SA
N. parvum	**CMW 46874**	*S. cordatum*	Lajuma, SA
N. parvum	**CMW 46875**	*S. cordatum*	Lajuma, SA
N. parvum	**CMW 46876**	*S. cordatum*	Lajuma, SA
N. parvum	**CMW 46877**	*S. cordatum*	Lajuma, SA
N. parvum	**CMW 31830**	*S. cordatum*	Malawi
N. parvum	**CMW 31824**	*S. cordatum*	Mozambique
N. kwambonambiense	***CMW 46862***	*S. cordatum*	New Agatha, SA
N. parvum	**CMW 31799**	*S. cordatum*	Tanzania
N. parvum	**CMW 31800**	*S. cordatum*	Tanzania
N. parvum	**CMW 31801**	*S. cordatum*	Tanzania
N. parvum	**CMW 31804**	*S. cordatum*	Tanzania
N. parvum	**CMW 31805**	*S. cordatum*	Tanzania
N. parvum	**CMW 31806**	*S. cordatum*	Tanzania
N. parvum	**CMW 31807**	*S. cordatum*	Tanzania
N. parvum	**CMW 31808**	*S. cordatum*	Tanzania
N. parvum	**CMW 31809**	*S. cordatum*	Tanzania
N. parvum	**CMW 31811**	*S. cordatum*	Tanzania

N. parvum	**CMW 31812**	*S. cordatum*	Tanzania				
N. parvum	**CMW 31813**	*S. cordatum*	Tanzania				
N. parvum	**CMW 46837**	*S. cordatum*	Grootbos				
*N. ribis**	CBS 121.26	*Ribes rubrum*	U.S.A.	AF241177	AY236908	AY236879	N/A
N. ribis	CBS 115475	*Ribes* sp.	U.S.A.	AY236935	AY236906	AY236877	N/A
N. umdonicola	**CMW 31822**	*S. cordatum*	Mozambique				
N. umdonicola	**CMW 31823**	*S. cordatum*	Mozambique				
N. umdonicola	**CMW 31826**	*S. cordatum*	Mozambique				
N. umdonicola	**CMW 31827**	*S. cordatum*	Mozambique				
*N. umdonicola**	CBS 123646	*S. cordatum*	South Africa	EU821905	EU821845	EU821875	N/A
N. umdonicola	CBS 123645	*S. cordatum*	South Africa	EU821904	EU821844	EU821874	N/A
***Neofusicoccum* hybrid**	**CMW 31825**	*S. cordatum*	Mozambique				
***Neofusicoccum* hybrid**	**CMW 31797**	*S. cordatum*	Tanzania				
***Neofusicoccum* sp. 1**	***CMW 46888***	*E. natalitia*	Grootbos, SA				
***Neofusicoccum* sp. 1**	***CMW 46887***	*E. natalitia*	Louis Trichardt, SA				
***Neofusicoccum* sp. 2**	**CMW 31802**	*S. cordatum*	Tanzania				
***Neofusicoccum* sp. 2**	**CMW 31803**	*S. cordatum*	Tanzania				
***Neofusicoccum* sp. 2**	**CMW 31810**	*S. cordatum*	Tanzania				
***Neofusicoccum* sp. 3**	***CMW 31796***	*S. cordatum*	Tanzania				
***Neofusicoccum* sp. 2**	**CMW 31798**	*S. cordatum*	Tanzania				
*L. citricola**	CBS 124707	*Citrus* sp.	Iran	GU945354	KU887505	GU945340	KU696351
L. citricola	CBS 124706	*Citrus* sp.	Iran	GU945353	KU887504	GU945339	KU696350
*L. euphorbicola**	CMW 33350	*Adansonia digitata*	Botswana	KU887149	KU887455	KU887026	KU696346
L. euphorbicola	CMM 3609	*Jatropha curcas*	Brazil	KF234543	KF254926	KF226689	N/A
*L. exigua**	CERC 1961	*Pistacia vera*	U.S.A	KP217059	KP217075	KP217067	N/A
L. exigua	CBS 137785	*Retama raetam*	Tunisia	KJ638317	KU887509	KJ638336	KU696355
Lasiodiplodia sp.	**CMW 46865**	*S. cordatum*	Fundudzi, SA				
Lasiodiplodia sp.	**CMW 46866**	*S. cordatum*	Fundudzi, SA				
Lasiodiplodia sp.	**CMW 46863**	*S. cordatum*	Georges valley, SA				
*L. gilanensis**	CBS 124704	Uknown	Gilan, Iran	GU945351	KP872411	GU945342	N/A
L. gilanensis	CBS 124705	Uknown	Gilan, Iran	GU945352	KP872412	GU945341	N/A
L. gonubiensis	**CMW 46908**	*E. camaldulensis*	Lajuma, SA				

L. gonubiensis	**CMW 46909**	*E. camaldulensis*	Lajuma, SA				
L. gonubiensis	**CMW 46910**	*E. camaldulensis*	Lajuma, SA				
L. gonubiensis	**CMW 46901**	*E. camaldulensis*	Louis Trichardt, SA				
L. gonubiensis	**CMW 46902**	*E. camaldulensis*	Louis Trichardt, SA				
L. gonubiensis	**CMW 46903**	*E. camaldulensis*	Louis Trichardt, SA				
L. gonubiensis	**CMW 46904**	*E. camaldulensis*	Louis Trichardt, SA				
L. gonubiensis	**CMW 46905**	*E. camaldulensis*	Louis Trichardt, SA				
L. gonubiensis	**CMW 46900**	*E. camaldulensis*	Louis Trichardt, SA				
L. gonubiensis	**CMW 46916**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46917**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46918**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46919**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46920**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46921**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46922**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46923**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46924**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46927**	*H. natalensis*	Georges valley, SA				
L. gonubiensis	**CMW 46930**	*H. natalensis*	Georges valley, SA				
***Lasiodiplodia* hybrid**	**CMW 46912**	*H. natalensis*	Lajuma, SA				
L. gonubiensis	**CMW 46913**	*H. natalensis*	Lajuma, SA				
L. gonubiensis	**CMW 46914**	*H. natalensis*	Lajuma, SA				
L. gonubiensis	**CMW 46915**	*H. natalensis*	Lajuma, SA				
L. gonubiensis	**CMW 46911**	*H. natalensis*	Lajuma, SA				
*L. gonubiensis**	CBS 115812	*S. cordatum*	South Africa	AY639595	DQ458860	DQ103566	KU696359
L. gonubiensis	CBS 116355	*S. cordatum*	South Africa	AY639594	EU673126	DQ103567	N/A
*L. hormozganensis**	CBS 124708	*M. indica*	Iran	GU945356	KU887514	GU945344	KU69360
L. hormozganensis	CBS 124709	*Olea* sp.	Iran	GU945355	KU887515	GU945343	KU696361
*L. iraniensis**	CBS 124711	*Juglans* sp.	Iran	GU945347	KU887517	GU945335	KU696362
L. iraniensis	CBS 124710	*Salvadora persica*	Iran	GU945346	KU887516	GU945334	KU696363
*L. lignicola**	CBS 134112	Dead wood	Thailand	JX646797	JX646845	KU887003	KU696364
*L. macrospora**	CMM 3833	*J. curcas*	Brazil	KF234557	KF254941	KF226718	N/A

*L. mahajangana**	CBS 124927	*Terminalia carpa*	Madagascar	FJ900597	KU887518	FJ900643	KU696365
L. mahajangana	CBS 124926	*T. carpa*	Madagascar	FJ900596	KU887519	FJ900642	KU696366
*L. margaritacea**	CBS 122519	*Adansonia gregorii*	Australia	EU144050	KU887520	EU144065	KU696367
*L. mediterranea**	CBS 137783	*Quercus ilex*	Italy	KJ638312	KU887521	KJ638331	KU696368
L. mediterranea	CBS 137784	*V. vinifera*	Italy	KJ638311	KU887522	KJ638330	KU696369
*L. missouriana**	CBS 128312	*V. vinifera*	U.S.A.	HQ288226	HQ288305	HQ288268	KU696371
L. missouriana	CBS 128311	*V. vinifera*	U.S.A.	HQ288225	HQ288304	HQ288267	KU696370
*L. parva**	CBS 456.78	Cassava field soil	Colombia	EF622083	KU887523	EF622063	KU696372
L. parva	CBS 494.78	Cassava field soil	Colombia	EF622084	EU673114	EF622064	KU696373
*L. plurivora**	CBS 120832	*Prunus salicina*	South Africa	EF445362	KU887524	EF445395	KU696374
L. plurivora	CBS 121103	*V. vinifera*	South Africa	AY343482	KU887525	EF445396	KU696375
*L. pseudotheobromae**	CBS 116459	*Gmelina arborea*	Costa Rica	EF622077	EU673111	EF622057	KU696376
L. pseudotheobromae	CMW 9074	*Pinus* sp.	Mexico	AY236952	KU8867526	AY236901	KU696377
*L. pyriformis**	CBS 121771	*Acacia mellifera*	Namibia	EU101308	KU887528	EU101353	KU696379
L. pyriformis	CBS 121770	*A. mellifera*	Namibia	EU101307	KU887527	EU101352	KU696378
*L. rubropurpurea**	CBS 118740	*E. grandis*	Australia	DQ103553	EU673136	EU673304	KU696380
L. rubropurpurea	CMW 15207	*E. grandis*	Australia	DQ103554	KU887530	DQ103572	KU696381
*L. subglobosa**	CMM 3872	*J. curcas*	Brazil	KF234558	KF254942	KF226721	N/A
L. subglobosa	CMM 4046	*J. curcas*	Brazil	KF234560	KF254944	KF226723	N/A
*L. theobromae**	CBS 164.96	Fruit on coral reef coast	New Guinea	AY640255	KU887532	AY640258	KU696383
L. theobromae	CBS 124.13	Unknown	Unknown	F622074	KU887531	EF622054	KU696382
*L. venezuelensis**	CBS 118739	*A. mangium*	Venezuela	DQ103547	KU887533	EU673305	KU696384
L. venezuelensis	CMW 13512	*A. mangium*	Venezuela	DQ103548	KU887534	DQ103569	N/A
*Lasiodplodia viticola**	CBS 128314	*V. vinifera*	U.S.A.	HQ288228	HQ288307	HQ288270	KU696386
L. viticola	CBS 128313	*V. vinifera*	U.S.A.	HQ288227	HQ288306	HQ288269	KU696385
***Lasiodiplodia* hybrid**	**CMW 31829**	*S. cordatum*	Malawi				
Lasiodiplodia sp. 1	***CMW 46870***	*S. cordatum*	Fundudzi, SA				
Lasiodiplodia sp. 1	**CMW 46943**	*E. grandis*	New Agatha, SA				
Lasiodiplodia sp. 1	**CMW 46891**	*P. guajava*	Fundudzi, SA				
Lasiodiplodia sp. 1	**CMW 46894**	*P. guajava*	Georges valley, SA				
Lasiodiplodia sp. 1	**CMW 46895**	*P. guajava*	Georges valley, SA				
Lasiodiplodia sp. 1	**CMW 46892**	*P. guajava*	Lajuma, SA				

Lasiodiplodia sp. 1	**CMW 46893**	*P. guajava*	Lajuma, SA				
Lasiodiplodia sp. 1	***CMW 46854***	*S. cordatum*	Grootbos, SA				
Lasiodiplodia sp. 1	**CMW 46855**	*S. cordatum*	Grootbos, SA				
Lasiodiplodia sp. 1	**CMW 46859**	*S. cordatum*	Grootbos, SA				
Lasiodiplodia sp. 1	**CMW 46860**	*S. cordatum*	Grootbos, SA				
Lasiodiplodia sp. 1	**CMW 46861**	*S. cordatum*	New Agatha, SA				
Lasiodiplodia sp. 1	**CMW 46896**	*S. gerrardii*	Grootbos, SA				
Lasiodiplodia sp. 1	**CMW 46897**	*S. gerrardii*	Grootbos, SA				
Lasiodiplodia sp. 1	**CMW 46898**	*S. gerrardii*	Grootbos, SA				
Diplodia sp.	**CMW 46899**	*S. gerrardii*	Grootbos, SA				
Diplodia sp.	**CMW 46906**	*E. camaldulensis*	Louis Trichardt, SA				
*D. africana**	CBS 121104	*Prunus persica*	South Africa	EF44534	N/A	EF445383	N/A
D. africana	CBS 120835	*P. persica*	South Africa	EF445343	N/A	EF445382	N/A
*D. agrifolia**	UCROK 1429	*Quercus agrifolia*	U.S.A.	JQ411412	N/A	JQ512121	N/A
D. agrifolia	CBS 132777	*Q. agrifolia*	U.S.A.	JN693507	N/A	JQ517317	N/A
*D. alatafructa**	CBS 124931	*Pterocarpus angolensis*	South Africa	FJ888460	N/A	FJ888444	N/A
D. alatafructa	CBS 124933	*P. angolensis*	South Africa	FJ888478	N/A	FJ888446	N/A
*D. allocellula**	CBS 130410	*Acacia karroo*	South Africa	JQ239399	N/A	JQ239386	N/A
D. allocellula	CBS 130408	*A. karroo*	South Africa	JQ239397	N/A	JQ239384	N/A
*D. bulgarica**	CBS 124135	*Malus sylvestris*	Bulgaria	GQ923852	N/A	GQ923820	N/A
D. bulgarica	CBS 124254	*M. sylvestris*	Bulgaria	GQ923853	N/A	GQ923821	N/A
*D. corticola**	CBS 112549	*Quercus suber*	Portugal	AY259100	N/A	AY573227	N/A
D. corticola	CBS 112546	*Q. suber*	Portugal	AY259110	N/A	DQ458872	N/A
*D. crataegicola**	MFLUCC 15-1311	*Crataegus* sp.	Italy	KT290244	N/A	KT290248	N/A
*D. cupressi**	CBS 168.87	*Cupressus sempervirens*	Israel	DQ458893	N/A	DQ458878	N/A
D. cupressi	CBS 261.85	*C. sempervirens*	Israel	DQ458894	N/A	DQ458879	N/A
*D. fraxinii**	CBS 136013	*Fraxinus angustifolia*	Italy	KF307710	N/A	KF318757	N/A
D. fraxinii	CBS 136010	*F. angustifolia*	Portugal	KF307700	N/A	KF318747	N/A
*D. galiicola**	MFLUCC 15-1310	*Galium* sp.	Italy	KT290245	N/A	KT290249	N/A
*D. insularis**	BL99	*Pistacia lentiscus*	Italy	KX833074	N/A	KX833075	N/A
D. insularis	CBS 140350	*P. lentiscus*	Italy	KX833072	N/A	KX833073	N/A
*D. intermedia**	CBS 124462	*M. sylvestris*	Portugal	GQ923858	N/A	GQ923826	N/A

D. intermedia	CBS 124134	*M. sylvestris*	Portugal	AY259096	N/A	GQ923851	N/A
*D. neonjuniperi**	CPC 22754	*Juniperus chinensis*	Thailand	KM006432	N/A	KM006463	N/A
D. neonjuniperi	CPC 22753	*J. chinensis*	Thailand	KM006431	N/A	KM006462	N/A
*D. pseudoseriata**	CBS 124906	*B. salicifolius*	Uruguay	EU080927	N/A	EU863181	N/A
D. pseudoseriata	CBS 124907	*B. salicifolius*	Uruguay	EU080922	N/A	EU863179	N/A
*D. quercivora**	CBS 133853	*Quercus canariensis*	Tunisia	JX894206	N/A	JX894230	N/A
D. quercivora	CBS 133852	*Q. canariensis*	Tunisia	JX894205	N/A	JX894229	N/A
*D. rosulata**	CBS 116472	*Prunus africana*	Ethiopia	EU430266	N/A	EU430268	N/A
D. rosulata	CBS 116470	*P. africana*	Ethiopia	EU430265	N/A	EU430267	N/A
*D. sapinea**	CBS 109725	*Pinus patula*	Indonesia	DQ458896	N/A	DQ458881	N/A
D. sapinea	CBS 393.84	*P. nigra*	Netherlands	DQ458895	N/A	DQ458880	N/A
*D. scrobiculata**	CBS 118110	*P. banksiana*	U.S.A	KF766160	N/A	KF766399	N/A
D. scrobiculata	CBS 109944	*P. greggii*	Mexico	DQ458899	N/A	DQ458884	N/A
*D. seriata**	CBS 119049	*V. vinifera*	Italy	DQ458889	N/A	DQ458874	N/A
D. seriata	CBS 112555	*V. vinifera*	Portugal	AY259094	N/A	AY573220	N/A
*D. tsugae**	CBS 418.64	*Tsuga heterophylla*	Canada	DQ458888	N/A	DQ458873	N/A

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[1] Culture collections: CERC: Culture collection of China Eucalypt Research Centre, Chinese Academy of Forestry, ZhanJiang, GuangDong, China; CMM: Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes”, Universidade Federal Rural de Pernambuco, Recife, Brazil; CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; BL: Personal number of B.T. Linaldeddu; CPC: Working collection of P.W. Crous, housed at CBS; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand; WAC: Department of Agriculture Western Australia Plant Pathogen Collection, South Perth, Western Australia; UCROK: Culture collection, University of Riverside, California, U.S.A.

[2] ID numbers for isolates sequenced in this study are given in bold. Species names in bold are novel species and hybrid species identified in this study.

[3] Isolates used in the pathogenicity tests are given in italics.

[4] SA: South Africa

*Ex-type strain.

Table 2. Primers used to amplify the four gene regions

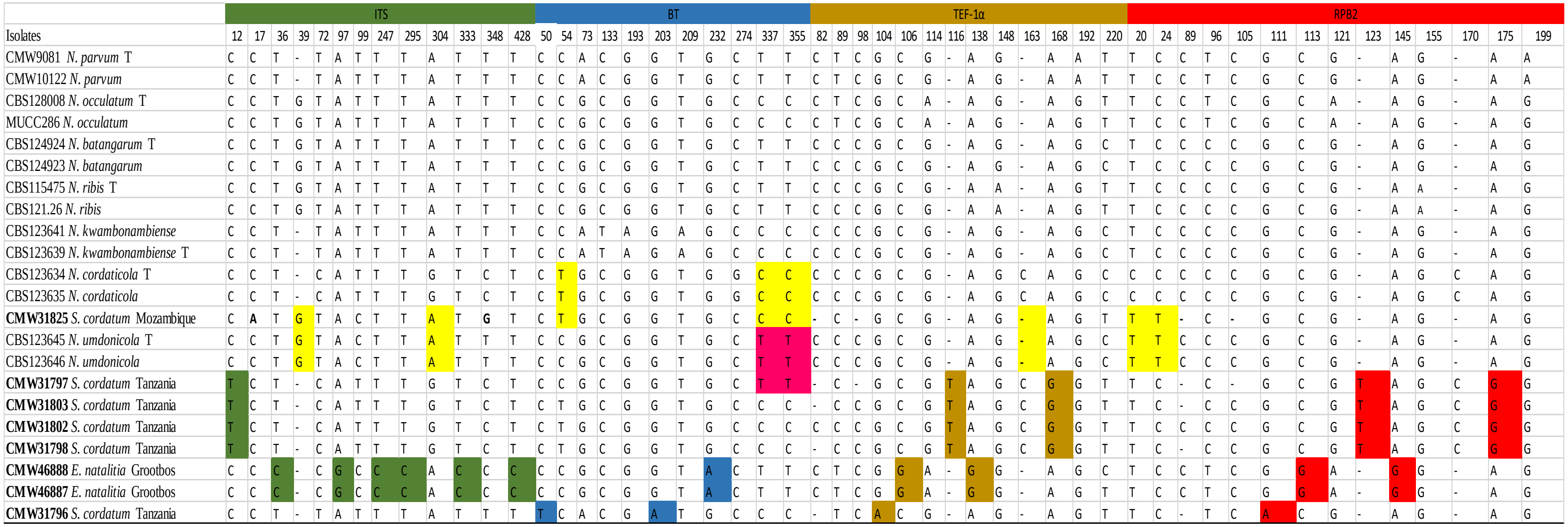
Region	Oligos	Oligo Sequences	AT (°C)[1]	References
ITS	ITS1	5' TCCGTAGGTGAACCTGCGG	55	White *et al*. 1990
	ITS4	5' TCCTCCGCTTATTGATATGC		
BT	BT2a	5' GGTAACCAAATCGGTGCTGCTTTC	55	Glass and Donaldson 1995
	BT2b	5' ACCCTCAGTGTAGTGACCCTTGGC		
TEF1-α	EF1-728F	5' CATCGAGAAGTTCGAGAAGG	55	Carbone *et al*. 1999
	EF1-986R	5' TACTTGAAGGAACCCTTACC		
RPB2	LAS F	5' GGTAGCGACGTCACTCCT	57	Crywagen *et al.* 2017
	LAS R	5' GCGCAAATACCCAGAATCAT		

[1] Annealing Temperature (AT) (°C)

Table 3. Data for phylogenetic analyses of individual trees

Dataset	**Total characters**	**Constant characters**	**Parsimony uninformative**	**Parsimony informative**	**Tree length**	**Consistency index**	**Retention index**	**Homoplasy index**
ITS *Diplodia*	521	415	7	99	170	0,753	0,893	0,247
TEF-1α *Diplodia*	471	155	8	108	191	0,754	0,905	0,246
ITS, TEF-1 α *Diplodia*	652	221	11	156	271	0.644	0.928	0.320
ITS *Neofusicoccum*	428	361	16	51	82	0,72	0,923	0,28
BT *Neofusicoccum*	383	277	43	63	91	0,813	0,944	0,187
TEF-1α *Neofusicoccum*	256	158	7	91	149	0.765	0.919	0.235
ITS *Lasiodiplodia*	433	387	2	44	60	0.850	0.946	0.150
BT *Lasiodiplodia*	378	318	3	57	71	0,873	0,97	0,127
ITS, BT *Lasiodiplodia*	903	461	46	857	441	0.689	0.926	0.341
TEF-1α *Lasiodiplodia*	211	115	1	95	146	0,842	0,916	0,158
RPB2 *Lasiodiplodia*	530	379	54	97	173	0,653	0,872	0,347
ITS, BT, TEF-1α, RPB2 *Lasiodiplodia*	645	445	21	389	210	0.712	0.974	0.312

Table 4: Single nucleotide polymorphisms (SNPs) shared between the closely related species in *Neofusicoccum* and novel species obtained in this study



	ITS													BT											TEF-1α													RPB2													
Isolates	12	17	36	39	72	97	99	247	295	304	333	348	428	50	54	73	133	193	203	209	232	274	337	355	82	89	98	104	106	114	116	138	148	163	168	192	220	20	24	89	96	105	111	113	121	123	145	155	170	175	199
CMW9081 *N. parvum* T	C	C	T	-	T	A	T	T	T	A	T	T	T	C	C	A	C	G	G	T	G	C	T	T	C	T	C	G	C	G	-	A	G	-	A	A	T	T	C	C	T	C	G	C	G	-	A	G	-	A	A
CMW10122 *N. parvum*	C	C	T	-	T	A	T	T	T	A	T	T	T	C	C	A	C	G	G	T	G	C	T	T	C	T	C	G	C	G	-	A	G	-	A	A	T	T	C	C	T	C	G	C	G	-	A	G	-	A	A
CBS128008 *N. occulatum* T	C	C	T	G	T	A	T	T	T	A	T	T	T	C	C	G	C	G	G	T	G	C	C	C	C	T	C	G	C	A	-	A	G	-	A	G	T	T	C	C	T	C	G	C	A	-	A	G	-	A	G
MUCC286 *N. occulatum*	C	C	T	G	T	A	T	T	T	A	T	T	T	C	C	G	C	G	G	T	G	C	C	C	C	T	C	G	C	A	-	A	G	-	A	G	T	T	C	C	T	C	G	C	A	-	A	G	-	A	G
CBS124924 *N. batangarum* T	C	C	T	G	T	A	T	T	T	A	T	T	T	C	C	G	C	G	G	T	G	C	T	T	C	C	C	G	C	G	-	A	G	-	A	G	C	T	C	C	C	C	G	C	G	-	A	G	-	A	G
CBS124923 *N. batangarum*	C	C	T	G	T	A	T	T	T	A	T	T	T	C	C	G	C	G	G	T	G	C	T	T	C	C	C	G	C	G	-	A	G	-	A	G	C	T	C	C	C	C	G	C	G	-	A	G	-	A	G
CBS115475 *N. ribis* T	C	C	T	G	T	A	T	T	T	A	T	T	T	C	C	G	C	G	G	T	G	C	T	T	C	C	C	G	C	G	-	A	A	-	A	G	T	T	C	C	C	C	G	C	G	-	A	A	-	A	G
CBS121.26 *N. ribis*	C	C	T	G	T	A	T	T	T	A	T	T	T	C	C	G	C	G	G	T	G	C	T	T	C	C	C	G	C	G	-	A	A	-	A	G	T	T	C	C	C	C	G	C	G	-	A	A	-	A	G
CBS123641 *N. kwambonambiense*	C	C	T	-	T	A	T	T	T	A	T	T	T	C	C	A	T	A	G	A	G	C	C	C	C	C	C	G	C	G	-	A	G	-	A	G	C	T	C	C	C	C	G	C	G	-	A	G	-	A	G
CBS123639 *N. kwambonambiense* T	C	C	T	-	T	A	T	T	T	A	T	T	T	C	C	A	T	A	G	A	G	C	C	C	C	C	C	G	C	G	-	A	G	-	A	G	C	T	C	C	C	C	G	C	G	-	A	G	-	A	G
CBS123634 *N. cordaticola* T	C	C	T	-	C	A	T	T	T	G	T	C	T	C	T	G	C	G	G	T	G	G	C	C	C	C	C	G	C	G	-	A	G	C	A	G	C	C	C	C	C	C	G	C	G	-	A	G	C	A	G
CBS123635 *N. cordaticola*	C	C	T	-	C	A	T	T	T	G	T	C	T	C	T	G	C	G	G	T	G	G	C	C	C	C	C	G	C	G	-	A	G	C	A	G	C	C	C	C	C	C	G	C	G	-	A	G	C	A	G
CMW31825 *S. cordatum* Mozambique	C	**A**	T	G	T	A	C	T	T	A	T	**G**	T	C	T	G	C	G	G	T	G	C	C	C	-	C	-	G	C	G	-	A	G	-	A	G	T	T	T	-	C	-	G	C	G	-	A	G	-	A	G
CBS123645 *N. umdonicola* T	C	C	T	G	T	A	C	T	T	A	T	T	T	C	C	G	C	G	G	T	G	C	T	T	C	C	C	G	C	G	-	A	G	-	A	G	C	T	T	C	C	C	G	C	G	-	A	G	-	A	G
CBS123646 *N. umdonicola*	C	C	T	G	T	A	C	T	T	A	T	T	T	C	C	G	C	G	G	T	G	C	T	T	C	C	C	G	C	G	-	A	G	-	A	G	C	T	T	C	C	C	G	C	G	-	A	G	-	A	G
CMW31797 *S. cordatum* Tanzania	T	C	T	-	C	A	T	T	T	G	T	C	T	C	C	G	C	G	G	T	G	C	T	T	-	C	-	G	C	G	T	A	G	C	G	G	T	T	C	-	C	-	G	C	G	T	A	G	C	G	G
CMW31803 *S. cordatum* Tanzania	T	C	T	-	C	A	T	T	T	G	T	C	T	C	T	G	C	G	G	T	G	C	C	C	-	C	C	G	C	G	T	A	G	C	G	G	T	T	C	-	C	C	G	C	G	T	A	G	C	G	G
CMW31802 *S. cordatum* Tanzania	T	C	T	-	C	A	T	T	T	G	T	C	T	C	T	G	C	G	G	T	G	C	C	C	C	C	C	G	C	G	T	A	G	C	G	G	T	T	C	C	C	C	G	C	G	T	A	G	C	G	G
CMW31798 *S. cordatum* Tanzania	T	C	T	-	C	A	T	T	T	G	T	C	T	C	T	G	C	G	G	T	G	C	C	C	-	C	C	G	C	G	T	A	G	C	G	G	T	T	C	-	C	C	G	C	G	T	A	G	C	G	G
CMW46888 *E. natalitia* Grootbos	C	C	C	-	C	G	C	C	C	A	C	C	C	C	C	G	C	G	G	T	A	C	T	T	C	T	C	G	G	A	-	G	G	-	A	G	C	T	C	C	T	C	G	G	A	-	G	G	-	A	G
CMW46887 *E. natalitia* Grootbos	C	C	C	-	C	G	C	C	C	A	C	C	C	C	C	G	C	G	G	T	A	C	T	T	C	T	C	G	G	A	-	G	G	-	A	G	T	T	C	C	T	C	G	G	A	-	G	G	-	A	G
CMW31796 *S. cordatum* Tanzania	C	C	T	-	T	A	T	T	T	A	T	T	T	T	C	A	C	G	A	T	G	C	C	C	-	T	C	A	C	G	-	A	G	-	A	G	T	T	C	-	T	C	A	C	G	-	A	G	-	A	G

*Yellow shows the shared polymorphisms between the hybrid species (CMW31825) and the two parental species, *Neofusicoccum umdonicola* and *N. cordaticola*.

*Pink colour shows the shared SNPs between the potential hybrid CMW31797 and *N. umdonicola*, therefore separating it from the three isolates CMW31798, CMW31802 and CMW31803 grouping with *N. cordaticola* in the BT gene region.

Figure 1. Map of Africa, indicating areas sampled in eastern and southern Africa.

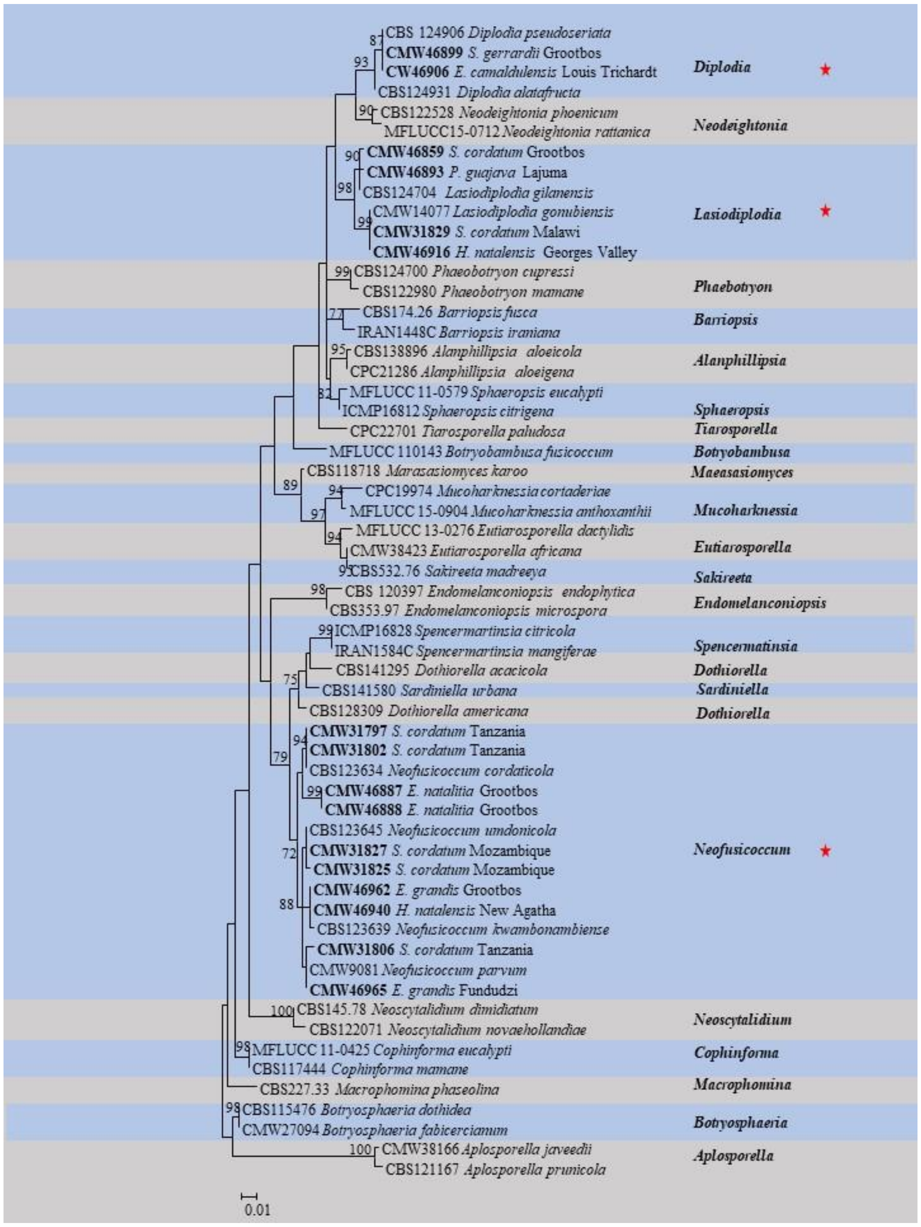


Figure 2. Maximum Likelihood (ML) tree of the ITS1 and 4, and 5.8S rRNA regions showing the placement of *Botryosphaeriaceae* isolates collected from five countries in southern and eastern Africa. Bootstrap values above 70% are represented at the tree nodes. Isolates collected in this study appear in bold and genera in which they group are marked by a star. The tree was rooted to outgroup taxa *Aplosporella javeedii* (CMW31866) and *Aplosporella prunicola* (CBS121167).

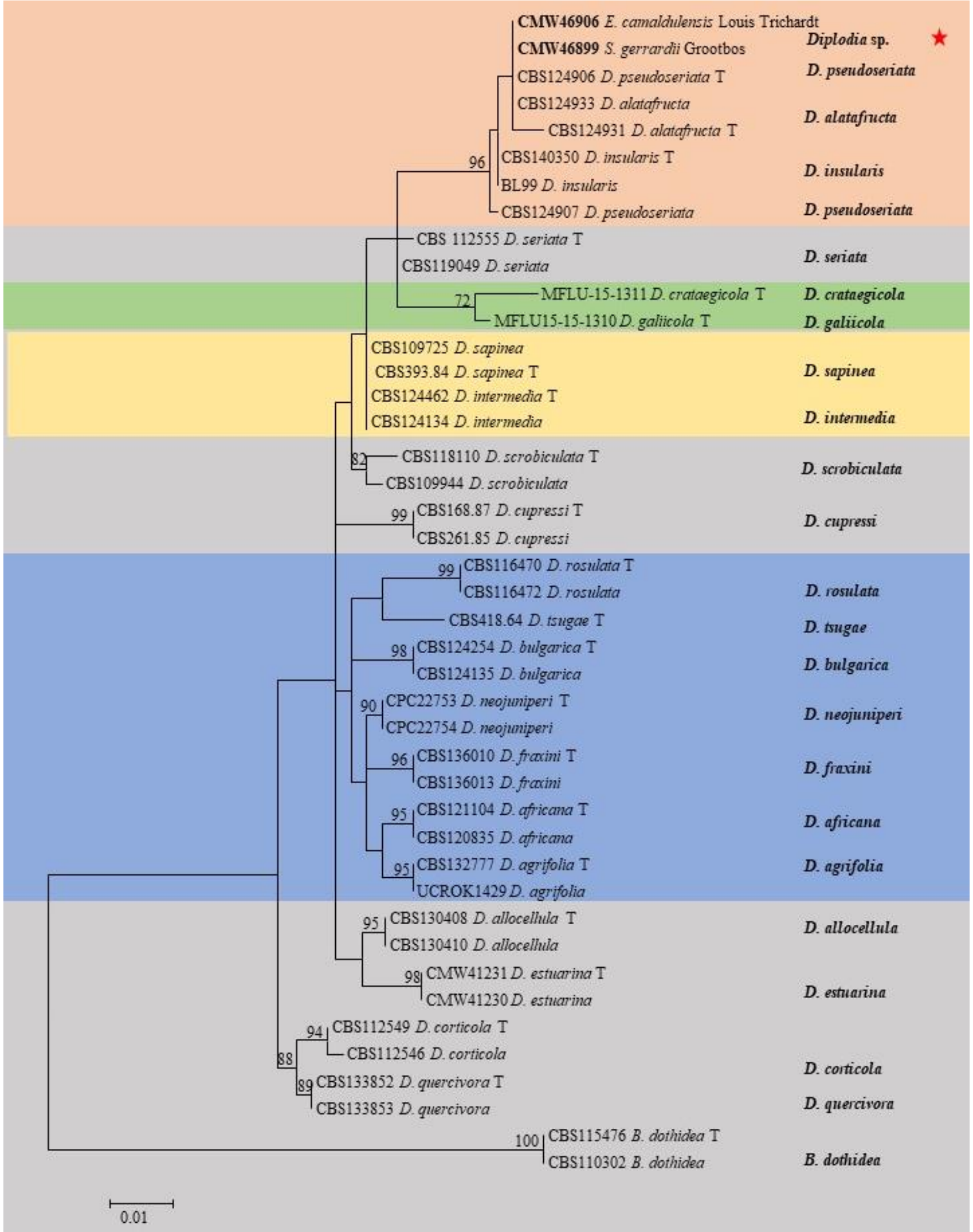


Figure 3. Maximum Likelihood (ML) tree of the genus *Diplodia* produced with ITS sequence data, showing the phylogenetic placement of two *Diplodia* isolates collected in this study. Bootstrap values above 70% are given at the nodes. Isolates sequenced in this study appear in bold and the red stars indicate their taxonomic positions. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

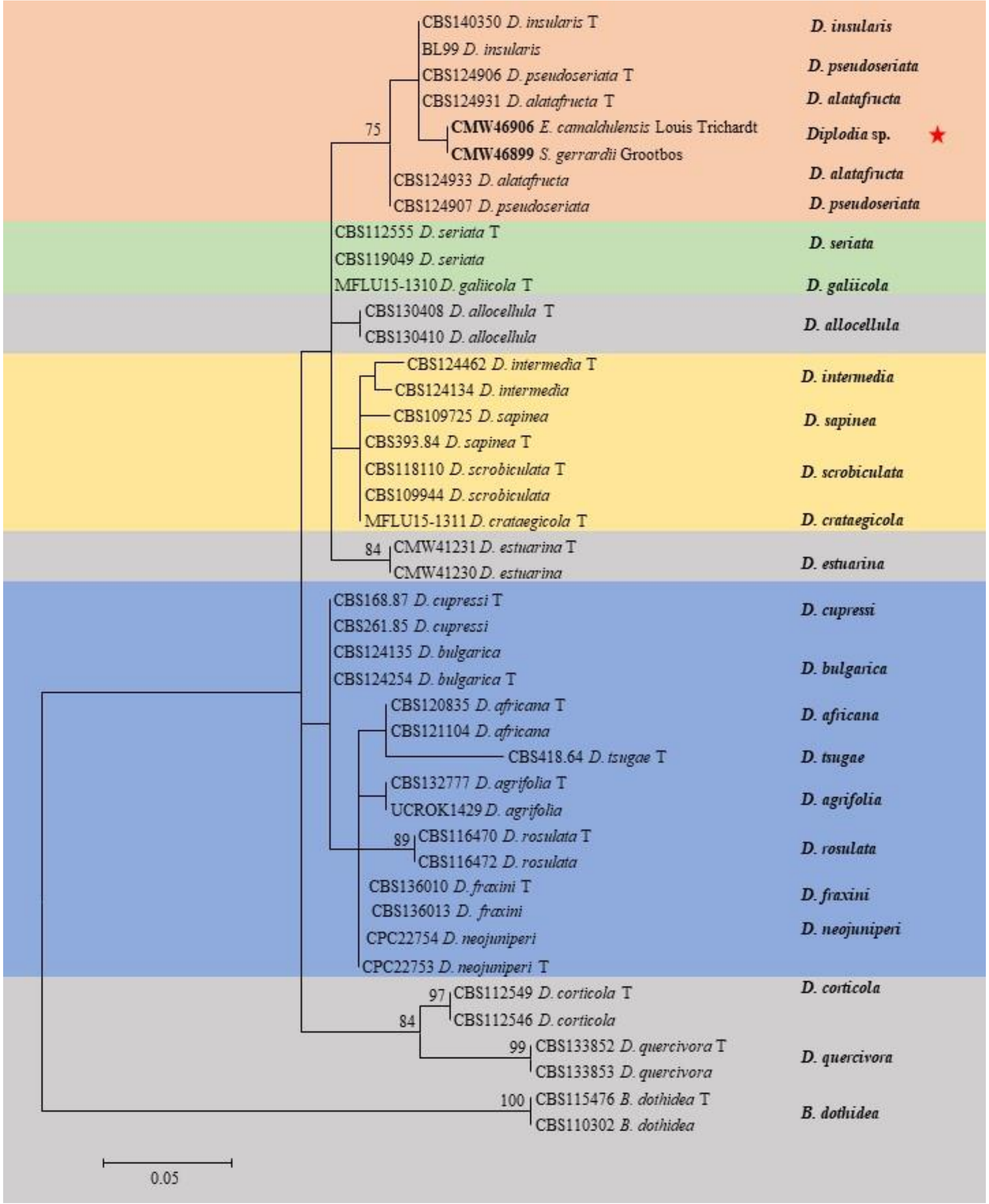


Figure 4. Maximum Likelihood (ML) tree of the genus *Diplodia* produced with TEF-1α sequences, showing the phylogenetic placement of two isolates. Bootstrap values above 70% are given at the nodes. The red stars represent their phylogenetic placement. Isolates sequenced in this study appear in bold. The tree was rooted to the outgroup taxon *Botryosphaeria dothidea* (CBS115476 and CBS110302).

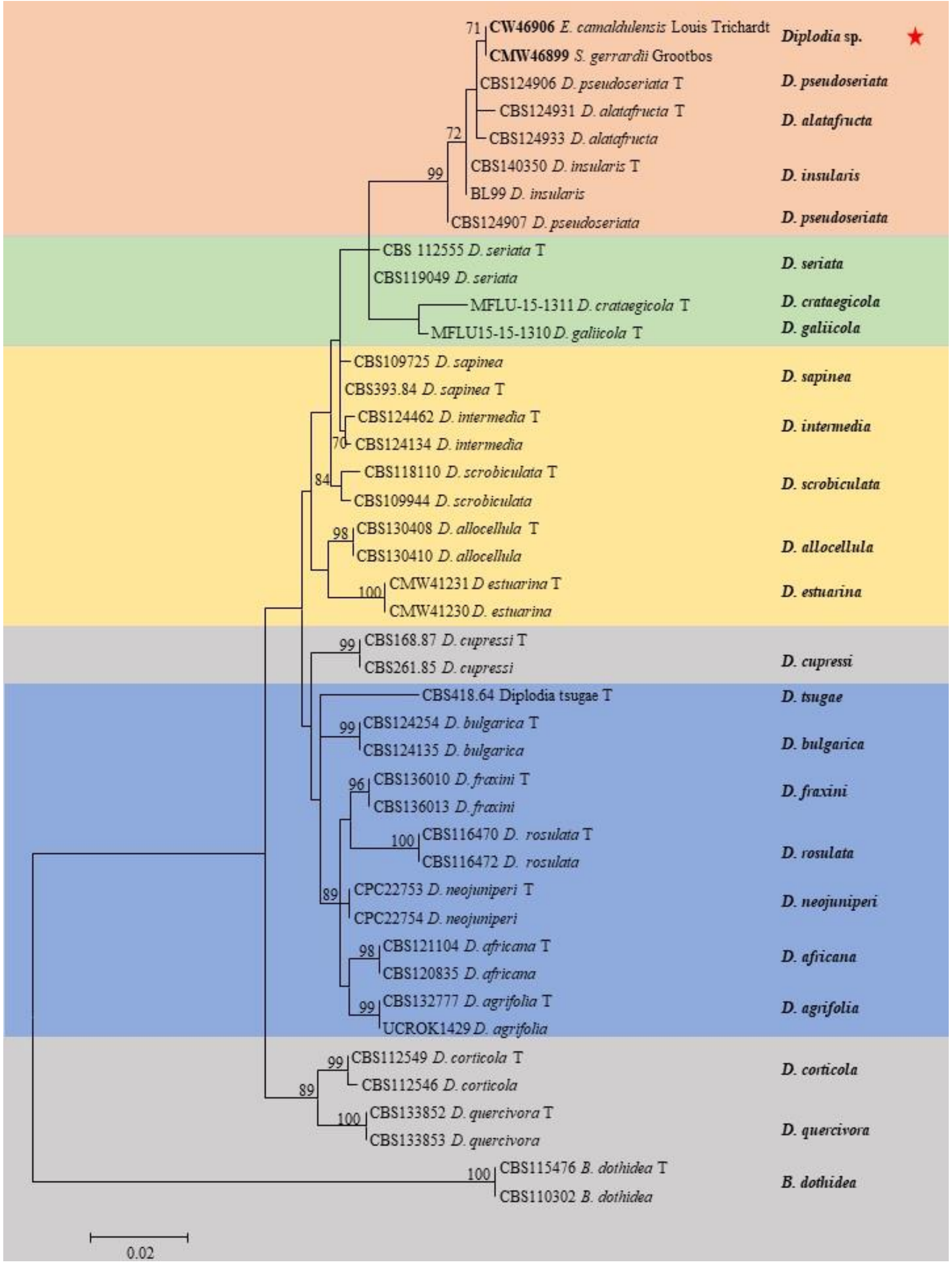


Figure 5. Maximum Likelihood (ML) tree from the combined sequence datasets of the ITS rDNA and TEF-1α loci for *Diplodia* species. Sequences in bold represent isolates obtained in this study, and red stars indicate their taxonomic positions. Bootstrap values above 70% are given at the nodes. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

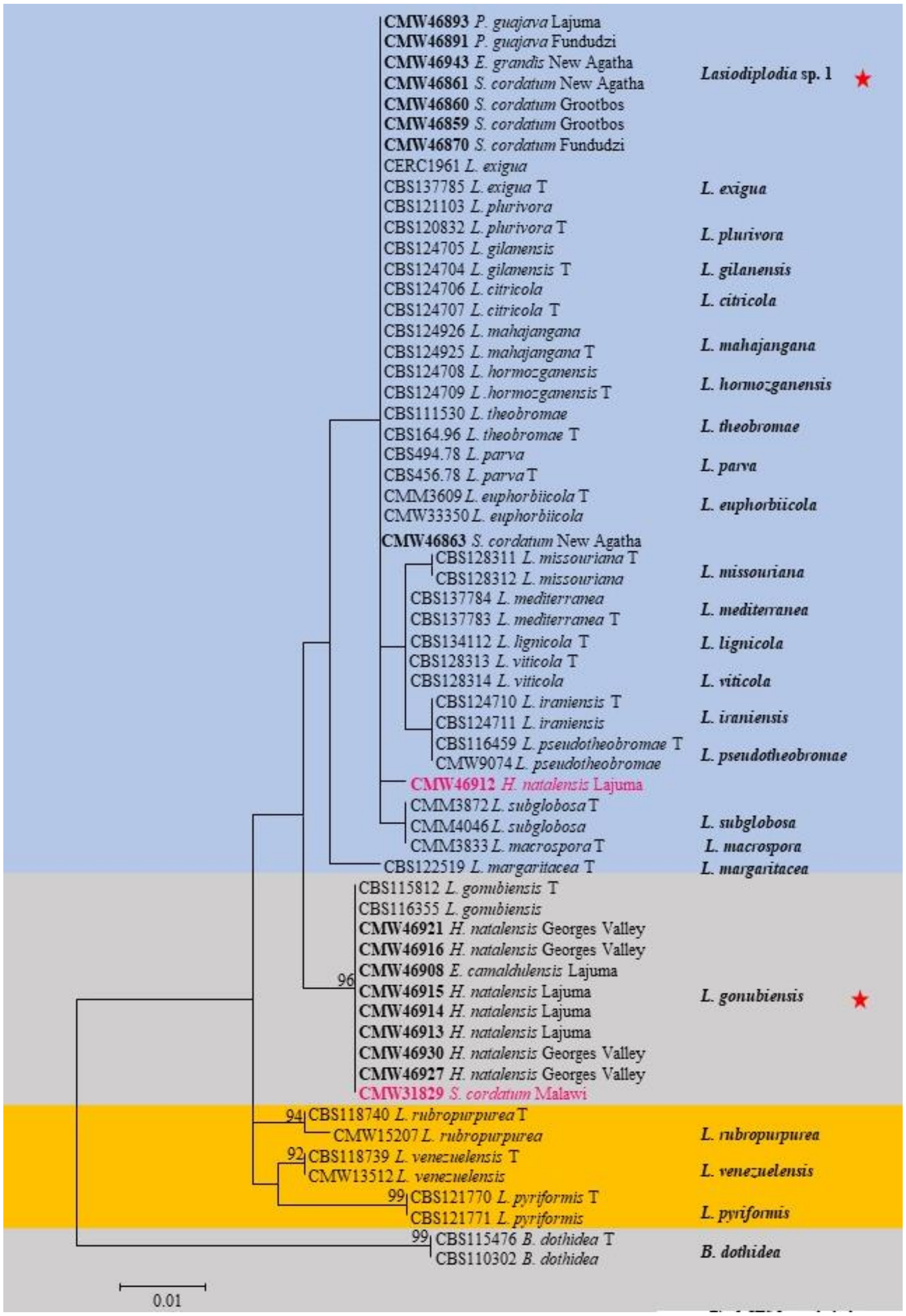


Figure 6. Maximum Likelihood (ML) tree of the genus *Lasiodiplodia* obtained from ITS sequence data, showing the phylogenetic placement of isolates obtained from eastern and southern Africa. Bootstrap values above 70% are given above the nodes. Isolates sequenced in this study appear in bold and their taxonomic positions are indicated with red stars. Potential hybrids are in a pink colour text. Bootstrap values above 70% are shown above the nodes. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

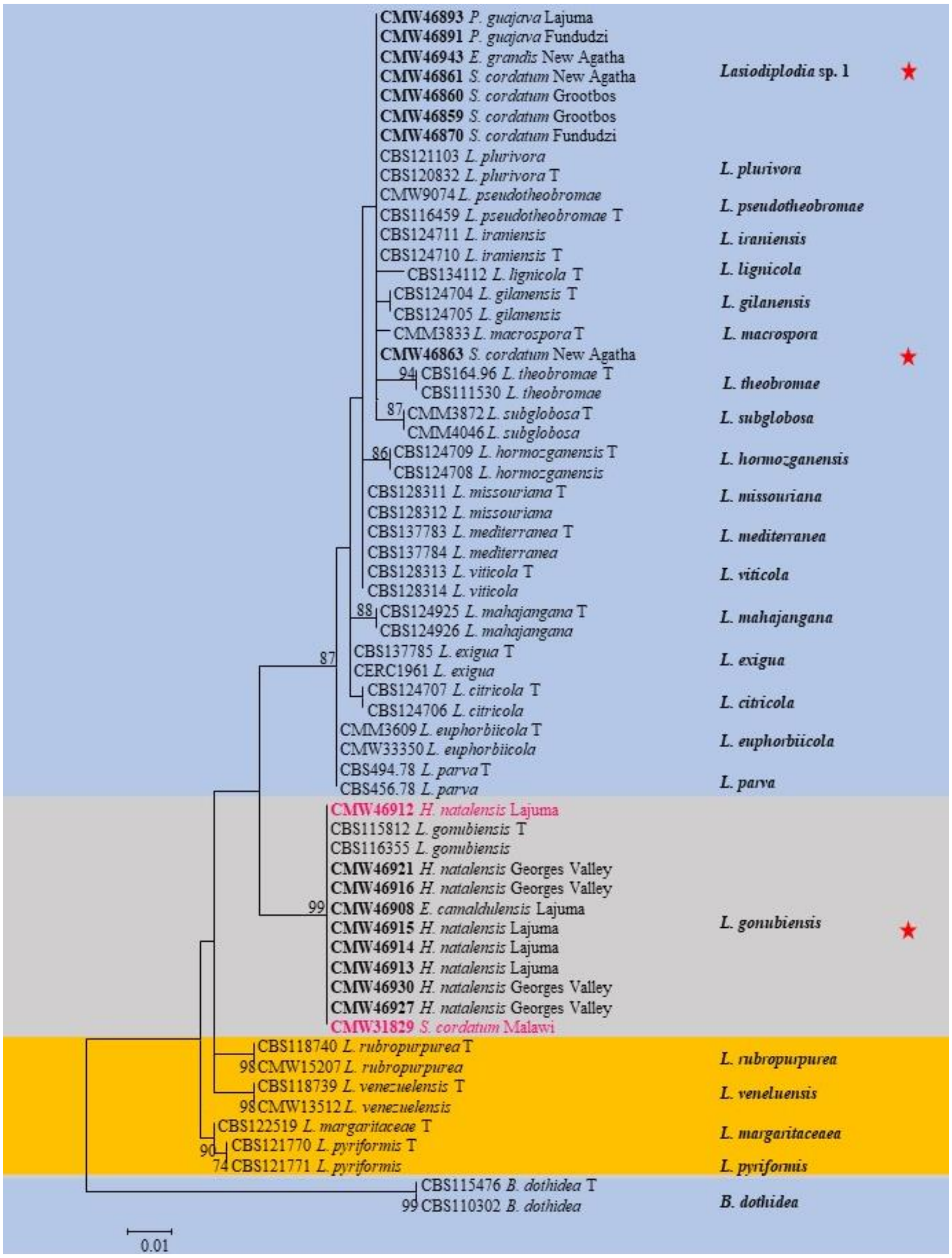


Figure 7. Maximum Likelihood (ML) tree of the genus *Lasiodiplodia* obtained from BT sequences, showing the phylogenetic placement of isolates grouping with *L. gonubiensis* and *L. gilanensis*. Bootstrap values above 70% are given above the nodes. The isolates sequenced in this study are represented in bold letters and their taxonomic position indicated with red stars. Potential hybrids are indicated in a pink colour text. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

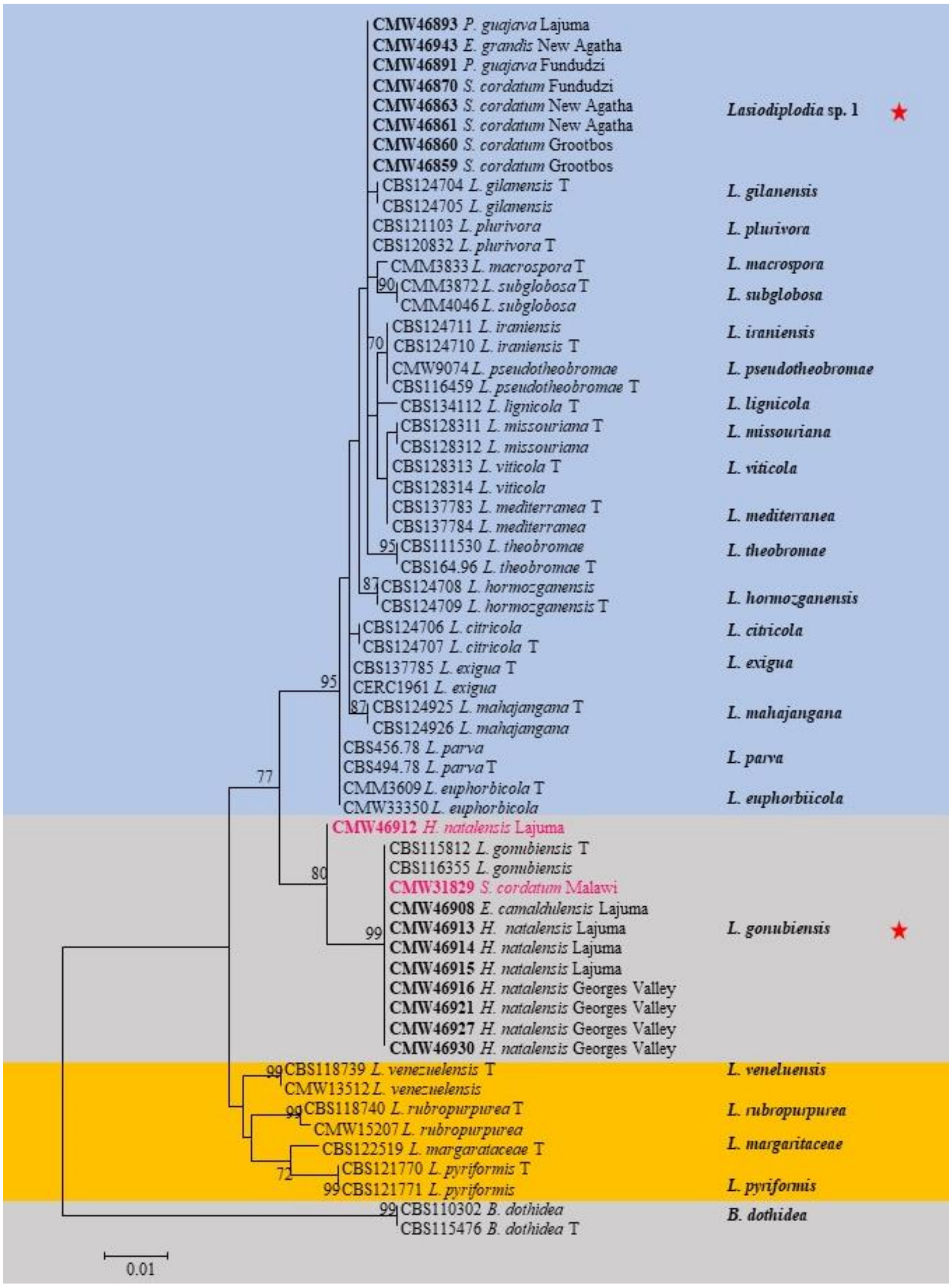


Figure 8. Maximum Likelihood (ML) tree obtained from the combined ITS and BT sequences of the genus *Lasiodiplodia* showing the placement of isolates collected in this study. Bootstrap values above 70% are given at the nodes. Isolates sequenced in this study appear in bold and their taxonomic positions are indicated with red stars. Potential hybrids are indicated in a pink colour text. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

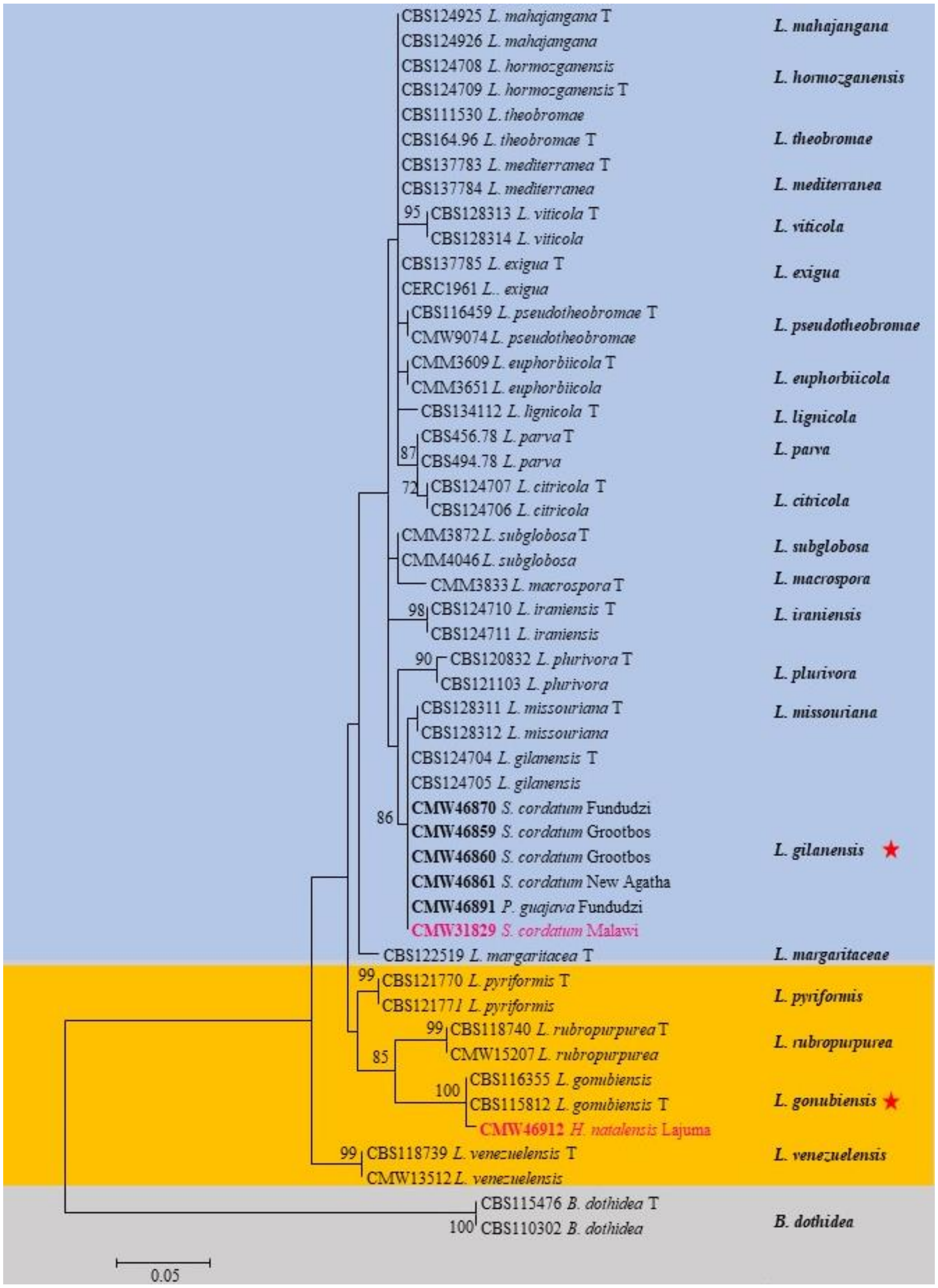


Figure 9. Maximum Likelihood (ML) tree obtained from TEF-1α sequences of the genus *Lasiodiplodia* showing the placement of isolates collected in this study. Bootstrap values above 70% are given at the nodes. Isolates sequenced in this study appear in bold. The red stars indicate their taxonomic positions and the potential hybrids are indicated in a pink colour text. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

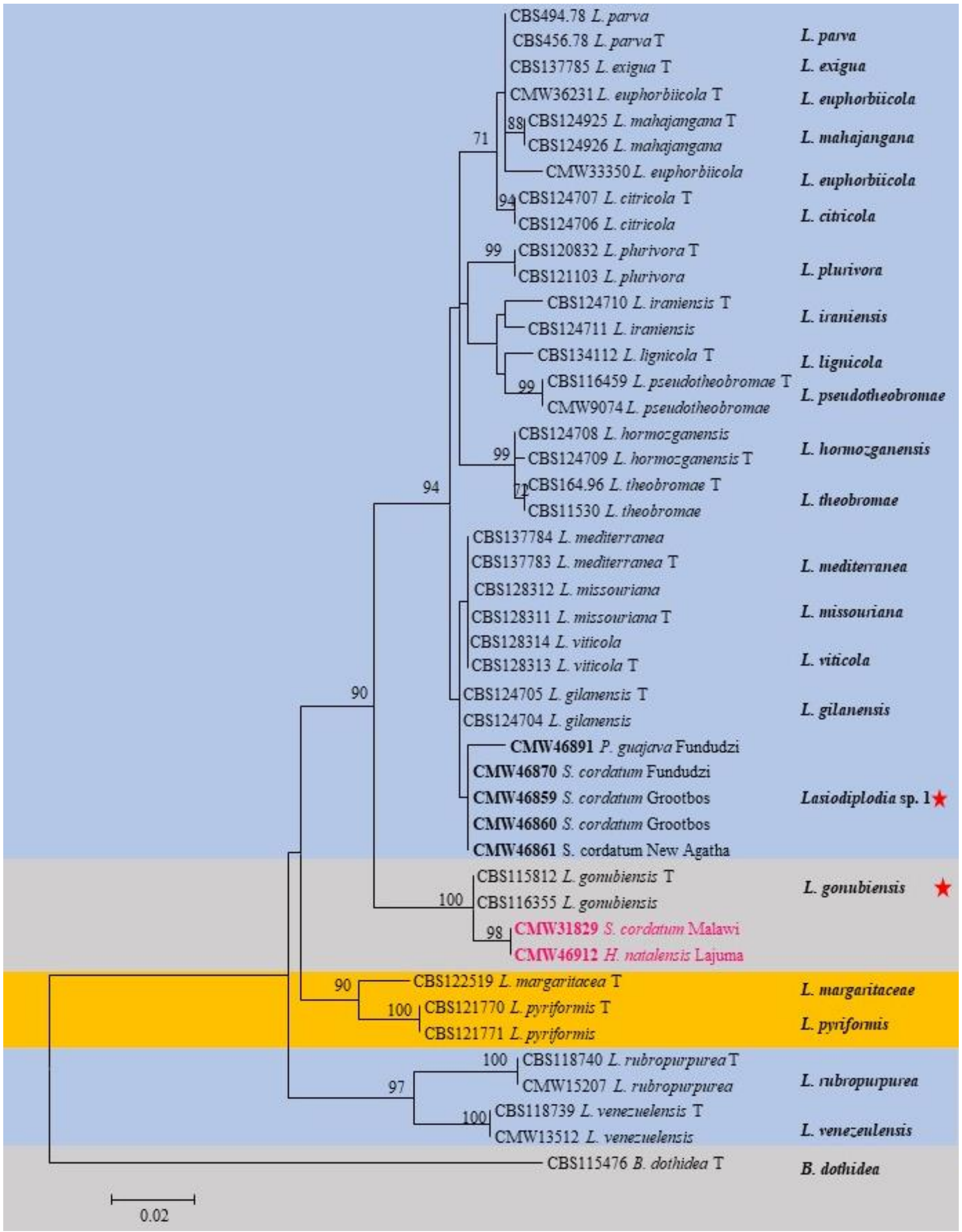


Figure 10. Maximum Likelihood (ML) tree obtained from RPB2 sequence data of all known *Lasiodiplodia* species, including isolates from this study. Bootstrap values above 70% are given at the nodes. Isolates sequenced in this study appear in bold and the red stars represent their taxonomic positions. Potential hybrids are indicated in a pink colour text. The tree was rooted to *Botryosphaeria dothidea* (CBS115476).

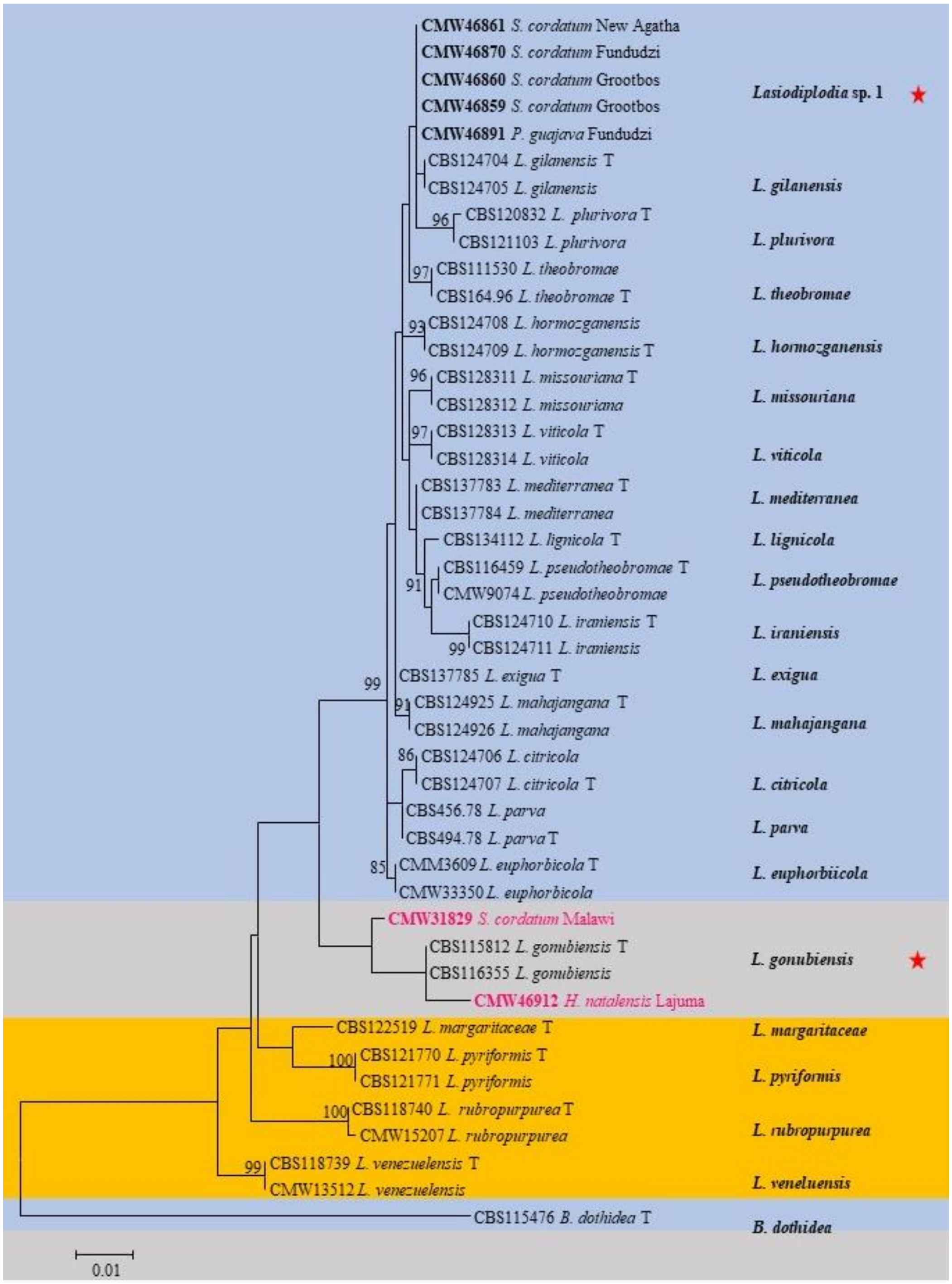


Figure 11. Maximum Likelihood (ML) tree produced from a combined ITS, BT, TEF-1α and RPB2 sequences of the genus *Lasiodiplodia*. Bootstrap values above 70% are given at the nodes. Isolates sequenced in this study appear in bold and the red stars represent their taxonomic positions. Potential hybrids are indicated in different colour text. The tree was rooted to *Botryosphaeria dothidea* (CBS115476).

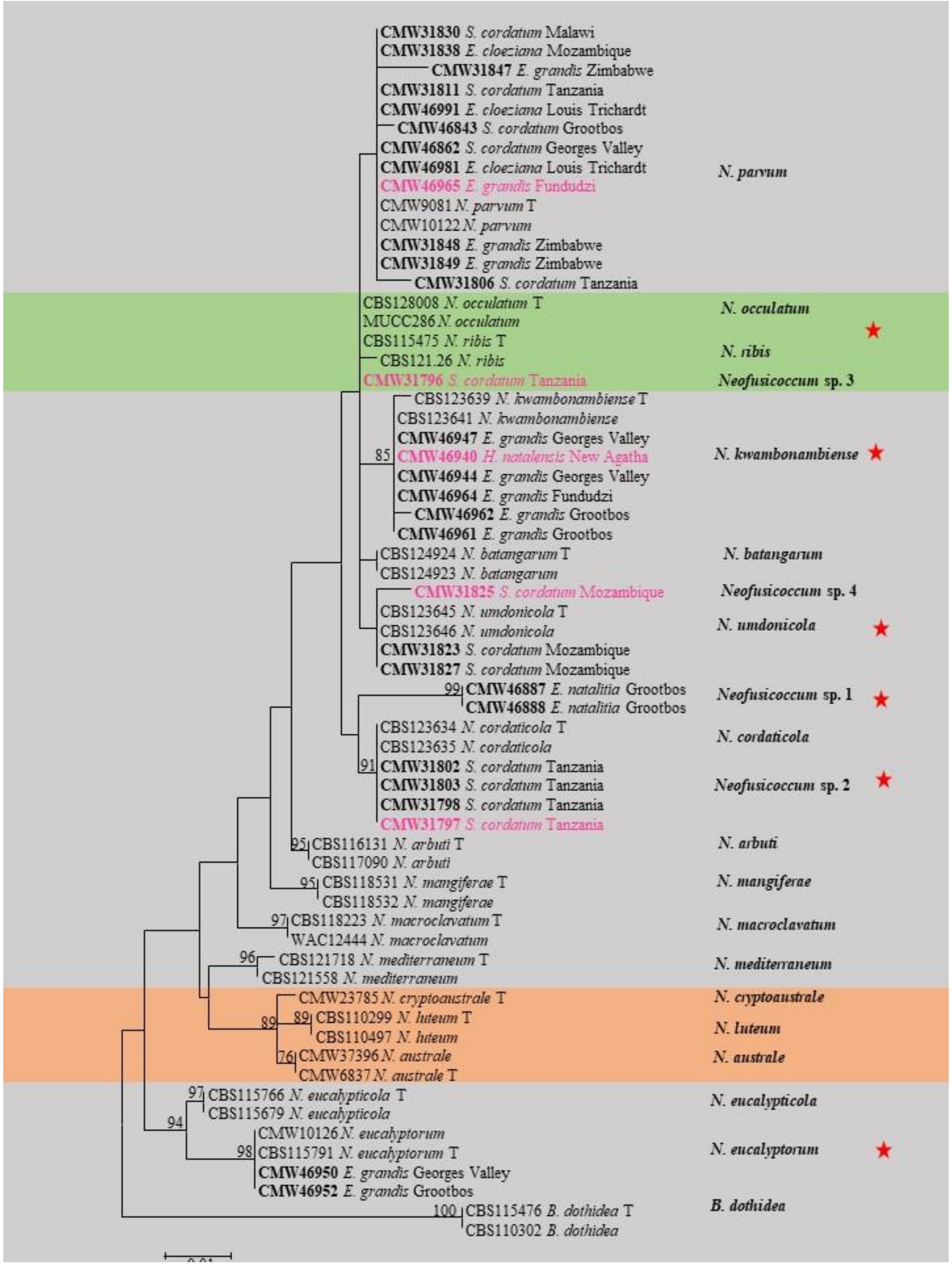


Figure 12. Maximum Likelihood (ML) tree of the genus *Neofusicoccum* resulting from the analyses of ITS sequence data, showing the phylogenetic placement of isolates obtained in this study. Isolates sequenced in this study appear in bold and the red stars represent their taxonomic positions. Potential hybrids are in a pink colour text. Bootstrap values above 70% are shown above the nodes. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

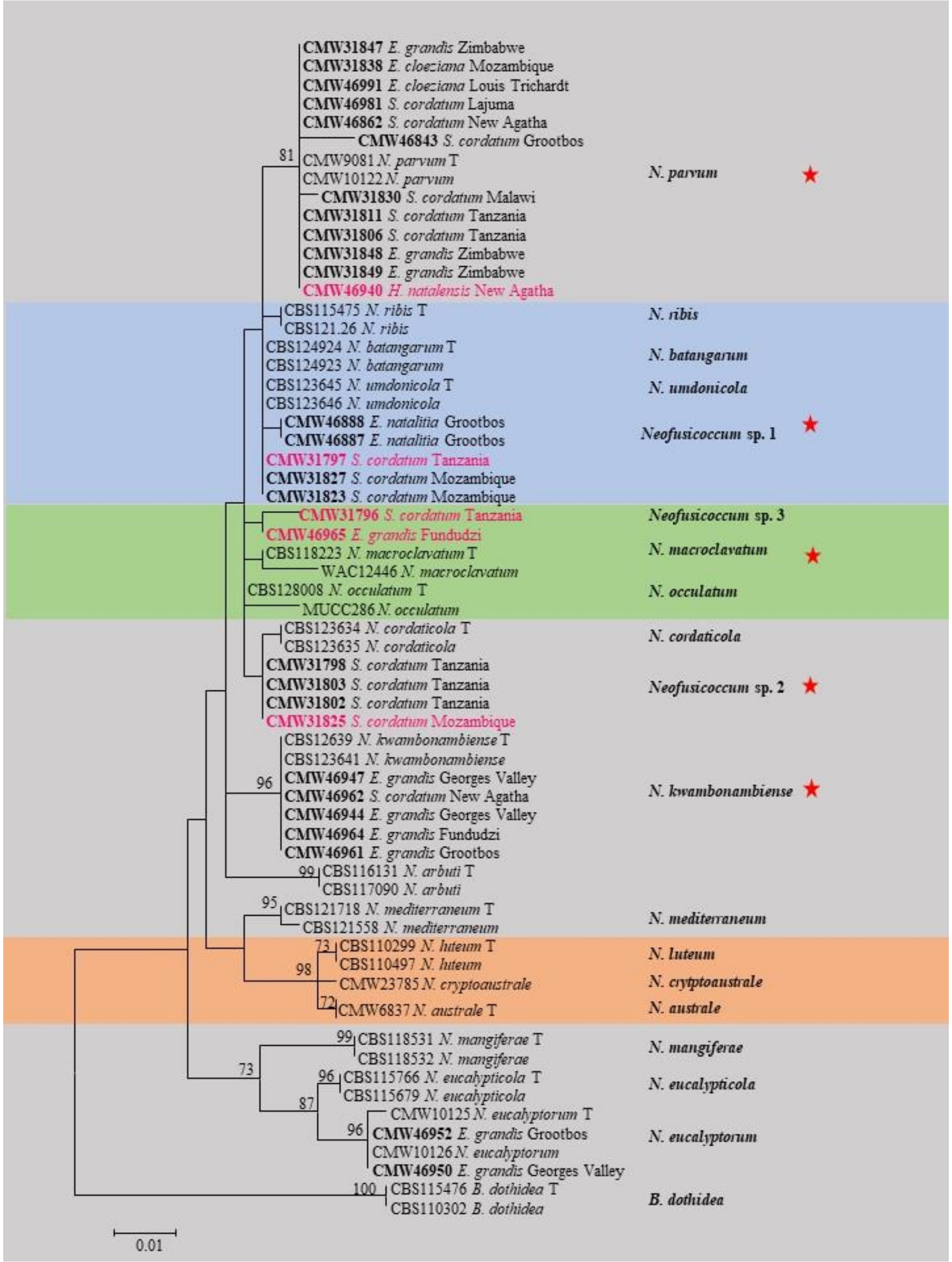


Figure 13. Maximum Llikelihood (ML) tree of the genus *Neofusicoccum* produced with BT sequence data, showing the phylogenetic placement of isolates from southern and eastern Africa. Bootstrap values above 70% are shown above the nodes. Isolates sequenced in this study appear in bold letters and the red stars indicate their taxonomic position. Potential hybrids are in a pink colour text. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

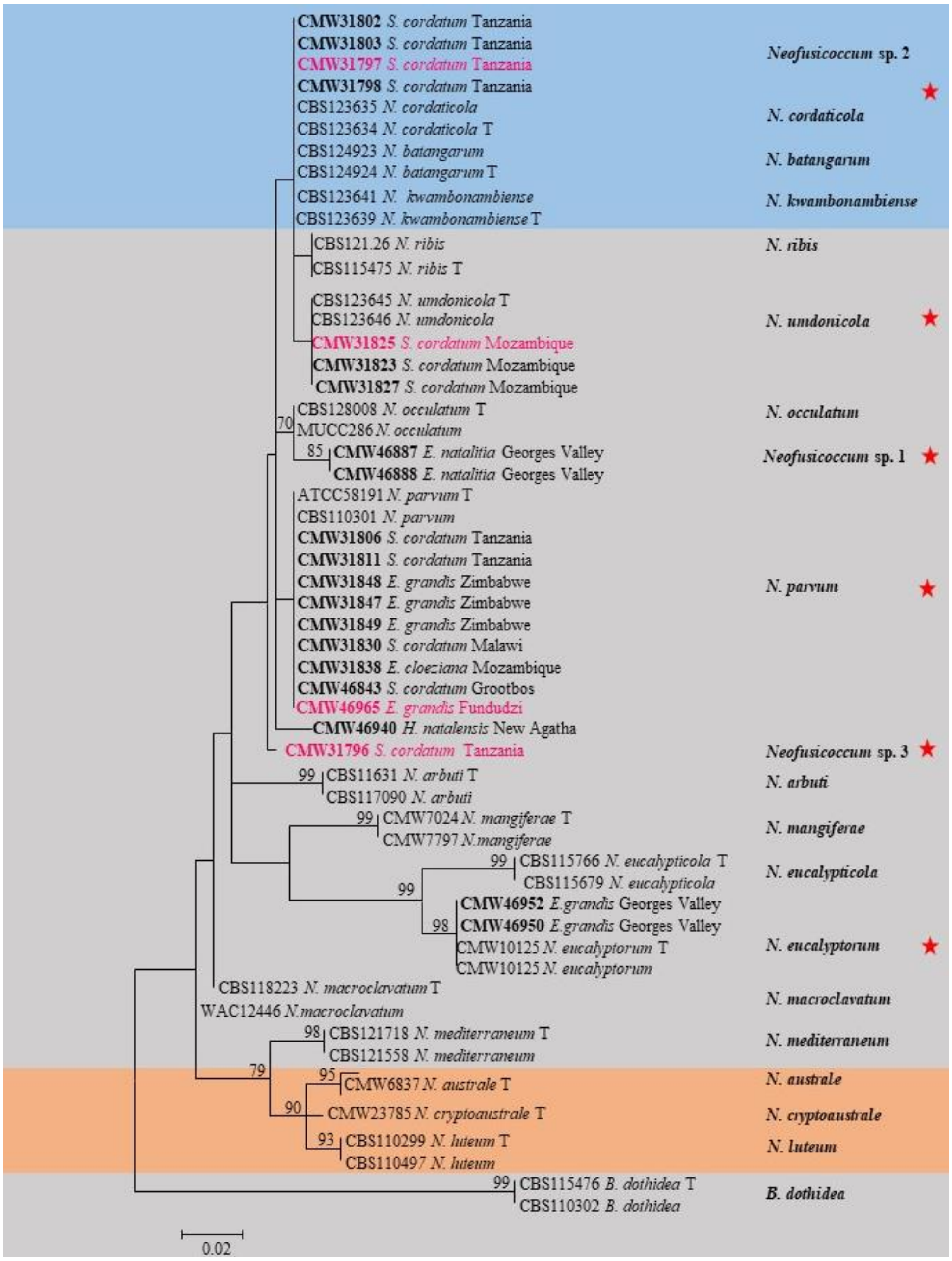


Figure 14. Maximum Likelihood (ML) tree obtained from the TEF-1α sequences of the currently known *Neofusicoccum* species, showing the phylogenetic position of isolates collected from eastern and southern Africa. Bootstrap values above 70% are shown above the nodes. Isolates sequenced in this study appear in bold and the red stars represent their taxonomic positions. Potential hybrids are in a pink colour text. The tree was rooted to *Botryosphaeria dothidea* (CBS115476 and CBS110302).

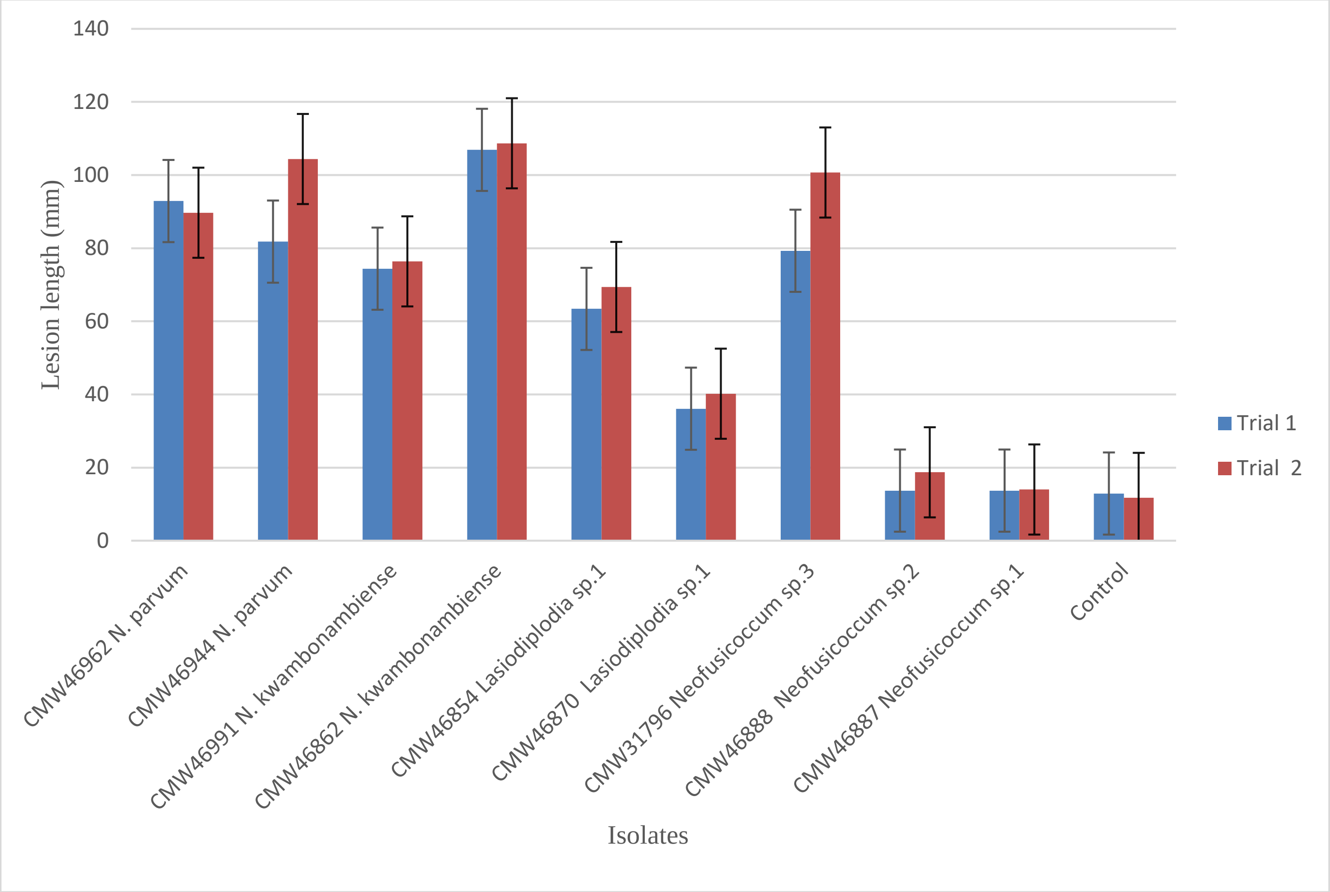


Figure 15. Mean lesion lengths (mm) obtained for each isolate of different *Botryosphaeriaceae* species six weeks after inoculation on *Eucalyptus*. Bars represent 95% confidence limits for each isolate. Mean leasion lengths produced by *Neofusicoccum* sp. 2 are not significantly different from the control ($P>0.05$), while the rest of the isolates produced lesions significantly different from the control ($P<0.05$). Control inoculations were done with 2% MEA agar.

Summary

Despite the importance of fungi in the *Botryosphaeriaceae* as plant pathogens and the multitude of studies on these fungi novel species are described annually. Many regions of the world and host plants remain unexplored in terms of the *Botryosphaeriacae*. This study represents the first investigation of *Botryosphaeriaceae* species on native *Myrtaceae* trees, other than *Syzygium cordatum,* in South Africa and a first look at these species on the *Myrtaceae* in Malawi, Mozambique, Tanzania, and Zimbabwe. Thirteen *Botryosphaeriaceae* species were identified using multi-gene DNA sequence data. These include *Diplodia* sp., *Neofusicoccum cordaticola, N. eucalyptorum, N. kwambonambiense, N. umdonicola, N. parvum,* three novel *Neofusicoccum* species, two *Neofusicoccum* hybrids, *Lasiodiplodia gonubiensis,* one *Lasiodiplodia* hybrid and *Lasiodiplodia* sp. 1. Some species in this study could not be classified to the species level due to icomplete data bases for some gene regions, or incongruency between different gene regions used. This study suggests that two gene regions are not sufficient for the identification of some species in the *Diplodia* genus. Furthermore, it suggests that for every novel species identified in the *Botryosphaeriaceae* a standard of four gene regions should be used to avoid incomplete data bases in the NCBI. Additonally, the study reports hybridization in the *Neofusicoccum* group for the first time.

Several novel host associations and and new geographical records of *Botryosphaeriaceae* species on native and non-native *Myrtaceae* were identified. *Neofusicoccum cordaticola* and *N. umdonicola* are reported for the first time on *S. cordatum* outside of southern Africa. Of the thirteen species identified in this study, *N. cordaticola, N. umdonicola* and the four novel *Neofusicoccum* species were found occurring exclusively on native *Myrtaceae* while the other species were isolated from both native and non-native hosts. Some of the species identified in this study are pathogenic to *Eucalyptus*, including *N. parvum* and *N. kwambonambiense* as shown in pathogenicity trials. Future studies should be focused on extensive sampling of *Botryosphaeriaceae* species that occur on other native *Myrtaceae* trees that are planted closely to *Eucalyptus* plantaions, particularly in regions outside South Africa.