Characterization of *Fusarium* species from *Pinus* and *Eucalyptus* nurseries in Colombia and South Africa

by

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“I have had dreams and I have had nightmares, but I have conquered my nightmares because of my dreams”

Jonas Edward Salk (1914 – 1995)

Declaration

I, the undersigned, declare that the work in this thesis is the result of my own investigation and that this is not previously in its entirety or partially been submitted for a degree at any other University.

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Preface

The genus *Fusarium* Link is one of the most well-known fungal genera in the world. It contains a number of toxigenic and pathogenic species. This is particularly true for an array of economically important plants including agricultural crop species, ornamentals, and forestry tree species. In forestry, the most important species is *Fusarium circinatum* Nirenberg & O'Donnell emend. Britz, Coutinho, Wingfield & Marasas, which is the causal agent of pitch canker of *Pinus* L. species. Since its discovery in 1946, this pathogen has spread to more than ten different countries on four different continents and infects more than 57 species of *Pinus* as well as *Pseudotsuga menziesii* (Mirb.) Franco. Besides the pitch canker pathogen, a number of other *Fusarium* species also cause diseases on *Pinus* and other forestry tree species, especially in the nursery environment. These include among others *Fusarium oxysporum* Schlechtendahl emend. Snyder and Hansen, *Fusarium solani* (Mart.) Appel & Wollenweber emend. Snyder & Hansen and *Fusarium proliferatum* (Matsushima) Nirenberg.

Compared to the agricultural and ornamental nursery environments, much less is known about the *Fusarium* species that occur in forestry plantations and nurseries. This is mainly because of the large number of devastating diseases known to be caused by *Fusarium* species on agricultural crop and ornamental plant species. **Chapter 1** therefore represents a critical review of the current knowledge of pathogenic *Fusarium* species in the commercial forestry environment. The review focusses on the three primary genera of exotic species utilized in plantation forestry in South Africa. They are *Pinus*, *Eucalyptus* L'Héritier and *Acacia* De Wild. This chapter also addresses the general control strategies for pathogenic *Fusarium* species in forestry and finally touches on the challenges climate change may pose to disease management in the future.

When *Pinus* species are infected with *Fusarium circinatum*, the pathogen can readily be recovered from the tissues associated with resinous cankers on trunks and branches, as well as from diseased roots and root collars of seedlings. Although no other fungus typically shows consistent association with these symptoms, a number of

other *Fusarium* species that are related to the pitch canker fungus were recently isolated from diseased and dying *Pinus* trees and seedlings in Colombia. The research presented in **Chapter 2** aimed to identify the *Fusarium* species associated with these diseased plants by making use of phylogenetic analyses based on partial sequences for the genes encoding translation elongation factor 1α (TEF) and β-tubulin. Isolates representing new species were further characterized and described based on their morphology and cultural characteristics. The possible association of these new species with the disease symptoms observed were evaluated using pathogenicity tests on *Pinus* seedlings.

Most plant pathogens are only noticed when disease symptoms start to develop on the host. In some cases pathogens can have an apparently 'latent' phase during which they are somehow associated with the host without inducing symptom expression. *Fusarium circinatum* is a well-known example of a species that can exist inside its *Pinus* host without causing disease. In cases such as these, the presence of the pathogen goes undetected. The research presented in **Chapter 3**, aimed to determine the diversity of *Fusarium* species associated with *Pinus* and *Eucalyptus* plants in the nursery environment by making use of DNA-based identifications. Isolates of *Fusarium* were collected from five South African nurseries located in KwaZulu-Natal and Mpumalanga. Isolates were sampled mainly from healthy *Eucalyptus* and *Pinus* species, although some isolates were recovered from *Pinus patula* seedlings showing signs of wilt and collar rot. The isolates were identified by making use of TEF-based phylogenetic analyses. In this way an inventory of the *Fusarium* species associated with *Eucalyptus* and *Pinus* seedlings/cuttings was generated, although their presence would have been masked by the healthy appearance of their hosts.

Chapter 1

Diversity of *Fusarium* species in the commercial forestry environment of South Africa

Abstract

The success of the forestry industry in the world and particularly the Southern hemisphere can be attributed to the choice of fast and easy-growing exotic pine and eucalypt tree species, which have been planted separated from their natural enemies. In South Africa, species of *Pinus*, *Eucalyptus* and an *Acacia* species have been planted to sustain commercial forestry. This industry in South Africa is at risk, however, because of pest and pathogen movement around the world. Various species of *Fusarium* represent some of the most serious threats to the forestry industry. The genus includes a large number of species, many of which are important plant pathogens with host ranges that include species in *Pinus*, *Eucalyptus* and *Acacia*. An important and well known example in South African commercial forestry is *Fusarium circinatum*, which causes the disease known as pitch canker. Research on this pathogen has advanced our understanding and ability to identify the pathogen rapidly and to establish measures that will contain its spread. Identification of pathogens such as species of *Fusarium* represents a first step towards developing control measures. Diseases caused by emerging pathogens are becoming more complex due to exacerbating factors such as the effect that climate change might have on host-pathogen interactions. Understanding of all these factors should contribute to an enhanced capacity to protect forest plantations in the future.

Keywords**:** *Acacia***,** climate change, control**,** disease, *Eucalyptus*, *Pinus*, pathogens, taxonomy.

1. Introduction

Commercial forestry began in the northern hemisphere and plays an important role in many economies of the world (Mather 1993). It was only towards the start of the twentieth century that countries in the southern hemisphere began planting large numbers of trees over great areas and distant from their natural environments (Zobel et al. 1987). In 2005, the Food and Agriculture Organization of the United Nations (FAO) estimated a total of 3, 952 million hectares of forests covered the earth, of which 2.8% (109 million ha) represent commercial plantations (FAO 2006).

Globally, a relatively small group of tree species form the basis for commercial forestry (Zobel et al. 1987). These include members of the genera *Pinus* L., *Eucalyptus* L'Heritier*, Tectona* L. and *Acacia* Mill. (Lamb and Tomlinson 1994; Sedjo 1999; Kelty 2006).The South African forestry industry is based primarily on species of *Pinus*, *Eucalyptus* and *Acacia* (Wingfield 1990; Geldenhuys, 1997; Wingfield et al. 2001a; Anonymous 2005a; Anonymous 2009).These all represent exotic species that have been selected for planting based on their superior performance under prevailing environmental conditions (Hellmers and Rook 1973; Teskey et al. 1987; Sutton, 1999).

There are a number of advantages to using non-native or exotic species for commercial forestry (Zobel et al. 1987; Mather, 1993). Amongst others, these species are typically less affected by diseases and pests, because they are planted outside of their natural ranges (Wingfield et al. 2001a, b). Seed of genetically improved exotic trees are easier to obtain than native species (Zobel et al. 1987; Mather 1993). Also, these tree species are usually easy to manage, fast growing and adapted to a variety of environments (Zobel et al. 1987; Mather, 1993; Wingfield, 2003).

Despite the advantages of planting non-native species, there are important disadvantages. Because of the wide use of monoculture practices (Gibson and Jones 1977; Wingfield 1990; Chou 1991), plantation forestry in many areas of the world is threatened by the introduction of pests and pathogens from the native range of the tree species (Wingfield et al. 2008b). Also, pests and pathogens may also jump from indigenous hosts to these planted exotic hosts, which could potentially have devastating

consequences (Wingfield et al. 2001b; Slippers et al. 2005; Paine et al. 2011). In fact, such host jumps have already been reported from South Africa and the rest of the world (Slippers et al. 2005; Paine et al. 2011) and they appear to be of growing importance (Woolhouse et al. 2005; Desprez-Loustau et al. 2007). Examples include Asian pine rust (*Cronartium ribicola* JC Fisch.: Rabenh.) on *Pinus* species in Europe and North America (Maloy 1997; Vogler and Bruns 1998; Kinloch 2003), Cryphonectria canker [*Cryphonectria cubensis* (Bruner) Hodges] on *Eucalyptus* in South Africa (Wingfield 2003; Gryzenhout et al. 2003) and Eucalyptus rust (*Puccinia psidii* Winter.) on *Eucalyptus* (Coutinho et al. 1998; Glen et al. 2007) in Central and South America.

Most forestry pathogens are fungi, which includes a diverse group of organisms encompassing a vast breadth of morphologies, taxa, ecologies and evolutionary histories (Mueller and Schmit 2007). There have been many disputes over how many fungi there really are in the world (Bisby and Ainsworth 1943; Martin 1951; May 1988; Smith and Waller 1992). Two of the commonly applied contemporary estimates are those of Hawksworth (1991) and Heywood (1995), suggesting that three are 1.5 million fungi on earth and that of these, only 5% have been identified (Hawksworth and Rossman 1997). In South Africa, the conservative estimates are about 170, 000 species (Crous et al. 2006a). However, such estimates are confounded by the fact that they are based on studies in particular environments. More importantly, many fungi represent large species complexes whose members are only diagnosable with specialised procedures. For example, studies on the diversity of species in the genus *Fusarium* Link. revealed that conventional species inventory approaches would have grossly underestimated the diversity of species in the environment examined (O'Donnell et al. 2008a; O'Donnell et al. 2009). The global and local fungal species estimates thus generally err on the low side (Blackwell 2011), which suggests that far fewer than the estimated 5% have actually been identified.

The genus *Fusarium* has a rich history in forestry and is associated with many different forestry species. There are also many diseases and disease symptoms associated with *Fusarium* species ranging from root rot and damping-off to cankers and wilts (reviewed by Bloomberg 1981). Species of *Fusarium* have been recorded on all

continents and in all climatic regions from tropical to temperate regions (Bloomberg 1981; Burgess 1981). Because of their common occurrence in the soil (Snyder 1981), *Fusarium* species are commonly isolated from nursery soils (Booth 1971; Burgess 1981; Burgess et al. 1988), and in many instances are responsible for the diseases of plants/crops in nurseries (Bloomberg 1981; Keane et al. 2000). However, most *Fusarium* species, like many other fungi have not been characterized (Hawksworth 2001), nor have they been tested for pathogenicity on hosts in the nursery environment.

The aim of this review is to consider the current and future problems faced by the plantation industry, primarily in South Africa, due to the presence of *Fusarium* species. A large portion of this review considers the diversity of *Fusarium* species causing disease in the South African plantations, particularly on *Pinus*, *Eucalyptus* and *Acacia* species. The taxonomy of these pathogens is also discussed, as well as the control measures available for these fungi. Finally, I consider how environmental dynamics such as climate change may affect these species and their ability to cause disease in the future.

2. South African Forestry

South Africa started using fast-growing introduced tree species towards the end of the 19th century to provide the country with timber (King 1943; Dovers et al. 2003; Olivier, 2009). The use of exotics was necessary because of the country's poor timber species diversity and rapidly increasing timber needs (Van der Zel and Brink 1980; Scholes 1995). Today, the South African forestry industry relies primarily on *Pinus* species, *Eucalyptus* species and wattle (*Acacia* species) (Anonymous 2005a; Anonymous, 2009). Combined they represent the third largest crop planted in South Africa (Van der Zel, 1989). To date, South Africa has established, and continues to manage, over 1.2 million hectares of plantation forestry (Anonymous 2005b; http://forestry.daff.gov.za/webapp/FactsForests.aspx). Between 1980 and 2008, the average afforested area was expanding at 4 234 ha a year and is still increasing in some provinces (Godsmark 2008; Anonymous 2009).

2.1 *Pinus* species

A small number of *Pinus* species were first brought to South Africa in the late 17th century with many more species being introduced and planted after that up until 1875 (Van der Zel and Brink 1980; Richardson et al. 1994; Richardson and Higgins 2000). *Pinus* species provide South Africa with the majority of its softwood with 53.2% (~660,000 ha) of the forestry industry based on pine. The most commonly used species is *P. patula* Schlecht. & Cham, followed by *P. elliottii* Engelm.,*P. radiata* D. Don. and *P. taeda* L. (Roux et al. 2007). These species are planted throughout South Africa, but primarily in Mpumalanga (47.6%), Western Cape (28.1%), KwaZulu Natal (20.2%) and the Limpopo province (4.1%) (Godsmark 2008).

In addition to the environmental factors that influence the yield of timber and other wood products in South Africa, the most important and most damaging are insect pests and pathogens (reviewed by Wingfield et al. 2001b; FAO 2007; Roux et al. 2012). Some examples of fungal pathogens include *Rhizina undulata* Fr., which infects and kills many trees after plantation fires; *Dothistroma septosporum* Doroguine, which infects the needles of susceptible *Pinus* species and *Diplodia pinea* Desm., which causes die-back. Currently, the most important pathogen and pestof *Pinus* species in South Africa is the pitch canker fungus *Fusarium circinatum* Nirenberg et O'Donnell. (Nirenberg and O'Donnell 1998; Wingfield et al. 2008a) and the wood boring wasp *Sirex noctillio* F., which is unquestionably the most important insect pest affecting pine forestry in the country (reviewed by Slippers et al. 2001).

2.2 *Eucalyptus* species

The use of *Eucalyptus* species in South Africa as well as many other parts of the world has been extremely successful and these trees represent one of the most important sources of fibre (Wingfield et al. 2008b). The genus *Eucalyptus* includes more than 700 species (Potts and Pederick, 2000) and is the most commonly planted hardwood species across the globe (Flynn 2009). In 2009, it was estimated that *Eucalyptus* species globally occupied between 16 and 19 million ha (Flynn 2009). In 2008, approximately half a million ha (39.1% of the 1.2 million ha commercial plantations) in

South Africa were planted with *Eucalyptus* (Anonymous 2009). The most commonly planted species of *Eucalyptus* include *E. grandis* W. Hill: Maiden and hybrids of *E. grandis* and *E.urophylla* S.T. Blake. They are planted mainly in the KwaZulu Natal province (55%), but are also found in the Western and Eastern Cape (4.6%), Limpopo (4.0%) and Mpumalanga (36.4%) provinces (Anonymous 2009).

Although *Eucalyptus* species have existed in South Africa for a long period of time without suffering major attacks from pests or pathogens, this situation appears to be changing. There have been two recent reports of emerging pests that are causing damage to eucalypt plantations. These are the sap-sucking insect *Thaumastocoris peregrinus* Carpintero and Dellap., which has been gaining importance in South Africa since its first report in December 2003 (Carpintero and Dellapé 2006; Nadel et al. 2010; Wilcken et al. 2010) and the gall forming wasp *Leptocybe invasa* Fisher & La Salle., which was first discovered in South Africa in 2007 (Mendel et al. 2004; Neser et al. 2007; Wingfield et al. 2008b). Other important pests of *Eucalyptus* species occurring in South Africa have been reviewed by Wingfield et al. (2008b) and Roux et al. (2012).

In addition to the insect pests, a variety of important pathogenic fungi and bacteria have also been associated with *Eucalyptus* species in South Africa. The diseases and symptoms they cause are diverse and affect different parts of the tree, from the roots to the leaves. The pathogens often found include those causing cankers of *Eucalyptus* such as *Holocryphia eucalypti* M. Venter & MJ Wingf. (previously known as *Cryphonectria eucalypti*) (Van der Westhuizen et al. 1993; Gryzenhout et al. 2003, 2006) and *Chrysoporthe austroafricana* Gryzenh.& MJ Wingf. (Hodges et al. 1979; Wingfield 2003); *Teratosphaeria nubilosa* Cooke., which causes *Mycosphaerella* leaf disease on *Eucalyptus* species (Hunter et al. 2004; Park et al. 2002), as well as many others which have been reviewed by Wingfield et al. (2008b) and Roux *et al*. (2012).

2.3 *Acacia* species

Compared to *Pinus* and *Eucalyptus*, *Acacia* species contribute a small percentage to commercial timber production in South Africa as only about 95 500 ha are planted to these trees (Godsmark 2008). They are, however, important because their timber is

utilized for poles and pulp production, and extractives from their bark can be used for leather tanning (Brown and Ko 1997). *Acacia* species were brought into South Africa at the beginning of the 19th century (Dennill and Donnelly 1991) where they were used for fuel wood, windbreaks and tannin production (Acland 1971; Sherry 1971). Today, many of these so-called wattle species are considered as invasive weeds (Van Wilgen et al. 2001), and mainly *A. mearnsii* de Wild. and *A. melanoxylon* R. Br. are utilized for commercial purposes (Sherry 1971; Geldenhuys 1986). Eighty percent of the wattle plantations are found in the KwaZulu Natal province, which is also where over 40% of all South Africa's plantations are found (Anonymous 2009) and this was where the first *Acacia* species were introduced (Sherry 1971).

Acacia species are affected by many different pests and pathogens worldwide. A study in South Africa by Govender (2007) established that of the insect pests occurring on *A. mearnsii* in South Africa, white grubs (larvae of *Coleoptera*: *Scarabaeidae*: *Rutelinae*, *Melolonthinae*) were responsible for the majority of damage caused to regenerating trees. Other insects often found on *A. mearnsii* included, cutworms (larvae of *Lepidoptera*: *Noctuidae*), termites (*Isoptera*: *Termitidae*, *Hodotermitidae*), grasshoppers (*Orthoptera*, *Acridiae*, *Pyrgomorphidae*), millipedes (*Diplopoda*: *Juliformia*), among others. These insects and many others have also been reviewed by Sherry (1971) and are known to cause damage to *A. mearnsii*.

A survey conducted by Roux and Wingfield (1997) showed that there are also a large number of microorganisms associated with *A. mearnsii* in South Africa. The most commonly isolated pathogens, isolated from lesions on mature *A. mearnsii* trees, from that survey were *Ceratocystis albifundus* Wingfield, De Beer and Morris, *Phytophthora parasitica* Dast., *Phytophthora boehmeriae* Sawada. and *Botryosphaeria dothidea* Moug.:Fr. Ces. and De Not., while other fungi such as *Fusarium* species and *Cylindrocladium candelabrum* Viegas. were also isolated. Pathogenicity assays in the study by Roux and Wingfield (1997) showed that all of these isolates produced lesions on 36 month old *A. mearnsii* trees and thus are all able to cause disease on *A. mearnsii*. It has also been reported by Hagemann and Rose (1988) that *Cylindrocladium scoparium* Morgan. caused a foliar disease on *A. longifolia* Andr. Willd. Many of these

pathogens and others have also been listed by See (1993). A recent review by Wingfield et al. (2011) also highlighted some of the most important examples of pest and diseases of *A. mearnsii* in South Africa and South-east Asia and their impacts on future plantation forestry. Two examples not mentioned previously included, *Phytophthora nicotianeae* (Dastur) Waterh. which causes the black butt disease and the rust fungus *Uromyces alpinum* McAlp.

3. The genus *Fusarium*

The genus *Fusarium* is one of the best known and widely recognized fungal genera in the world (Snyder 1981; Seifert 2001). *Fusarium* species are distributed across the globe, from the arctic to the Sahara, but are most commonly found in tropical and temperate soils (Booth 1971). The members of this genus thus represent a diverse and well adapted ascomycete assemblage, widely distributed in air, water, organic materials and soil, and in association with a range of other eukaryotes such as plants and animals (Snyder 1981; Nelson et al. 1983; Leslie and Summerell 2006; Gams 2007). In addition to being pathogens, *Fusarium* species can also represent beneficial saprophytes (Booth 1971; Snyder 1981; Leslie and Summerell 2006), while some can even be used as bio-control agents (e.g., Mandeel and Baker 1991; Nel et al. 2006; James and Dumroese 2007).

Fusarium's notoriety can be attributed to the fact that it includes a large variety of important plant pathogens (Booth 1971; Tousson 1981; Nelson et al. 1983; Leslie and Summerell 2006). Of all cultivated plants, approximately 20% are not associated with a species of *Fusarium* (Leslie and Summerell 2006; Kvas et al. 2009). Phytopathogenic *Fusarium* species are mainly responsible for two types of diseases: cortical rots and vascular wilts (Tousson 1981). *Fusarium oxysporum* Schlechtend.:Fr. is a good example of a species responsible for both cortical rots and vascular wilts of many agricultural crops including pepper, cucumber, tomato, oil palm and chickpea (Colhoun 1981; Nelson et al. 1983; Trapero-Casas and Jiménez-Díaz 1985; Vakalounakis 1996; Miller et al. 2002). However, there are many other diseases associated with *Fusarium*, including wheat head blight and maize ear rot caused by *Fusarium graminearum*

Schwabe. (Sutton 1982), crown rot of wheat caused by *Fusarium pseudograminearum* T. Aoki & O'Donnell and *Fusarium culmorum* WG Sm. (Burgess et al., 2001) and pitch canker on *Pinus* species (Wingfield et al. 2008a; Mitchell et al. 2011). These diseases are responsible for huge economic losses in both agriculture and forestry (Tousson 1981; Nelson et al. 1983; Johnson et al. 1998; Güldener et al. 2006; Leslie and Summerell 2006; Wingfield et al. 2008a).

Fusarium species are also known to affect humans and animals (Nelson et al. 1993; Nelson et al. 1994). In humans, disease is usually associated with immunocompromised patients (Anaissie 1992; Nucci and Anaissie 2002, 2007), while in some cases they can cause disease in domestic animals (Evans et al., 2004). *Fusarium* species also have the ability to produce mycotoxins, which are toxic secondary metabolites (Nelson et al. 1993; Nelson et al. 1994; Desjardins 2006). Some of the well-known mycotoxin producers include *F. moniliforme* Sheld., *F. graminearum* Schw., *F. culmorum* (WGSm.) Sacc., *F. poae* Peck., *F. verticillioides* (Saccardo) Nirenberg, *F. proliferatum* (Matsushima) Nirenberg (Marasas et al. 1984; Sydenham et al., 1990; Nelson et al. 1993; Nelson et al. 1994; Gilbert and Tekauz 2000). Compounds produced by these particular *Fusarium* species include moniliformin (MON), fumonisins (B1, B2), trichothecenes (nivalenol (NIV), deoxynivalenol (DON), T-2 toxin, tricarboxyllic acid (TCA) and zearalenone (ZON) (Nelson et al. 1994). Ingestion of food contaminated by these compounds by animals or humans can cause severe illness and even death (Marasas et al. 1984; Sydenham et al. 1990; Henry and Wyatt 1993; Nelson et al. 1993; Munkvold and Desjardins 1997; Desjardins 2006).

3.1 *Fusarium* taxonomy

The history of *Fusarium* and its taxonomy extends over 200 years. The work by Wollenweber and Reinking (1935), laid the foundation for *Fusarium* taxonomy. They used many specific morphological characters to discern species that ultimately provided a sound basis for *Fusarium* taxonomy. Based on their work, 16 sections, 65 species, and 77 forms and varieties were recognized (Nelson 1991; Nelson et al. 1994; Leslie and Summerell 2006). Their system was, however, problematic as it included the use of

non-single spored cultures, a general lack of understanding of cultural mutation, and the use of too few cultures for species descriptions.

In 1935, Raillo proposed that the form of the apical cell of the macroconidia and the curvature of conidia are the most important characters to separate members of the genus (Raillo 1935). In 1950, Raillo and Bilai also considered the variability of cultures (Raillo 1950; Bilai 1955). Later, Bilai (1970) also studied the cultural mutation and variability of individual isolates based on various external factors. Although as complex as previous taxonomic systems, Bilai (1970) recognized 26 species and 29 varieties (Nelson 1991; Nelson et al. 1994).

The creation and/or use of complex taxonomic systems is what lead to Snyder and Hansen (1941) to develop a simple taxonomic system for *Fusarium* that would be stable and practical (Nelson 1991; Nelson et al. 1994). As a result they focused on microconidial morphology, cultural variation and the general nature of *Fusarium*, instead of the many other secondary characteristics suggested by Wollenweber and Reinking (Nelson 1991; Nelson et al. 1994). Their simplified approach led to the lumping of many species under one name (Snyder and Hansen 1941**,** 1945**;** Tousson and Nelson 1976), for example those in the section Elegans were reduced to one species, *Fusarium oxysporum* (Snyder and Hansen 1941).

Gerlach and Nirenberg (1982) used Wollenweber and Reinking's system as the basis of their work (Gerlach 1981; Gerlach and Nirenberg 1982). However, Gerlach and Nirenberg (1982) made the same principle mistakes as Wollenweber and Reinking. For example, they described new species based on single cultures or at times on a single mutant, and focused on characteristic differences rather than similarities, which would lead to the establishment of new species based on small differences (Nelson et al. 1994). As a result, their taxonomic system was also complex, confusing and difficult to use (Gerlach 1981; Gerlach and Nirenberg 1982).

Booth (1971) introduced a taxonomic system based on conidial morphology, particularly that of the sporogenous cells (Booth 1971). Based on this system, 44 *Fusarium* species with 7 varieties were recognized. The taxonomic system of Booth

(1971) was a modification of others that came before it because he expanded the information available on important characters for species separation. His taxonomic system revealed the importance of specific morphological characters such as conidiogenous cells and conidiophores, as well as the presence of mono- versus polyphialides, which resulted in the separation of *F. moniliforme* from its variety *F. moniliforme* var. *subglutinans* (Booth 1971).

Nelson et al. (1983) produced a manual for the identification of *Fusarium* entitled "*Fusarium* species: An illustrated manual for identification". This manual was complete with species descriptions and illustrations, culturing techniques and synoptic keys for the various sections and is still widely cited today. The morphological characters captured in their manual are more complicated than the earlier taxonomic systems and included a greater number of recognized species than the earlier taxonomic system of Snyder and Hansen (1941, 1945). Nelson et al. (1983) also did not recognize some of the species recognized by Gerlach and Nirenberg (1982).

Although the application of morphological characters for *Fusarium* identification is important, the use of DNA-based markers to aid species descriptions has revolutionised *Fusarium* taxonomy. In the 1990s, DNA-based tools became readily available for fungal taxonomy. Techniques such as electrophoretic karyotyping (Boehm et al. 1994; Nazareth and Bruschi 1994; To-Anun et al. 1995), PCR restriction fragment length polymorphism (RFLP) profiles (Steenkamp et al. 1999; Llorens et al. 2006), random amplified polymorphic DNA (RAPD) (Ouellet and Seifert 1993; Achenbach et al. 1996) and amplified fragment length polymorphisms (AFLP) (Baayen et al. 2000; Marasas et al. 2001; Zeller et al. 2003) were used.

All contemporary species descriptions are based on DNA sequence information for specific genomic regions (O'Donnell 2000; O'Donnell et al. 2000a, b; Tautz et al. 2003; Geiser et al. 2004; O'Donnell et al. 2008b; Cook et al. 2010). These include genes encoding proteins such as beta-tubulin, calmodulin and translation elongation factor-1α (TEF), as well as the various spacer and gene regions of the nuclear and mitochondrial ribosomal RNA operons (e.g., O'Donnell and Cigelnik 1997; O'Donnell et al. 1998; 2000a; Geiser et al. 2004; Stenglein et al. 2010). Of these, TEF is most

popular and widely accepted as the so-called “barcoding” region for *Fusarium* species (Geiser et al. 2004).

Today, most taxonomists do not adhere to a single classification system for *Fusarium* as it is well-recognised that the great amount of variation in the genus complicates the emphasis of one or a few taxonomically important traits. As a result, today’s descriptions of *Fusarium* species rely on numerous phenotypic and genotypic characters and they incorporate approaches that were introduced by many previous workers in the field (Booth 1971; Burgess et al. 1988, Nelson et al. 1983; Nirenberg et al. 1998; Nirenberg and O’Donnell 1998). The descriptions and the histories of the various taxonomic systems for *Fusarium* taxonomy and the most comprehensive collection of species in the genus *Fusarium* are available in the *Fusarium* laboratory manual by Leslie and Summerell (2006).

3.2 Recognizing *Fusarium* species

The large number of characters available for *Fusarium* and the diversity within the genus make it difficult to use a single characteristic to group and separate species (Donoghue 1985). Therefore, to define *Fusarium* species, suites of different criteria are usually employed (Summerell et al. 2003). These need to be sufficiently robust to be applicable in many instances and in different settings, thus enabling scientists to make accurate species diagnoses (Leslie et al. 2001). For *Fusarium* species the Morphological, Biological and Phylogenetic species recognition (MSR, BSR and PSR, respectively) criteria are the most widely employed (Taylor et al. 2000; Leslie et al. 2001; Summerell et al. 2003; Kvas et al. 2009). However, in many instances, combinations of these recognition criteria are used to achieve a “holistic” species diagnosis that reveals not only well resolved taxa efficiently (Sites and Marshall 2004; Dayrat 2005; DeSalle et al. 2005), but also generates valuable biological information beyond that needed for taxonomy (Schlick-Steiner et al. 2010).

3.2.1 Morphological species recognition

Of all the species recognition criteria, the MSR has been the most widely used in fungi (Taylor et al. 2000; Leslie et al. 2001). The principle of MSR is that the morphology of one individual or "type" represents the overall variation within a particular species (Taylor et al. 2000). This view has worked well for many years with many different fungi, resulting in its invaluable use today.

The most characteristic features utilized by the MSR for *Fusarium* species relate to the shape and size of macroconidia (Snyder and Hansen 1945; Booth 1971; Nelson et al. 1983; Seifert 2001; Leslie and Summerell 2006), aerial arrangement of microconidia, the presence or absence of chlamydospores, and the morphology of conidiogenous cells (Booth 1971; Nelson et al. 1983; Seifert 2001; Leslie and Summerell 2006). It is nevertheless virtually impossible to describe a species of *Fusarium* using the MSR alone (Leslie et al. 2001; Seifert 2001), as there are not nearly enough differentiating features for each one of the hundreds if not thousands of *Fusarium* species that are known to exist (Guadet et al. 1989; Leslie et al. 2001; Kvas et al. 2009). Furthermore, morphological characters are often not stable as they are prone to change depending on environmental conditions (Leslie et al. 2001).

3.2.2 Biological species recognition

Towards the end of the twentieth century, it became clear that many species defined using the MSR included a number of different reproductively isolated entities (Leslie et al. 2007). Exploitation of sexual compatibility among heterothallic species, led to the application of the BSR in *Fusarium* (Leslie et al. 2007). According to the BSR, two individuals are able to mate with one another to produce fertile offspring when they belong to the same species and/or reproductive community (Dobzhansky 1950; Mayr 1963; Taylor et al. 2000).

Applying the BSR in order to identify *Fusarium* species can be time consuming, labour intensive and may not always generate unambiguous results. Not all species of *Fusarium* will produce a teleomorph in culture (Booth 1981; Kvas et al. 2009; Summerell

et al. 2010), while some are able to interbreed thus producing hybrid progeny (Desjardins et al. 2000; Steenkamp et al. 2001; Leslie et al. 2004, 2007). Also, routine use of the BSR requires development of female-fertile tester strains to be used in matings with unknowns (Klittich and Leslie 1992; Klaasen and Nelson 1996; Britz et al. 1999; Zeller et al. 2003). Should the existing DNA-based approaches for diagnosing the mating type (e.g., Covert et al. 1999; Kerényi et al. 1999; Steenkamp et al. 2000) of *Fusarium* isolates fail, application of the BSR becomes even more complex, as all the unknowns must then be paired with both of the testers and not only those of opposite mating type (Leslie and Summerell 2006).

The inclusion of the BSR in the taxonomy of *Fusarium* has contributed to the problems surrounding the dual nomenclature system for fungi. In the sexual species of *Fusarium*, both the anamorph (asexual state = *Fusarium*) and teleomorph (sexual state = *Gibberella*, *Nectria*, *Albonectria*, *Haematonectria*) stages are described and named (Booth 1971, Nelson et al. 1983; Samuels et al. 2001). But this is beginning to change, with the newly accepted approach of "One Fungus = One Name". The need for one fungus, one name has led to a number of decisions made at the XVIII International Botanical Congress which is providing options and action toward resolving the dual nomenclature system (Greuter et al. 2011; Hawksworth 2011a,b; McNeill et al. 2011; Norvell 2011, Rossman and Seifert, 2011; Wingfield et al. 2012) and which has already occured for *Fusarium* (Geiser et al. 2013), allowing the exclusive use and description of a species with just the anamorph name, even when a sexual structure is identified.

In a recent paper, Taylor (2011) outlines two important studies where species were described with just one name, regardless of the existence of both sexual states (Crous et al. 2006b; Houbracken et al. 2010). In both of these studies, authors believe that "One Fungus = One Name" is required to repair the taxonomic "mess" created by the combination of mycological nomenclature with that of botanical nomenclature. The problems with classification are further complicated by the increasing reliance on DNA-based techniques to identify both culturable and unculturable fungi, and their subsequent classification without a specimen demands the need to move away from

dual nomenclature and towards a new naming system with a single name such as a universal BioCode (Hawksworth et al. 2011).

3.2.3 Phylogenetic species recognition

The PSR identifies and separates species based on the reconstruction of evolutionary relationships in the form of a hierarchy, and subsequently interprets these relationships into a taxonomic system (Davis, 1996). The main principle behind the PSR is to represent the relatedness or evolutionary history between taxa (Leslie et al. 2001). The PSR thus does not consider the character itself but rather the history underlying its presence or loss (Baum and Donoghue 1995). Although a large number of different versions of the PSR are available (reviewed by Mayden 1997), Nixon and Wheeler's (1990) version of the 'diagnostic' PSR is most commonly employed in *Fusarium* (e.g., O'Donnell et al. 1998, 2000b; Steenkamp et al. 1999, 2000).

The PSR works well in almost all situations. Unlike the BSR, both homothallic and heterothallic species can be diagnosed (Leslie and Summerell 2006). The same is also true for apparently asexual species (Leslie and Summerell 2006) and species that can hybridize under laboratory conditions (Bowden and Leslie 1999; Leslie and Summerell 2006). Unlike the MSR, the genome sequences harbour an almost inexhaustible number of characters that can be used to delineate species. However, some authors argue that the results of the PSR may not always be biologically meaningful (Coyne and Orr 2004; Dayrat 2005). In such cases, the inclusion of data collected using the MSR and BSR, *i.e.*, the application of a so-called polyphasic approach, would aid the species identification and/or delineation process (Nirenberg and O'Donnell 1998; Britz et al. 2002; Summerell et al. 2003).

4. *Fusarium* diversity in the commercial forestry environments

Although there are not many *Fusarium* species that cause serious diseases of pine seedlings in South Africa, many are known from other parts of the world (Bloomberg 1981; Huang and Kuhlman 1990; Viljoen et al. 1992; Dick and Dobbie 2002). The large

majority of the *Fusarium* species occurring in the commercial forestry environment represent both saprotrophs or “non-pathogens”, but some are also pathogens (Nelson et al. 1983). This is true for various strains of species such as *F. oxysporum* (Booth 1971; Edel et al. 1995). The nature of many *Fusarium* species and their association with plants in forestry environments remains largely unknown. In the following sections, the *Fusarium* species associated with *Pinus*, *Eucalyptus* and *Acacia* in the commercial forestry environments are considered.

4.1 *Fusarium* species associated with *Pinus*

4.1.1 *Fusarium circinatum*

Fusarium circinatum (teleomorph = *Gibberella circinata*) is the causal agent of pitch canker (Dwinell et al. 1985; Correll et al. 1991; Viljoen et al. 1994; Nirenberg and O’Donnell 1998; Wingfield et al. 2008a). The fungus was originally referred to as an undescribed *Fusarium* species in 1946 and has since been renamed, a number of times, based on an increasing number of isolates, morphology, host association, biological crosses and phylogenetics (reviewed by Wingfield et al. 2008a). The first record of the disease was from the south-eastern United States on *Pinus virginiana* Mill. (Virginia pines) (Hepting and Roth 1946). Since then, pitch canker has spread to eleven countries on four continents including Haiti (Hepting and Roth 1953), Japan (Kobayashi and Muramoto 1989), Mexico (Santos and Tovar 1991), South Africa (Viljoen et al. 1994), Korea (Lee 2000), Chile (Wingfield et al. 2002b), Spain (Landeras et al. 2005), Italy (Carlucci et al. 2007), Uruguay (Alonso and Bettucci 2009), Portugal (Bragança et al. 2009) and Colombia (Steenkamp et al. 2012). Globally, *F. circinatum* is one of the most important and destructive pathogens of pines.

Fusarium circinatum can infect at least 57 susceptible species in the *Pinaceae* including *P. radiata, P. patula, P. elliottii,* and many others (Hepting and Roth 1953; Storer et al.1994; Hodge and Dvorak 2000; Wingfield et al. 2008a). The only non-pine host known for this pathogen is *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) (Gordon 2006). Reduced yields and high levels of tree mortality are commonly associated with pitch canker and this can lead to great economic losses.

The name “pitch canker” is derived from the symptoms usually observed on diseased trees. These are typically the large, dark resinous cankers that form on the main stems and lateral branches (Barnard and Blakeslee 1980). Cankers are formed as the result of the tree’s response to the attack of the pathogen by forming large amounts of (pitch) resin (Hepting and Roth 1946; Hodge and Dvorak 2000). Infection of susceptible pine tree by *F. circinatum* can affect both the reproductive and vegetative parts, in particular the woody bole, cones, seeds, shoots, roots and needle tissue (Dwinell et al. 1985; Viljoen et al. 1994; Hodge and Dvorak 2000; Wingfield et al. 2008a).

The pitch canker pathogen causes damping-off and post emergence root disease on seedlings (Viljoen et al. 1994; Dwinell et al. 1985; Wingfield et al. 2008a; Mitchell et al. 2011). In these nursery situations, infection greatly reduces seedling emergence (Wingfield et al. 2008a). Although seeds may appear pathogen-free, they can habour the fungus internally, and once germinated, the fungus can be isolated from the seedling shoots (Storer et al. 1998; Mitchell et al. 2011). Pre- and post-emergence damping-off of seedlings can cause mortality in established seedlings (Viljoen et al. 1994; Mitchell et al. 2011). The disease usually results in the wilting of seedlings and the under-development of new roots (Viljoen et al. 1994; Mitchell et al. 2011). In some cases, seedlings may be apparently asymptomatic, and only start expressing symptoms after experiencing stress (Mitchell et al. 2011). The latter is particularly a problem during post planting establishment of seedlings in plantations (Mitchell et al. 2011).

4.1.2 *Fusarium oxysporum*

Fusarium oxysporum is an asexual fungus that includes pathogens and so called “non-pathogens” (Burgess 1981; Appel and Gordon 1994; Edel et al. 1995; Fravel etal. 2003). This fungus is very common in soil (Burgess 1981; Appel and Gordon 1994; Kistler 1997; Baayen et al. 2000) and gained notoriety when it was found commonly occurring on agricultural crops (Gordon and Martyn 1997). Non-pathogenic *F. oxysporum* isolates are usually found on the cortex of a plant’s roots causing no disease and can also be found living in dead organic matter (Appel and Gordon 1994). Many

pathogenic *F. oxysporum* isolates produce typical wilt symptoms on their hosts (Edel et al. 1995) and is the result of the pathogen occluding the vascular tissues of the host plants which eventually kills the plant (Beckman 1987).

Fusarium oxysporum is commonly implicated in disease of *Pinus* species and Douglas fir. One of the first reports of *F. oxysporum* occurring in the forestry environment was in a British Colombia nursery (Bloomberg 1972) on Douglas fir seedlings (Bloomberg1981; Weiland et al. 2009). In pines, *F. oxysporum* has been reported as a causal agent of root rot in seedlings of *Pinus resinosa* Ait.(Farqguhar and Peterson 1989); eastern white pine (*Pinus strobes* L.) in Wisconsin, USA (Riffle and Strong 1960); damping-off in *P. ponderosa* Laws.(Salerno and Lori 2007); Caribbean pine (*Pinus caribaea* Morelet) and *P. kesiya* Royle. in Tanzania (Hocking 1968); and necrosis and wilting of *P. caribaea* (Mohali 1996). *Fusarium oxysporum* has also been reported as a pathogen on *P. patula* (Hocking et al. 1968), and shown to infect the roots of *Pinus contorta* Dougl.: Loud. (James and Gillian 1984), *P. ponderosa* Dougl.:P. and C. Laws. (James and Gillian 1988), *P. taeda* and *P. elliottii* Engelm. (Fraedrich and Dwinell 2003). In addition, it has been isolated from the soils of 30 nurseries across ten states of the USA (Hodges 1962) and in *P. radiata* bare-root nurseries in New Zealand (Dick and Dobbie 2002). However, from the literature available, it seems that the pathogen is more important in agriculture when compared with forestry, as it is associated with a variety of different ornamental, vegetable and fruit crops and causes greater damage (Nelson et al. 1981; Gordon and Martyn 1997; Leslie and Summerell 2006).

4.1.3 Other *Fusarium* species

There are a number of well-known cosmopolitan species of *Fusarium* that are often encountered in the pine forestry environment (James, 1984; Burgess et al. 1988; Carey and Kelley 1994). These species usually cause minimal damage or none at all and do not limit pine production significantly. These include, but are not limited to *F. avenaceum* Fr., *F. solani* and a few others.

Like *F. solani*, *F. avenaceum* is more important in the agricultural sector than forestry. This fungus predominantly infects grains (oats, barley and wheat) where it is important due to the mycotoxins it produces (Bottalico and Perone 2002; Parry et al. 1995; Uhlig et al. 2007). Furthermore, it causes post-emergence damping-off of young *Pinus* seedlings (Bloomberg 1981), such as Scots pine (*Pinus sylvestris* L.) (Lilja et al. 1995) and is associated with root necrosis (Asiegbu et al. 1999). *F. avenaceum* has also been implicated in root disease in western white pine (*Pinus monticola* Dougl.) nurseries in the United States (James 2007).

Fusarium solani is a well-known soil-borne fungus capable of causing disease on over 100 plant species in over 80 genera (Kolattukudy and Gamble 1995). Although there have been a number of reports, since 1931 of *F. solani* causing disease on pines and other conifers (reviewed by Bloomberg 1981), the frequency with which this pathogen has been encountered in the nursery environment is much lower than that for *F. oxysporum* (Hocking 1968; Bloomberg 1981). Nevertheless, *F. solani,* along with other *Fusarium* species, was isolated from damped-off and healthy seedlings of *P. caribaea* and *P. kesiya* (Hocking 1968).The fungus has also been reported from seedlings of Douglas fir (James 1983), white pine (*Pinus albicaulis* Engelm.) (James and Burr 2000), in Monterey pine bare-root nurseries (Dick and Dobbie 2002), as well as being a causal agent of pre- and post-emergence diseases of Colorado Blue Spruce (*Picea pungens* Engelm.) (James 1985), and chlorosis, root necrosis and wilting of Caribbean pine (*Pinus caribaea*) (Mohali 1996).

Other *Fusarium* species reported from *Pinus* species include *F. moniliforme*, *F. equiseti* Corda. Sacc., *F. sambucinum* Fuckel., and *F. proliferatum*. *Fusarium moniliforme* and *F. equiseti*, were isolated from diseased *P. caribaea* and *P. kesiya* nursery seedlings from East Africa (Hocking 1968) and the diseased roots of *P. ponderosa* (Salerno and Lori, 2007). It is important to record here that *F. moniiforme*, represents an obsolete taxon that can refer to any number of species in *Fusarium* section Liseola or the so-called *Gibberella fujikuroi* complex (Seifert et al. 2004). *Fusarium equiseti* has been isolated from diseased *P. taeda* (Solel et al. 1988), and damping-off *P. ponderosa* seedlings (Salerno and Lori 2007). *Fusarium proliferatum* has been found colonizing the seedlings

and germlings of white pine (*P. albicaulis*) (James and Burr 2000), while *F. sambucinum* and *F. acuminatum* Ellis and Everhart. have been reported from *P. ponderosa, P. contorta* (James and Gillian 1984) and from root rot symptoms on *P. pondersoa* (Salerno and Lori 2007).

4.2 *Fusarium* species associated with *Eucalyptus*

4.2.1 *Fusarium graminearum*

Fusarium graminearum (teleomorph: *Gibberella zea*) usually occurs on maize and wheat plants (Sutton 1982; Ouellet and Seifert 1993; Desjardins and Hohn 1997) and was first reported from *Eucalyptus grandis* in South Africa in 2001 (Roux et al. 2001). The pathogen was isolated from diseased seedlings and used in pathogenicity tests to show that it can cause disease. However, the possible role of this wheat and maize pathogen on *Eucalyptus* species remains unknown. In some studies, the occurrence of *F. graminearum* on *Eucalyptus* has only been mentioned, although no pathogenicity tests were performed (Bettucci and Alonso 1997; Gezahgne et al. 2003).

4.2.2 *Fusarium oxysporum*

Despite the prevalence of *F. oxysporum* in soil, especially in plant nursery environments (Burgess 1981), this fungus is not commonly associated with *Eucalyptus* species. *F. oxysporum* has, however, been associated with eucalypts (Keane et al. 2000), including reports of root rot and stem cankers (Sharma et al. 1984) and root rot and necrosis in *Eucalyptus* nurseries (Salerno et al. 2000).

4.2.3 Other species of *Fusarium*

In the published literature, there are many reports of *Fusarium* on *Eucalyptus* species, although the available information on the specific species of the fungus is limited or questionable. Also, little is known about *Fusarium* species occurring on *Eucalyptus* in South Africa (Viljoen et al. 1992). Many of the reports from around the world were published before 1990 and there has been little or no mention of these fungi on *Eucalyptus* hosts subsequently (Keane et al. 2002). In this regard, the motivation is that

these *Fusarium* species, at the time, were considered unimportant or regarded as secondary colonists and as mentioned above, their taxonomy was also confused and poorly defined.

Fusarium solani has been associated with vascular wilt of *Eucalyptus* species (Kumar and Nath 1988) and damping-off of *Eucalyptus* species in Argentina (Salerno et al. 2000), while *F. equiseti* has been associated with damping-off of young *Eucalyptus* cuttings in Argentina (Salerno et al. 2000). Most other reports dated before 1990 and have been summarized in a book by Keane et al. (2000), which considers all of the diseases occurring on *Eucalyptus* and includes mention of *F. equiseti*, *F. graminearum, F. moniliforme*, *F. oxysporum*, *F. poae*, *F. semitectum* Berk. and Rav., *F. solani* as well as other *Fusarium* species.

4.3 *Fusarium* associated with Acacia species

Particular diseases associated with *A. mearnsii,* both locally and globally, were reviewed by Roux et al. (1995). In 1997, a number of *Fusarium* species were isolated from various diseased *Acacia* species. These included *F. acuminatum*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani* and *F. subglutinans* (Roux and Wingfield 1997). The results of the pathogenicity studies have, however, indicated that none of these represent the primary causes of the observed disease symptoms. Later, in 2001, it was determined that *F. graminearum* was the causal agent of the cankers and die-back symptoms on *A. mearnsii* (Roux et al. 2001), although the exact role of this pathogen on *Acacia* is still unknown.

In Hawaii, *F. oxysporum* f. sp. *koae* Gardner is the causal agent of koa die-back and wilt of *Acacia koa* trees (Gardner 1980). Later studies on *A. koa* have shown that multiple species of *Fusarium* could be isolated from this host (James, 2004; James et al. 2006). These included *F. oxysporum*, *F. subglutinans*, *F. semitectum*, *F. equiseti*, *F. solani*, and the less frequently *F. avenaceum*, *F. acuminatum*, *F. sambucinum* and *F. sporotrichioides* Sherb. were also isolated (James et al. 2006). Of these, only *F. oxysporum* was shown to represent the primary pathogen, while the others are thought to be secondary pathogens or non-pathogenic fungal endophytes (James et al. 2006).

5. Control of *Fusarium*

In the commercial forestry environment, losses due to disease can only be prevented or restricted with effective control of pathogens. However, *Fusarium* diseases are more difficult to manage than others because many species (i) thrive in soils, (ii) have broad host ranges and (iii) produce reproductive structures that can survive in harsh environments. Nevertheless, like the pitch canker pathogen, *Fusarium* species can be controlled by applying an integrated management approach (Wingfield et al. 2008a; Mitchell et al. 2011).

There are three main strategies for the control of pathogens in the nursery, i.e., cultural, biological, and chemical methods (Viljoen et al. 1992; Mitchell et al. 2011). Although a number of agricultural crop diseases may be controlled using biological control or biocontrol agents (i.e., formulations of non-pathogenic microorganisms used to control pathogens), little or no success has been achieved using this approach in commercial forestry nurseries (Kelly 1976; Huang and Kuhlman 1991; Nel et al. 2006). Cultural and chemical control techniques thus provide a better alternative for disease management.

Cultural methods for pathogen control involve prevention of disease by creating an environment that is unfavourable for the pathogen, for infection, or for disease development (Civerolo 1982). This typically means reducing stress to the plants and eradicating conditions that would otherwise allow for disease development (Gordon et al. 2001; Wingfield et al. 2008a). Good nursery practices and hygiene thus aim to keep pathogens out and seedlings healthy. The latter approach is particularly important in terms of *Fusarium* species, as many occur commonly in soil where they persist as saprophytes that can become pathogenic when they happen to encounter a plant host with reduced resistance or under stress (Nigh 1990; Fisher and Pertrini 1992).

In the nursery environment, the aim of chemical control is usually to prevent the establishment of disease (Viljoen et al. 1992). The use of chemical control has always been popular because of its easy use and rapid deployment. Mitchell *et al*. (2011) recently reviewed some of the popular means for controlling *Fusarium* before it

becomes established and include methods such as soaking seed in diluted solutions of ethanol, hydrogen peroxide and hypochlorite, the sterilization of irrigation water using calcium hypochlorite (chlorine), steam treating seedling trays to list but a few approaches.

Once *Fusarium* has become established in a nursery, fungicides are often used to control the fungus. Although effective at controlling the target organism, there are many environmental concerns *i.e.*, fungicide resistance (Staub and Sozzi 1984) and toxicity (Wingfield et al. 2008a). Before it was banned by the Forestry Stewardship Council (FSC 2002), effective fungicides usually contained the active ingredient Benomyl (Mitchell et al., 2011). Although there are alternatives to Benomyl, they have all been shown to be ineffective, while others remain untested (reviewed by Mitchell et al. 2011).

Control of pathogens in the field can best be achieved by deploying resistant planting stock (Wingfield et al. 2008b; Mitchell et al. 2011). Improvement of silvicultural practices can also reduce the amount of stress on planted tree species and improve yield, which is necessary for long term forestry management (Allen 1998, 2001; Pallet and Sale 2004; Wingfield et al. 2008a,b). Today, the trees in plantations are bred for resistance in the form of hybrids and clones, which is the best line of defence against an attacking pest or pathogen of *Pinus* (Carson and Carson 1989; Burdon 2001; Sniezko 2006), *Eucalyptus* (Carson and Carson, 1989; Namkoong 1991) and *Acacia* species (Vengadesan et al. 2002; Midgley and Turnbull 2003).

6. Climate change and its impact on *Fusarium* species

The development of any disease on a plant host is the direct result of favourable environmental conditions, susceptibility of the host and pathogenicity of the disease causing agent. Therefore, environmental and host factors influence the dissemination, growth and survival of *Fusarium* species, thus also influencing their incidence in the environment (Doohan et al. 2003). There have been many studies on the influence that host species have on the toxicity and pathogenicity of *Fusarium* species (Miedaner 1997; Miedaner et al. 2001; Magg et al. 2002). The role that climate has on *Fusarium*

and its biology, however, is difficult to interpret as *Fusarium* species can cause disease in complex infections or on their own (Doohan et al. 1998). There are also a number of reports on the different responses of *Fusarium* species to environmental variation, particularly humidity and temperature (Doohan et al. 2003). Although Doohan et al. (2003) stated that temperature and humidity are the main factors influencing the development of *Fusarium* diseases on cereal crops, the same effects can, conceivably, be associated with other *Fusarium* species and other hosts.

Environmental conditions have an obvious effect on a plant host because of the plant's physiology which in turn determines a plant's distribution (Coakley et al. 1999). In addition, climate can also affect the plant's distribution indirectly by affecting a pathogen or pest (Coakley et al. 1999; Ayres and Lombardero 2000). The outcome of such an interaction could be either positive or negative. A positive affect may increase the aggressiveness of a pest or pathogen, while a negative affect could make the environment more unfavourable preventing the pest or pathogen from spreading, which will affect a host's population and distribution (Coakley et al. 1999; Ayres and Lombardero 2000).

It has been predicted that global warming will lead to an increase in pathogen numbers (Coakley et al. 1999; Ayres and Lombardero 2000; Dale et al. 2001; Harvell et al. 2002), because of longer growth seasons and higher reproduction rates, especially in currently colder environments (Roy et al. 2004). This could in turn increase the impact of pests and pathogens (Chakraborty and Datta 2003), the effects of which would be even more pronounced with changes in environmental conditions and host resistance. However, more research in this area is needed, particularly to determine (i) the capacity of pathogens to adapt to new environments (ii) if this would be a global trend or if it would be confined to particular pathogens and their respective plant hosts, (iii) the damage one might expect from these environmental changes, and (iv) how the host and pathogen interaction would change over time.

The elevation in CO_2 levels that is likely to accompany global warming will have profound effects on the growth of pathogens. This idea is well illustrated in the results of a study on *F. pseudograminearum* Aoki and O'Donnell, which is responsible for the

crown rot disease on wheat (Burgess et al. 2001). Under laboratory conditions, Melloy et al. (2010) showed that elevated levels of CO_2 increased the biomass of the pathogen per unit host tissue but that it did not affect the saprophytic fitness of *F. pseudograminearum*. Also, with the use of various linked models for *Fusarium* head blight (caused by *F. graminearum* and *F. culmorum*) it has been reported that the incidence of *Fusarium* head or ear blight on wheat will increase in the future, more dramatically than has been recorded in the past (Fernandez et al. 2004; Madgwick et al. 2011).

On the tropical pasture legume *Stylosanthes scabra* Vog., elevated levels of CO_2 and a favourable microclimate for anthracnose development could accelerate the evolution of the pathogen *Colletotrichum gloeosporioides* (Penz.) Sacc. (Chakraborty and Datta 2003). In contrast, it is widely expected that elevated levels of CO_2 will increase crop yield and photosynthesis (Cure and Acock, 1986). This has been demonstrated for various agriculturally important crops (Cure and Acock 1986; Ainsworth and Long 2005), as well as *Quercus coccifera* L. (scrub oak) (Reynolds et al. 1992), *Pinus taeda* (Tissue et al. 1997) and *S. scabra* (Chakraborty et al. 2000), amongst others. Thus, while plants might grow more effectively, they will also need to concurrently deal with pathogens in the changing environment. Overall, changing climates will affect plants, their physiology, their resistance to invasion and their distribution. Increased temperatures and rising CO_2 levels may increase photosynthetic activity and improve crop yield in certain parts of the world, but it may also be counteracted by plant stress, for example drought or heat stress (Semenov 2009).

The increased temperatures will influence the rate of pathogen reproduction, which will increase the number of generations a pathogen can undergo thus increasing the evolutionary rates (Garrett et al. 2006). Therefore, the most important factor for plants in response to climate change will be adaption (Davis et al. 2005), which might be difficult, especially in the case of trees that grow very slowly (Etterson and Shaw 2001). It thus seems that plant pathogen evolution will leap ahead of the evolution of host plant resistance, resulting in more severe disease symptoms. Although this is a

commonly held generalisation, it may not be true for every pathosystem (Garrett et al. 2006).

In a recent review, Sturrock et al. (2011) considered some well-known forest pathogens and the influence that warmer, drier or warmer, wetter weather could have on them and the diseases they cause. In terms of *Fusarium, these authors* used *Fusarium* circinatum, a "group 2" pathogen and thus one where moisture and temperature more directly affect the susceptibility of the plant host and not the pathogen. Other *Fusarium* species associated with forestry will also most likely behave in this way as they too are influenced by plant stress. Much of this is speculation and there are many factors involved that influence both plant and pathogen alike and the resulting situations will only be fully understood in the future.

7. Conclusions

The commercial forestry industry contributes to the GDP (Gross Domestic Product) in many countries around the world (Sedjo 1999). The lack of native, fast growing trees suitable for commercial plantation in South Africa led to the planting of non-native, fast growing trees such as species of *Pinus*, *Eucalyptus* and *Acacia*, which formed the foundation of commercial forestry in South Africa (Van der Zel and Brink 1980; Geldenhuys 1997; Sedjo 1999). The advantages of using these species, particularly non-natives, is the short rotation times and the fact that they are planted away from the pest and pathogens occurring in their native ranges (Wingfield et al. 2001a, b). The distances between South Africa and their native ranges are no longer sufficient to shield these trees from their native pests and pathogens, with humans moving increasingly rapidly between these boundaries (Levine and D' Antonio 2003).

The genus *Fusarium* includes some of the world's most important plant pathogens and thus plays an important role in both the agricultural and forestry industries (Bloomberg 1981). The most important example of a *Fusarium* species in forestry is the pitch canker pathogen *F. circinatum*. Since its discovery in 1946 as an unknown *Fusarium* sp. (Hepting and Roth 1946), the pitch canker pathogen has changed its name five times, when in 1998 it was finally described as a member of the

so-called *Gibberella fujikuroi* species complex (Nirenberg and O'Donnell 1998). The name changes, over the last 40 years, were the result of changing taxonomic concepts. Importantly, DNA-based techniques and phylogenetic species concepts (reviewed by Wingfield et al., 2008a; Kvas et al. 2009.) have had huge influence in this process. The use of DNA-based methods has shown that it improves the identification of species, both rapidly and accurately, which is important for diagnosis and quarantine.

The world's climate is changing more rapidly than originally expected. This complicates predictions of pathogen emergence and spread, although climate change will undoubtedly have a huge effect on plant-pathogen interactions. Some believe that these changes will favour pathogens. For the genus *Fusarium,* this is important as many of these species, although not serious pathogens in commercial forestry, can cause disease when host plants are under stress. It is, therefore, necessary to determine the diversity of *Fusarium* in these environments, focusing not only on the known species but also the unknowns and the uncommon species, and to characterize them with the most rapid and precise means possible. Information regarding these issues will be valuable for the development of effective disease management strategies and to more accurately understand and predict pathogen emergence.

This review has attempted to consider all *Fusarium* species associated with *Pinus*, *Eucalyptus* and *Acacia* species, but it is clear that available information is quite limited. To improve knowledge, studies need to be conducted in environments not only where important pathogens are involved, but also in environments where the diversity of various saprophytic, secondary and opportunistic species are unknown. This will be important for the commercial forestry industry in the future, especially when considering climate change.

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Chapter 2

Novel *Fusarium* species in the *Gibberella fujikuroi* species complex from Pinus spp. in Colombia

Abstract

Devastation in many parts of the world caused by the pitch canker fungus *Fusarium circinatum* has drawn attention to the potential importance of *Fusarium* species in forestry. In this study, we explored the diversity of *Fusarium* species associated with diseased *Pinus patula, P. tecunumanii*, *P. kesiya* and *P. maximinoi* in Colombian plantations and nurseries. Plants displaying symptoms typical of *F. circinatum* infection including stem cankers and branch die-back on trees in plantations and root or collar rot of seedlings were targeted for sampling. A total of 57 isolates were collected and characterized based on analyses of DNA sequence data for the TEF and β-tubulin gene regions. Ten species where recovered including *F. circinatum, F. oxysporum*, *F. solani* and five novel species described here as *Fusarium parvisorum* sp. nov., *F. fracticaudum* sp. nov., *Fusarium marasasianum* sp. nov., *F. pinemorale* sp. nov. and *F. sororula* sp. nov. Selected isolates of the five new species were tested for their pathogencity on *Pinus patula*. *F. pinemorale* sp. nov. and *F. parvisorum* sp. nov. displayed levels of pathogenicity to *Pinus patula*, that were comparable with that of *F. circinatum*. These are apparently emerging pathogens that are potentially important to forestry in Colombia. They could also cause damage similar to that associated with the pitch canker pathogen should they move to a new environment. These species are an excellent example of an emerging pathogen which poses a significant risk to forestry in Colombia and other parts of the world.

Key words: *F. fracticaudum*, *F. marasasianum*, *Fusarium parvisorum*, *F. pinemorale*, *F. sororula*, morphology, pathogenicity, phylogenetics, *Pinus kesiya*, *P. maximinoi*, *P. patula, P. tecunumanii*

1. Introduction

During the course of the past decade, the incidence of plant diseases in forest ecosystems has increased dramatically (Orwig 2002). This is primarily due to anthropogenic activities (e.g., Anagnostakis 2001; Wingfield, et al. 2001; Wingfield et al. 2008b; Garnas et al. 2012) and the disruption of forest ecosystems (Liebhold et al. 1995; Jactel et al. 2009). The emergence of disease is particularly emphasized where native ecosystems have been disrupted by the planting of extensive areas to forest monocultures, especially exotic species (Chou 1991; Bradshaw et al. 2000; Wingfield et al. 2001, Scholthof 2006, Jactel et al. 2009). For example, in the Southern hemisphere large areas are planted to monocultures of exotic *Pinus* L. and *Eucalyptus* L'Heritier species (Wingfield 2003), which are typically located within or near natural woodlands and forests (Richardson et al. 1994; Ayala et al. 2005; Sano et al. 2010; da Silva et al. 2011). In such areas where native and commercial forestry ecosystems co-occur, the risks associated with the emergence of new plant diseases are significantly increased (Perkins and Matlack 2002; Tommerup et al. 2003; Wingfield et al. 2010; Blitzer et al. 2012).

The forests in Colombia, together with those in Brazil, Peru, Bolivia and Venezuela make up approximately 84% of South America's total afforested area (FAO 2012). Of the *ca.* 60.5 million ha of forests in Colombia, only 405,000 ha represent commercial plantations (FAO 2005; FAO 2010). *Pinus* species represent approximately 35% of the commercially planted species in Colombia (IDEAM 2009). Although the forestry industry of this country is relatively young, a number of diseases and pests associated with *Pinus* have been reported. For example, Rodas (1998) recorded 30 different native species of defoliating insects occurring on exotic plantation species in the Andean region of Colombia. More recently, *Fusarium circinatum* Nirenberg and O'Donnell emend. Britz, Coutinho, Wingfield and Marasas, the causal agent of pitch canker, was also reported from diseased seedlings and established *Pinus* species in Colombia (Steenkamp et al. 2012). As time passes, the number of emerging pests and pathogens will likely increase in Colombia as native organisms adapt to infest/infect non-native trees and as new organisms are accidentally introduced into the country.

The economically important genus *Fusarium* Link has a global distribution (Leslie and Summerell 2006) that includes numerous species which produce toxic secondary metabolites and cause disease of plants, animals and humans (Leslie and Summerell 2006). Notable examples include *Fusarium solani* (Mart.) Appel & Wollenweber emend. Snyder & Hansen and *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen. Apart from the human diseases these species cause, both are commonly found in soil and have been associated with a range of diseases of various plants (Matuo and Snyder 1973; Nucci and Anaissie 2002; Pietro et al. 2003; Zhang et al. 2006). However, other than the one report of *F. circinatum* associated with *Pinus* species in Colombia (Steenkamp et al. 2012), limited information is available regarding the *Fusarium* species and the diseases they cause on commercial forestry hosts, especially *Pinus* species. Most previous reports and studies have focused on agricultural crops and ornamentals; e.g., *F. oxysporum* causing disease on potatoes (Bayona et al. 2011), carnations (Arbeláez 1987; Brayford 1996; Garibaldi et al. 2011) and coffee (Bustillo et al. 1998) and *Fusarium* species causing disease on African oil palm (Stover 1981).

The study by Steenkamp et al. (2012) explored the presence of the pitch canker fungus, *F. circinatum*, on *Pinus* species in Colombia but also found a number of other *Fusarium* spp. that were frequently and/or consistently encountered (unpublished data). All of these other fungi were also isolated from *Pinus* seedlings or established plantation trees, showing symptoms typical of infection with *F. circinatum*. On seedlings, the symptoms included wilting, root and collar rot, and on established plantation trees they included stem cankers, branch and tip-die back (Steenkamp et al. 2012). These symptoms, particularly those on established or mature *Pinus* trees, are uniquely associated with the pitch canker fungus, and have never been shown to be the result of infection with another fungal species. Knowledge regarding the identity and pathogenicity of these isolates is thus crucial to realistically quantify the risks they pose to *Pinus*-based plantation forestry in Colombia and other parts of the world, as well as where *Pinus* species occur naturally.

The first aim of this study was to identify the *Fusarium* species associated with diseased *P. patula* seedlings and with *P. patula*, *P. tecunumanii, P. kesiya* and *P.*

maximinoi trees in plantations showing symptoms of pitch canker in Colombia. This was accomplished using conventional morphology and culture-based approaches together with the DNA sequence information for the genes encoding the translation elongation factor 1 alpha (TEF) and β-tubulin (BT). These data were also used to provide descriptions for the new *Fusarium* species recognized. An additional aim of this study was to determine the pathogenicity of the identified fungi on *Pinus* and to determine whether they could have been responsible for the symptoms observed.

2. Materials and Methods

2.1 Collection of Fungal isolates: The *Fusarium* isolates used in this study were collected from a number of different locations and hosts in Colombia (Table 1). These included *Pinus tecunumanii*, *P. maximinoi, P. kesiya* and *P. patula* trees showing signs of pitch canker-like infections in plantations in or near Calima, El Darién (Valle del Cauca); Angela Maria (Risaralda), Volconda (Valle de Cauca), Campania, Riosucio (Caldas), Aguaclara, La Cumbre (Valle de Cauca), and El Guasimo (Antioquia). Isolates were also obtained from symptomatic (*i.e.*, wilting, root rot, root collar and stem discolouration) *P. patula* seedlings in nurseries in Vivero, Peňas Negra (Valle de Cauca), Vivero, Bandeja (Valle de Cauce) and Vivero, Canaleta (Valle de Cauca).

Diseased plant tissue was surface disinfected for 1 min in a solution containing 1.5 % (v/v) sodium hypochlorite, rinsed with sterile distilled water and immersed in 70% (v/v) ethanol for 1 min and air dried. Small pieces, cut from the leading edges of the symptomatic tissue, were plated directly onto half-strength potato dextrose agar medium (PDA; 20g/L PDA, 15g/L Agar; Biolab Diagnostics, Wadeville, South Africa) and *Fusarium* selective medium (FSM, Nash and Snyder, 1962). Following incubation at 27.5℃, isolates resembling *Fusarium*, according to Nelson et al. (1983), were transferred to fresh PDA and grown for seven days at 23℃. From these pure cultures, conidia were washed with a 2.5% (v/v) Tween 60 (Sigma-Aldrich, St Louis, Missouri, USA) solution and 1mL of the spore suspension was spread across a water agar medium (WA;20g/L PDA; Biolab Diagnostics). Following incubation at 16℃ for two days, single germinating conidia were transferred to fresh PDA and incubated for seven

days at 23℃. All of the cultures collected for this study are maintained in the *Fusarium* Culture Collection (CMWF) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

2.2 DNA Isolations, PCR amplification and sequencing: DNA was isolated from seven day old cultures using a modified CTAB (hexadecyltrimethylammonium bromide) method (Steenkamp et al. 1999). Briefly, fungal tissue was scraped directly off the surface of the growth media and homogenized in 500 µL DNA extraction buffer containing 100mM Tris-HCl (pH 8.0), 10 mM ethylenediaminetetra-acetic acid di-sodium salt (EDTA; pH 8.0), 2.0% (w/v) sodium dodecyl sulphate (SDS) and 0.2 µg/µL proteinase K (Sigma-Aldrich, St Louis, Missouri, USA). Following incubation for 1 h at -24℃ and then at 60℃ for 1h, 180µL of 5 M NaCl and 80µL 10% (w/v) CTAB solutions were added. Following a final incubation at 65℃ for 10 min, standard phenol-chloroform extractions (Sambrook et al. 1989) were performed. Nucleic acids were precipitated overnight at -20℃ in the presence of 0.6 volumes of isopropanol. Precipitates were harvested after centrifugation (10000 x g) at 4℃ for 30 min, washed with 70% ethanol, air dried and dissolved in 40 µL sterile distilled water. Extracted DNA was visualized and quantified, using agarose gel electrophoresis (Sambrook et al. 1989) and a Nanodrop spectrophotometer (Nanodrop, Wilmington, USA), and stored at -20℃ until further use.

Specific regions of TEF and BT were amplified with a Bio-Rad iCycler (Bio-Rad, California, USA) using, respectively, primers EF-1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and EF-2 (5'-GGA(G/A)GTACCAGT(G/C) ATCATGTT- 3') (O'Donnell et al. 1998; Geiser et al. 2004) and primers T-1 (5'-AACATGCGTGAGATTGTAAGT-3') and T-2 (5'-TAGTGACCC TTGGCCCAGTTG-3') (O'Donnell and Cigelnik 1997). Each amplification reaction contained 2-4 ng/µL DNA, 0.25 µM of each primer, 200 µM dNTPs (Fermentas, Nunningen, Germany), 2.5 mM $MgCl_2$, 0.04 U/µL of Supertherm *Taq* polymerase and reaction buffer with KCl (Southern Cross Biotechnology, Cape Town, South Africa). PCR cycling conditions consisted of 35 cycles at 94℃ for 30 seconds, 54℃ (BT) and 56℃ (TEF) for 45 seconds, 72℃ for 1 minute. Each PCR reaction followed an initial denaturation step at 95℃ for 3 minutes and was concluded by a final extension step at 72℃ for 10 minutes.

PCR products were purified using the polyethylene glycol method (Steenkamp et al. 2006) or G50 Sephadex columns (Sigma, Steinheim, Germany). The purified samples were then sequenced in both directions using the original PCR primers, an ABI PRISM Big Dye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and an ABI PRISM 3100™ DNA sequencer (Applied Biosystems). Electropherograms were examined and manually corrected where necessary using Chromas Lite 2.0 (Technelysium, Australia) and BioEdit version 7.0.5.2 (Hall 1999). The TEF nucleotide sequences were compared to those in the *Fusarium* identification database (Geiser et al. 2004; http://isolate.fusariumdb.org) using the basic local alignment search tool BLAST search program (Altschul et al. 1990).

2.3 Phylogenetic analyses: Multiple sequence alignments were generated with MAFFT version 6.0 (http://mafft.cbrc.jp/alignment/software/) with the L-INS-i option selected (Katoh et al. 2002, 2005; Katoh and Toh 2008) and corrected manually where needed. The two datasets constructed for TEF and BT, contained all the sequences generated in this study and the recognized species and phylogenetic lineages in the *Gibberella fujikuroi* complex, as well as the outgroup species *F. oxysporum* and *F. inflexum* (Table 2). The combinability of the datasets was tested using the partition-homogeneity test (Farris et al. 1995) implemented in PAUP version 4b10 (Swofford 2003) on parsimony informative sites only (Dolphin et al. 2000; Lee 2001; Darlu and Lecointre 2002) based on 1000 repartitions and heuristic searches using 100 rounds of random sequence additions with tree bisection reconnection branch swapping.

MrBayes version 3.2.1 (Heulsenbeck et al. 2001) and PhyML version 3.0 (Guindon et al. 2010) were used to generate phylogenies based on Bayesian inference (BI) and Maximum Likelihood (ML), respectively. The best-fit parameters, as indicated by jModelTest 0.1.1 (Posada 2008), for ML analyses of the TEF and β-tubulin datasets included gamma correction (G) to account for among site rate variation and the TIM2ef and TIM2 (Posada 2003) models, respectively. BI analysis of these datasets utilized the General Time Reversible (GTR) model (Tavare 1986) with G. BI and ML analysis of the combined dataset also utilized GTR+G. ML branch support was estmated using bootstrap analysis based on 1000 pseudo replicates and model parameters as before.

The BI analyses were based on 6 000 000 generations using one cold and three heated chains, and Bayesian posterior probabilities were calculated after discarding a burn-in corresponding to approximately 75 000 generations post-stationarity. BI-based analysis of the combined dataset utilized separate model parameters for each gene (Heulsenbeck et al. 2001). Phylogenetic trees were viewed and edited using MEGA 4 (Tamura et al. 2007).

2.4 Morphology: Isolates were examined using an Axioskop 2 plus microscope equipped with an ICc 3 Axiocam digital camera (Carl Zeiss Ltd, Germany). Images were captured and annotated with Axiovision SE64 Release 4.8 software (Carl Zeiss Ltd, Germany). The morphological characters of ten isolates, two for each of the five purportedly novel species as determined by phylogenetic analyses (see below), were studied. These isolates were as follows: Clade 1 (CMW 25237; CMW 25245), Clade 2 (CMW 25267; CMW 25268), Clade 3 (CMW 25253; CMW 25261), Clade 4 (CMW 25243; CMW 25244) and Clade 6 (CMW 25254; Colombia 21). The morphological characteristics examined included microconidia, macroconidia and conidiophores. Measurements of the micro- and macroconidia were done on 7 and 14-day-old cultures grown on carnation leaf agar (CLA; 20 g/L agar Biolab Diagnostics, 5-6 carnation leaf pieces). Microscope slides of each isolate were made with 85% (v/v) lactic acid (Sigma-Aldrich, St Louis, Missouri, USA) and 25 to 50 measurements were recorded. The sizes of the microconidia and macroconidia were recorded as minimum-maximum (average). The characteristics of the specimens were described based on the species descriptions of Leslie and Summerell (2006).

For all isolates, the colony reverse colour was observed on full-strength potato dextrose agar (PDA: 15 g/L potato dextrose agar, 20 g/L agar) after incubation at room temperature, either in the dark or under near-UV light. Colony colours (surface and reverse) were described using the colour charts of Rayner (1970). Growth rates were determined on full-strengh PDA in 90 mm Petri plates at 10–35 °C at 5 °C intervals after 8 days in the dark on PDA. The growth studies were performed in triplicate.

2.5 Pathogenicity trials: Two sets of pathogenicity tests were conducted. The first test included 12 isolates that were inoculated onto 4-month-old *P. patula* seedlings in a

glass house (Table 3). These isolates included at least one representative of each of the purported novel species or phylogenetic lineages discovered in this study. The inoculants for these tests were prepared by growing the isolates on full-strength PDA for 10 days at 25℃, after which the spores were washed off the cultures using sterile distilled water. These spore suspensions were filtered through cheese cloth and adjusted to a concentration of 1 x 10^6 spores/mL using a haemacytometer. Each isolate was inoculated onto 40 seedlings by first cutting the growth tips from the tops of the seedlings, approximately 1 cm from the top, and then placing a 1 mL drop of the spore suspension onto the cut end using a pipette (Porter et al. 2009). The seedlings used for the negative controls were treated in the same manner, except that 80 trees were used and distilled water replaced the spore suspension. The seedlings were arranged using a randomized block design and maintained in the greenhouse. After 8 weeks, disease severity was measured by measuring the lesion length from the inoculation site to the leading edge of the lesions down the stems. The entire trial was repeated once.

The second set of pathogenicity tests were conducted on 6-month old *P. patula* seedlings. For these tests, the four *Fusarium* isolates, CMW 25243, CMW 25247, CMW 25267, CMW 25269, were selected based on their results from the first test. For comparative purposes an aggressive strain of *Fusarium circinatum* (FCC 3579) was also included (Table 4). Inoculations, and the negative control, were performed as described for the first test on 20 seedlings for each treatment, using a randomized block design. The entire trial was repeated once.

An ANOVA (Analysis of Variation) was used to determine significant differences within and between treatments for the first pathogenicity test and the Duncan Multiple Range Test was used to compare treatment differences (SAS institute 2009). For the second test an ANOVA and a Duncan's Multiple Range Test was used with the EXCEL® Add-in macro DSAASTAT v 1.101 (Onofri 2006) to determine significant differences within and between treatments. After conclusion of the pathogenicity trials, Koch's postulates were confirmed by making use of re-isolations from the diseased seedling tissues and using TEF sequence data for a representative set of isolates to confirm that the inoculated fungi were responsible for the lesions. .

3. Results

3.1 Fungal isolates: A total of 57 isolates resembling those in the genus *Fusarium* were recovered from the diseased plant material examined in this study. These isolates were collected in Colombia from *P. patula* seedlings in nurseries, and from trees in established plantations of *P. tecunumanii, P. maximinoi* and *P. kesiya* (Table 1). All of the trees and seedlings sampled produced similar symptoms to trees and seedlings infected with *F. circinatum* (Wingfield et al. 2008a).

3.2 Sequence analysis: Comparison of the TEF sequences against those in the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the *Fusarium* Identification database (Fusarium-ID) (http://isolate.fusariumdb.org/) revealed that 49 of the 57 *Fusarium* isolates examined in this study represented members of the *G. fujikuroi* complex. Of these, 21 isolates displayed 97-99% TEF sequence similarity to *F. circinatum*. The sequences for six isolates were 98-99% similar to that of *F. begoniae* Nirenberg & O'Donnell and 22 isolates were 97-99% similar to that of *F. sterilihyphosum* Britz, Marasas & Wingfield. The remaining eight isolates including CMW 25503 and CMW 25504 shared a 98-100% TEF sequence similarity with members of the *F. oxysporum* species complex while CMW 25505, CMW 25506, CMW 25507, CMW 25509, CMW 25514, CMW 25515 shared a 98-100% TEF sequence similar to members of the *F. solani* species complex.

3.3 Phylogenetic analyses: The aligned TEF and BT datasets respectively consisted of 675 and 552 nucleotide bases. The partition-homogeneity test supported combination of the two datasets (p=0.01) with the TEF+BT dataset consisting of 1220 bases. ML and BI analyses of these datasets generated trees (Figure 1, 4 and 5) with topologies resembling those previously recovered from these gene regions (O'Donnell et al. 1998; O'Donnell et al. 2000; Geiser et al. 2005) in which the *G. fujikuroi complex* is separated into three large clades (*i.e.*, the so-called "American", "Asian" and "African" clades. All 49 of the *G. fujikuroi complex* isolates examined in this study formed part of the "American" clade.

Analysis of the combined TEF+BT dataset, separated the isolates from Colombia into 8 distinct groups. Of these, only one corresponded to a known species, *i.e.*, *F. circinatum*. The remaining 7 lineages appeared to represent novel species based on the fact that the isolates did not cluster with any known species in the *G. fujikuroi* complex. Because of the limited resolving power of most single gene phylogenetic analyses of the *G. fujikuroi complex*, all 8 of the groups were not recovered from the respective TEF and β-tubulin phylogenies (Figures 4-5), although neither supported groups that were incongruent with those supported by the combined dataset (Figure 1). Application of a modified version of Nixon and Wheeler's (1990) phylogenetic species concept, which is commonly employed for taxonomic studies on this complex (O'Donnell et al. 1998; O'Donnell et al. 2000; Geiser et al. 2005), indicated that the 7 lineages identified for the Colombian isolates should be recognized as distinct species. In this study, descriptions are provided for those groups (Groups 1, 2, 4, 5 and 7) that included multiple representatives (see below). Lineages 3 and 6 were represented by inordinately few isolates to justify describing them at the present time.

In general, the results of the BLAST analyses were not mirrored in the phylogenies, because isolates that had sequence similarity to *F. sterilihyphosum* did not group closely with it, rather they were scattered into phylogenetic groups/lineages (1, 2, 4, 5 and 7) throughout the American clade (Figure 1). Also, the isolates that had sequence similarity to *F. begoniae* all formed part of a group, which did not include this species. The isolates that had a 99 – 100% sequence similarity with *F. circinatum* were the only isolates that grouped with the type strain of any species. This general lack of consistency between the results of BLAST and phylogenetic analyses highlights the limitations associated with using sequence similarity alone for diagnosing novel species (e.g., Kang et al. 2010; Hibbet et al. 2011; Boykin et al. 2012)

3.4 Morphology: The morphological characters of the representative isolates for Groups 1, 2, 4, 5 and 7 were all different in terms of colony colour, size, shape and spore septation (Figures 2 and 3). Although they shared an optimum growth temperature (*i.e.*, 25 ºC), there were differences in their average growth/day, which

ranged from 6 to 15.4 mm/d (Table 5). The isolates also differed morphologically from *F. sterilihyphosum*, *F. begonia* and *F. circinatum*.

3.5 Taxonomy: Based on the results of the phylogenetic and morphological analyses, isolates in phylogenetic Groups 1, 2, 4, 5 and 7 could be treated as five distinct and novel species in the *G. fujikuroi* species complex. The new species are described as follows:

Fusarium fracticaudum Herron, Marincowitz, Wingfield sp. nov. Figs. 2a-c, 3a-j

Etymology: *fracti*, Latin for broken or bent; *caudum*, Latin for tail to describe the "broken tail" of the skewed macroconidial foot cell.

Macroconidia abundant, elongate, straight, 38.2–63.7 × 2.3–4.3 µm (avg. 47.55 × 3.26 µm), with 3–5 septa, apical cells tapering, curved, 8.8–15.2 µm long (avg. 12.15 µm), basal cells distinctly notched to foot-shaped, 9.2–14.5 µm long (avg. 11.77 µm). **Microconidia** abundant, fusiform to obovoid, occasionally curved, 7.8–13.2 × 1.6–3.0 µm (avg. 9.9 × 2.3 µm), with 0–1 septum. **Conidiogenous cells** monophialidic or polyphialidic, 10.9–23.3 µm long, arranged in false heads.

Cultural characteristics: Colonies showing the best growth at 25 ℃ with an average growth rate of 6.9 mm per d (CMW 25237) and 9.1 mm per d (CMW 25245). Colony reverse in the dark more or less uniformly fulvous (11'i or 15´) or in near UV uniformly buff (15'f or 15'd).

Substrate: Stem canker on mature *Pinus maximonoi* trees.

Distribution: Angela Maria (Santa Rosa), Risaralda, Colombia, South America

Specimens examined: COLOMBIA**,** from the stem cankers of *P. maximinoi*. Santa Rosa de Cabal, Risaralda, January 2007, M.J. Wingfield and C.A. Rodas, HOLOTYPE CMW 25245 = FCC 5385; Angela Maria (Santa Rosa), Risaralda, 2007. M.J. Wingfield and C.A Rodas, PARATYPE CMW 25237 = FCC 5377, living culture; Calima (Darien Valle), Colombia, 2007, M.J. Wingfield and C.A. Rodas.

Fusarium parvisorum Herron, Marincowitz, Wingfield sp. nov. Figs. 2d-f, 3k-t

Etymology: *parvi*, Latin for small; *sorum*, Latin for spore, describing the small macroconidia produced by this species.

Macroconidia not abundant, squat, straight, 12.6–29.3 × 1.6–3.0 µm (avg. 19.11 × 2.27 µm), with 1–3 septa, apical cells hooked, 4.6–10.3 µm long (avg. 7.05 µm), basal cells not well developed, barely to distinctly notched, 4.3–12.1 µm long (avg.7.56 µm). **Microconidia** not abundant, fusiform to obovoid, 7.2–13.0 × 1.7–3.2 µm (avg. 9.7 × 2.5 µm), with 0–1 septum. **Conidiogenous cells** monophialidic or polyphialidic, 5.6–27.3 x 1.6–3.2 µm long, arranged in false heads. **Other characteristics** include circinate hyphae present.

Cultural characteristics: Colonies showing the best growth at 25 ℃ with an average growth of 11.4 mm per d (CMW 25267) and 13.3 mm per d (CMW 25268). Colony reverse in the dark and near UV light unpigmented.

Substrate: Diseased roots of *P. patula* seedlings.

Distribution: Vivero, Peñas Negra, Valle del Cauca Colombia, South America

Specimens examined: COLOMBIA**,** from the diseased roots of *P. patula*. Peñas Negra, Colombia, January 2007, M.J. Wingfield and C.A. Rodas, HOLOTYPE CMW 25267 = FCC5407; Vivero, Peñas Negra, Valle del Cauca, Colombia 2007. M.J. Wingfield and C.A. Rodas, PARATYPE CMW 25268 FCC 5408, living culture; Peñas Negra, Colombia, 2007, M.J. Wingfield and C.A. Rodas.

Fusarium marasasianum Herron, Marincowitz, Wingfield sp. nov. Figs 2g-i, 3u-ad

Etymology: named for the late Professor W.F.O Marasas who dedicated most of his professional life to the study of Fusarium spp. and mentored many students of the subject including the authors of this study.

Macroconidia abundant, elongate, straight, 23.4–44.7 × 2.5–3.8 µm (avg. 34.8 × 3.1 µm), with 0–3 septa, apical cells tapering, curved or hooked, 7.1–14.0 µm long (avg. 10.44 µm), basal cells not well developed, barely to distinctly notched or foot-shaped,

6.8–11.9 µm long (avg. 9.2 µm). **Microconidia** scarce, fusiform to obovoid, 7.3–18.2 × 2.1–3.5 µm (avg. 11.4 × 2.7 µm), with 0–1 septum. **Conidiogenous cells** monohialidic or polyphialidic, 9.2–27.1 X 2.0–3.5 µm long, arranged in false heads.

Cultural characteristics: Colonies showing the optimum growth at 25 ℃ with an average growth rate of 11.4 mm per d (CMW 25253) and 15.4 mm per d (CMW 25261). Colony reverse in the dark unpigmented with spots of purple or in near UV light entirely dark purple but with less intensity.

Substrate: Diseased roots of *P. patula* seedlings.

Distribution: Vivero, Peňas Negra, Valle del Cauca, Colombia, South America

Specimens examined: COLOMBIA**,** from the diseased roots of *P. patula*. Peňas Negra, Colombia, January 2007, M.J. Wingfield and C.A. s Rodas, HOLOTYPE CMW 25261 = FCC 5401; Vivero, Peňas Negra, Valle del Cauca, Colombia 2007. M.J. Wingfield and C.A. Rodas, PARATYPE CMW 25253 = FCC 5393, living culture; Colombia, 2007, M.J. Wingfield and C.A.s Rodas.

Fusarium pinemorale Herron, Marincowitz, Wingfield sp. nov. Figs. 5j-l, 6 af-ao

Etymology: *pin*, from pine, the host of this species; *nemorale*, from *nemoralis* which is Latin for a “collection” or “group” thus describing the fact that this species was isolated from a group of pines or pine plantation.

Macroconidia abundant, elongate, straight, 35.1–52.2 × 2.0–3.5 µm (avg. 42.2 × 2.9 µm), with 3–4 septa, apical cells tapering, curved, 8.5–13.8 µm long (avg. 12.0 µm), basal cells foot-shaped, elongated foot shape, distinctly notched, barely to distinctly notched, 8.9–13.9 µm long (11.00 µm). **Microconidia** scarce, fusiform to obovoid, 5.3–16.3 × 1.6–3.0 µm (avg. 10.1 × 2.2 µm), 0–1 septa. **Conidiogenous cells** monophialidic or polyphialidic, 6.4–31.8 X 2.1–3.5 µm long, arranged in false heads.

Cultural characteristics: Colonies showing the best growth at 25 ℃ with an average growth rate of 6 mm per d (CMW 25243) and 10.2 mm per d (CMW 25244). Colony reverse in the dark and near UV light unpigmented.

Substrate: Stem canker on *P. tecunumanii*.

Distribution: Angela Maria (Santa Rosa), Risaralda, Colombia, South America

Specimen examined: COLOMBIA**,** from the diseased roots of *P. patula*. Santa Rosa de Cabal, Risaralda, January 2007, M.J. Wingfield and C.A. Rodas, HOLOTYPE CMW 25243 = FCC 5383; Angela Maria (Santa Rosa), Risaralda, 2007. Mike Wingfield and Carlos Rodas. PARATYPE CMW 25244 = FCC 5384, living culture; Colombia, 2007, M.J. Wingfield and C.A. Rodas.

Fusarium sororula Herron, Marincowitz, Wingfield sp. nov. Figs. 5m-o, 6 ap-ay

Etymology: *soror-*, Latin for sister, *sororula* (diminutive), little sister. This name depicts the fact that this specie produces small macroconidia similar to its sister species, *F. parvisorum*, also described in this study.

Macroconidia scarce, elongate, straight, 20.2–42.6 × 1.9–3.8 µm (avg. 28.65 × 2.91 µm), with 1–3 septa, apical cells hooked, 7.7–12.7 µm long (avg. 9.30 µm), basal cells foot-shaped, elongated foot shape, distinctly notched, barely notched, 7.1–12.4 µm long (avg. 9.11 µm), some producing secondary conidia. **Microconidia** abundant, fusiform to obovoid or pyriform, 5.8–15.6 × 1.7–3.1 µm (avg. 8.12 × 2.24 µm), with 0–1 septum. **Conidiogenous cells** monohialidic or polyphialidic, 10.9–34 µm long, arranged in false heads.

Cultural characteristics: Colonies showing the best growth at 25 ℃ at an average growth rate of 7.48 mm per d (CMW 25254) and 11.43 mm per d (CMWF 1653). Colony reverse in the dark with patches, sectors or entire area of purple or dark purple or in near UV light with patches of partly covered with purple or dark purple.

Substrate: Stem canker on *P. patula*.

Distribution: Angela Maria (Santa Rosa), Risaralda, Colombia, South America

Specimens examined: COLOMBIA**,** from the stem cankers of *P. patula*. Angela Maria, Colombia, January 2007, M.J. Wingfield and C.A. Rodas, HOLOTYPE CMWF 1653; Angela Maria (Santa Rosa), Risaralda, Colombia 2007. M.J. Wingfield and C.A. Rodas. PARATYPE CMW 25254 = FCC 5394, living culture; Colombia, 2007, M.J. Wingfield and C.A. Rodas.

3.6 Pathogenicity trials: From the first pathogenicity trial, only one of the 12 *Fusarium* isolates (CMW 25269), used to inoculate one-year old *P. patula* trees produced lesions that were significantly larger ($P \leq 0.0001$) than those recorded for the negative controls (Table 3). The isolates of *F. parvisorum* (CMW 25269 and CMW 25267) and *F. pinemorale* (CMW 25244) all produced larger lesions than the controls. All the isolates could be re-isolated from each of their respective treatments and not from the controls, which confirmed Koch's postulates that the observed lesions resulted from the inoculated isolates.

In the second pathogenicity trail, three isolates (CMW 25247, CMW 25267 and CMW 25269) representing *F. pinemorale* and *F. parvisorum* were compared with an aggressive strain of *F. circinatum* (FCC 3579) and an additional isolate of *F. pinemorale* (CMW 25243) was also included. The two *F. parvisorum* isolates (CMW 25267 and CMW 25269), again, produced the largest lesions of all isolates tested. Isolate CMW 25269 produced larger lesions than *F. circinatum* and both *F. parvisorum* isolates were significantly different from the control and *F. circinatum* treatments. The *F. pinemorale* isolates produced much smaller lesions that were not significantly different from each other or the control. All the isolates could be re-isolated from each of their respective treatments, confirming Koch's postulates. No *Fusarium* spp. were isolated from the controls.

4. Discussion

In this study, 10 distinct *Fusarium* species were recovered from *Pinus* tissue showing signs of infection by *F. circinatum*. These included the pitch canker fungus, the five newly described species *F. marasasianum*, *F. fracticaudum*, *F. pinemorale*, *F. sororula* and *F. parvisorum*, two as-yet un-described species and isolates belonging to the *F. oxysporum* (Baayen et al. 2000) and *F. solani* species complexes (O'Donnell 2000; Zhang et al. 2006). Of these species, only *F. circinatum* is known to represent a primary pathogen having an established association with *Pinus* (Nirenberg and O'Donnell 1998; Gordon 2006; Wingfield et al. 2008a), while *F. oxysporum* and *F. solani* probably represented secondary pathogens or saprophytes (Burgess 1981).

The distribution of the *Fusarium* species examined in this study varied in terms of the host and tissue type from which they were recovered. Like *F. circinatum, F. marasasianum* and *F. solani* were isolated from both nursery seedlings and from established plantation trees, while isolates of *F. fracticaudum*, *F. pinemorale* and *F. sororula* were isolated from plantation trees only and isolates of *F. parvisorum* and *F. oxysporum* from nursery seedlings only. Also, *F. circinatum*, *F. fracticaudum*, *F. marasasianum*, *F. sororula*, *F. solani* were isolated from multiple *Pinus* species, while *F. pinemorale* and *F. oxysporum* were isolated only from *P. tecunumanii*. Apart from the two novel *Fusarium* species represented by single isolates, all species examined here were also recovered from multiple locations in Colombia.

The recovery of isolates residing in the *F. oxysporum* and *F. solani* species complexes was not unexpected. There are a number of strains belonging to the *F. oxysporum* species complex (Baayen et al. 2000) and the *F. solani* species complex (O'Donnell et al. 2000; Zhang et al. 2006) that are regarded as plant pathogens. For example, strains of *F. oxysporum* and *F. solani* have been associated with diseased *Pinus strobus* seed and seedlings (Ocamb and Juzwik 1995; Ocamb et al. 2002) and *Pinus radiata* bare root nurseries (Dick and Dobbie 2002). However, the symptoms induced by *F. oxysporum* and *F. solani* typically do not resemble those of the pitch canker fungus (Ocamb and Juzwik 1995; Dick and Dobbie 2002; Ocamb et al. 2002;

Wingfield et al. 2008a). Their recovery from the *Pinus* tissues used in this study is, therefore, likely to be a consequence of the fact that members of these two species complexes are often saprophytes with ubiquitous distributions (Burgess 1981).

Apart from *F. oxysporum* and *F. solani*, all of the *Fusarium* species included in this study form part of the so-called "American Clade" (O'Donnell et al. 1998) of the *Gibberella fujikuroi* species complex. Therefore, based on the biogeographic hypothesis for this complex, all of the members of this clade are associated with hosts that have their centers of origin in the Americas (O'Donnell et al. 1998). For example, the American Clade species *F. circinatum* and *F. subglutinans* are thought to have co-evolved with their hosts (i.e., *Pinus* and Zea species, respectively) in Mexico and Central America (Gaut and Doebley 1997; Iltis 2000; Wikler and Gordon 2000). These fungi were then introduced with their hosts to other parts of the world as part of the development and expansion of agriculture and forestry (Desjardins et al. 2000; Wingfield et al. 2008a). Following this view, it is possible that the new *Fusarium* species identified in this study also originated from Mexico and Central America, because these regions represent centers of origin for many *Pinus* species (Millar 1993).

An alternative hypothesis, but consistent with the biogeographic hypothesis for the *G. fujikuroi* species complex, is that the new species recognized in this study are native on other host plant species in Colombia. This would then suggest that the *Fusarium* species jumped to *Pinus* species from these hosts. This is plausible as the phenomenon of host jumping (Slippers et al. 2005) occurs frequently in environments where native ecosystems and exotic monoculture-based forestry or agriculture exist in close association (Burgess and Wingfield 2004; Stenlid et al. 2011). Furthermore, these host jumps occur more readily when the host species are related *i.e.*, the canker pathogen *Chrysoporthe austroafricana* (Gryzenhout et al. 2004). This fungus is native to southern Africa and associated with native Myrtales (Heath et al. 2006); however, it can also cause comparable (and often more severe) symptoms on exotic *Eucalyptus* species planted in commercial forestry plantations (Nakabonge et al. 2006). Another example is guava rust or Eucalyptus rust caused by *Puccinia psidii,* which is native on many Myrtales but has jumped to *Eucalyptus* planted as exotics in commercial forestry

industries (Coutinho et al. 1998). Future studies should thus seek to understand the host range, especially in terms of conifers and other gymnosperms native to Colombia, of newly identified species from both this study and future studies. From these investigations it will be possible to address the hypothesis that these species have truly jumped or, alternatively, adapted to a specific host i.e., *formae specialis*.

The results of this study showed that the new species, *F. parvisorum*, is pathogenic when inoculated onto *P. patula* seedlings. This fungus induced lesions on the seedlings that were as large as or larger than those associated with a virulent isolate of *F. circinatum*. The association of *Pinus* species with a pathogen as aggressive as *F. circinatum* has important implications for commercial forestry in Colombia and elsewhere where *Pinus* is planted or occurs naturally. In general, the susceptibility of planting stock to this new pathogen will have to be evaluated, by following approaches similar to those used for *F. circinatum* (e.g., Roux et al. 2007; Mitchell et al. 2011). Suitable control strategies will also have to be developed, although this will require detailed knowledge regarding the distribution, host range and ecology of this newly recognized pathogen.

Studies such as this are important for the discovery of new pathogens and vital for improving quarantine and limiting/reducing their spread. For example, strategies can now be developed to identify and track the possible movement of *F. parvisorum* in Colombia and possibly elsewhere in the world. The active monitoring of these areas are of particular importance in forestry industries where the rotation periods are especially long. This implies that they are exposed to pests and pathogens for long periods and where problems emerge, the consequences can be dire. But even where early detection is achieved, the appearance of new tree disease is difficult to treat. In the case of *F. circinatum*, 70 years after its discovery it has spread to more than ten countries on five different continents (reviewed by Steenkamp et al. 2012) and there are still no effective means to eradicate or effectively control the disease it causes on seedlings or mature trees. Preventing the establishment of new pathogens provides the best options to restrict the spread of pathogens and can be fortified by enforced quarantine procedures.

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Table 1: Host and geographic origin of the *Fusarium* isolates used in this study.

Isolate number	*Fusarium* species [b]	*Pinus* species [a]	Area in Colombia	Provenance	GPS Co-ordinates	Phylogenetic Group [c]	Other number [d]
CMW 25237	*F. fracticaudum* sp. nov.	*P. tecunumanii* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	1	FCC 5377
CMW 25238	*F. fracticaudum* sp. nov.	*P. tecunumanii* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	1	FCC 5378
CMW 25241	*F. fracticaudum* sp. nov.	*P. maximinoi* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	1	FCC 5381
CMW 25242	*F. fracticaudum* sp. nov.	*P. maximinoi* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	1	FCC 5382
CMW 25245	*F. fracticaudum* sp. nov.	*P. maximinoi* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	1	FCC 5385
CMW 25249	*F. fracticaudum* sp. nov.	*P. maximinoi* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	1	FCC 5389
CMW 25250	*F. fracticaudum* sp. nov.	*P. maximinoi* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	1	FCC 5390
CMW 25511	*F. fracticaudum* sp. nov.	*P. tecunumanii* (T)	Volconda (Calima El Darién)	Valle del Cauca	76°25'06" W 4°01'47" N	1	FCC 5423
CMW 25267	*F. parvisorum* sp. nov.	*P. patula* (S)	Vivero, Peñas Negra	Valle del Cauca	76°29'49" W 3°51'45" N	2	FCC 5407
CMW 25268	*F. parvisorum* sp. nov.	*P. patula* (S)	Vivero, Peñas Negra	Valle del Cauca	76°29'49" W 3°51'45" N	2	FCC 5408
CMW 25269	*F. parvisorum* sp. nov.	*P. patula* (S)	Vivero, Peñas Negra	Valle del Cauca	76°29'49" W 3°51'45" N	2	FCC 5409
CMW 25246	*F. marasasianum* sp. nov.	*P. tecunumanii* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	4	FCC 5386
CMW 25248	*F. marasasianum* sp. nov.	*Pinus* sp. (T)	Colombia	n/a	n/a	4	FCC 5388
CMW 25252	*F. marasasianum* sp. nov.	*Pinus* sp. (T)	Colombia	n/a	n/a	4	FCC 5392

Isolate number	*Fusarium* species [b]	*Pinus* species [a]	Area in Colombia	Provenance	GPS Co-ordinates	Phylogenetic Group [c]	Other number [d]
CMW 25253	*F. marasasianum* sp. nov.	*Pinus* sp. (T)	Colombia	n/a	n/a	4	FCC 5393
CMW 25261	*F. marasasianum* sp. nov.	*P. patula* (S)	Vivero, Peñas Negra	Valle del Cauca	76°29'49" W 3°51'45" N	4	FCC 5401
CMW 25512	*F. marasasianum* sp. nov.	*P. tecunumanii* (T)	Volconda (Calima, El Darién)	Valle del Cauca	76°25'06" W 4°01'47" N	4	FCC 5424
CMW 25243	*F. pinemorale* sp. nov.	*P. tecunumanii* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	5	FCC 5383
CMW 25244	*F. pinemorale* sp. nov.	*P. tecunumanii* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	5	FCC 5384
CMW 25247	*F. pinemorale* sp. nov.	*P. tecunumanii* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	5	FCC 5387
CMW 25254	*F. sororula* sp. nov.	*Pinus* sp. (T)	Colombia	n/a	n/a	7	FCC 5394
CMW 25513	*F. sororula* sp. nov.	*P. tecunumanii* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	7	FCC 5425
CMW 25520	*F. sororula* sp. nov.	*P. patula* (T)	El Guasimo (Santa Rosa de Osos)	Antioquia	75°26'30" W 6°52'04" N	7	FCC 5432
Colombia 8	*F. sororula* sp. nov.	*P. patula* (T)	Volconda (Calima, El Darién)	Valle del Cauca	76°25'06" W 4°01'47" N	7	n/a
Colombia 19	*F. sororula* sp. nov.	*P. patula* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	7	n/a
CMWF 1653	*F. sororula* sp. nov.	*P. patula* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	7	n/a
CMW 25516	*Fusarium* sp.	*P. patula* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	6	FCC 5428
Colombia 18	*Fusarium* sp.	*P. patula* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	3	n/a
CMW 25239	*F. circinatum*	*P. tecunumanii* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	n/a	FCC 5379

Isolate number	*Fusarium* species [b]	*Pinus* species [a]	Area in Colombia	Provenance	GPS Co-ordinates	Phylogenetic Group [c]	Other number [d]
CMW 25240	*F. circinatum*	*P. tecunumanii* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	n/a	FCC 5380
CMW 25251	*F. circinatum*	*P. maximinoi* (T)	Calima, El Darién	Valle del Cauca	76°26'03" W 3°56'57" N	n/a	FCC 5391
CMW 25255	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5395
CMW 25256	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5396
CMW 25257	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5397
CMW 25258	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5398
CMW 25259	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5399
CMW 25260	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5400
CMW 25262	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5402
CMW 25263	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5403
CMW 25264	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5404
CMW 25265	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5405
CMW 25266	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5406
CMW 25271	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5411
CMW 25272	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5412

Isolate number	*Fusarium* species [b]	*Pinus* species [a]	Area in Colombia	Provenance	GPS Co-ordinates	Phylogenetic Group [c]	Other number [d]
CMW 25273	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5413
CMW 25274	*F. circinatum*	*P. patula* (S)	Vivero, Peñas Negras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5414
CMW 25517	*F. circinatum*	*P. patula* (T)	Campania, Riosucio	Caldas	75°49'18" W 5°21'45" N	n/a	FCC 5429
CMW 25518	*F. circinatum*	*P. kesiya* (T)	Aguaclara, La Cumbre	Valle del Cauca		n/a	FCC 5430
CMW 25519	*F. circinatum*	*P. patula* (T)	Angela Maria (Santa Rosa)	Risaralda	75°36'21" W 4°49'18" N	n/a	FCC 5431
CMW 25503	*F. oxysporum*	*P. tecunumanii* (S)	Vivero Eras	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5415
CMW 25504	*F. oxysporum*	*P. tecunumanii* (S)	Vivero Bandeja	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5416
CMW 25505	*F. solani*	*P. tecunumanii* (S)	Vivero Bandeja	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5417
CMW 25506	*F. solani*	*P. maximinoi* (S)	Vivero Canaleta	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5418
CMW 25507	*F. solani*	*P. maximinoi* (S)	Vivero Canaleta	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5419
CMW 25509	*F. solani*	*P. maximinoi* (S)	Vivero Canaleta	Valle del Cauca	76°29'49" W 3°51'45" N	n/a	FCC 5421
CMW 25514	*F. solani*	*P. tecunumanii* (T)	La Suiza, Restrepo	Valle del Cauca	76°29'33" W 3°50'55" N	n/a	FCC 5426
CMW 25515	*F. solani*	*P. tecunumanii* (T)	La Suiza, Restrepo	Valle del Cauca	76°29'33" W 3°50'55" N	n/a	FCC 5427

[*] - CMWF: Fusarium culture collection at the FABI, University of Pretoria, South Africa. [a] The names of the *Fusarium* sp. nov. were designated from this study. [b] The species of the host *Pinus* species where the *Fusarium* species were isolated from. Not all of the host species are known. The letters in brackets indicate whether the isolates came from seedlings (S) or mature trees (T). [c] The Phylogenetic group is the designation given in Figure 1 in the combined dataset. [d] FCC Original numbers of the Fusarium culture collection at FABI, University of Pretoria, South Africa. CBS: Culture collection at the CBS-KNAW Fungal diversity Centre, Utrecht, Netherlands; PREM: Culture collection at the National Fungus Collection, Pretoria, South Africa.

Table 2: The species names and their GenBank accession numbers for all the *Fusarium* isolates included in the phylogenetic analyses.

Species	Host/Substrate	Origin	Culture Collection[a]	GenBank accession[b]	
				β-tubulin	TEF
F. acutatum	Unknown	India	NRRL 13308	U34431[1]	AF160276[2]
F. ananatum	*Ananas comosus*	England	NRRL 22945	U34420[1]	AF160297[2]
F. anthophilum	*Hippeastrum sp.*	Germany	NRRL 13602	U61541[1]	AF160292[2]
F. bactridioides	*Cronartium conigenum*	USA	NRRL 20476	U34434[1]	AF160290[2]
F. begoniae	*Begonia elatior*	Germany	NRRL 25300	U61543[1]	AF160293[2]
F. brevicatenulatum	*Striga asiatica*	Madagascar	NRRL 25446	U61545[1]	AF160265[2]
F. bulbicola	*Nerine bowdenii*	Netherlands	NRRL 13618	U61546[1]	AF160294[2]
F. circinatum	*Pinus radiata*	USA	NRRL 25331	U61547[1]	AF160295[2]
F. concentricum	*Musa sapientum*	Costa Rica	NRRL 25181	U61548[1]	AF160282[2]
F. denticulatum	*Ipomoea batatas*	USA	NRRL 25302	U61550[1]	AF160269[2]
F. dlaminii	Soil	South Africa	n/a	n/a[10,11]	n/a[10,11]
F. fractiflexum	*Cymbidium* sp.	Japan	NRRL 28852	AF160315[3]	AF160288[3]

Species	Host/Substrate	Origin	Culture Collection[a]	GenBank accession[b]	
				β-tubulin	TEF
F. fujikuroi	*Oryza sativa*	Taiwan	NRRL 13566	U34415[1]	AF160279[2]
F. globosum	*Zea mays*	Central America	NRRL 26131	U61557[2]	AF160285[2,12]
F. guttiforme	*Ananas comosus*	South America	NRRL 22945	U34446[2]	AF160297[2, 12]
F. inflexum	*Vicia faba*	Germany	NRRL 20433	U34435[1]	AF8479[2]
F. konzum	*Andropogon gerardii*	North America	MRC 8854	EU220234[10]	EU220235[10]
F. lactis	*Ficus carica*	USA	NRRL 25200	U61551[1]	AF160272[2]
F. lyarnte	Soil	Australia	F19374	EF107122[6]	EF107118[6]
F. mangiferae	*Mangifera indica*	India	NRRL 25226	U61561[1]	AF160281[2]
F. mexicanum	*Mangifera indica*	Mexico	NRRL 53147	GU737494[5]	GU737282[5]
F. musae	*Musa* sp.	Honduras	MUCL 52574	FN545368[8]	FN552086[8]
F. napiforme	*Pennisetum typhoides*	South Africa	NRRL 13604	U34428[1]	AF160266[2]
F. nygamai	*Sorghum bicolor*	Australia	NRRL 13448	U34426[1]	AF160273[2]
F. oxysporum	*Pseudotsuga menziesii*	USA	NRRL 22902	U34424[1]	AF160312[2]

Species	Host/Substrate	Origin	Culture Collection[a]	GenBank accession[b]	
				β-tubulin	TEF
F. phyllophilum	*Dracaena deremensis*	Italy	NRRL 13617	U34432[1]	AF160274[2]
F. proliferatum	*Cattleya* sp.	Germany	NRRL 22944	U34416[1]	AF160280[2]
F. pseudoanthophilum	*Zea mays*	Zimbabwe	NRRL 25206	U61553[1]	AF160264[2]
F. pseudocircinatum	*Solanum* sp.	Zimbabwe	NRRL 22946	U34427[1]	AF160271[2]
F. pseudonygamai	*Pennisetum typhoides*	Ghana	NRRL 13592	U34421[1]	AF160263[2]
F. ramigenum	*Ficus carica*	Nigeria	NRRL 25208	U61554[1]	AF160267[2]
F. sacchari	*Saccharum officinarum*	USA	NRRL 13999	U34414[1]	AF160278[2]
F. sterilihyphosum	*Mangifera indica*	India	MRC 2802	AF160316[2]	AF160300[2]
F. subglutinans	*Zea mays*	USA	NRRL 22016	U34417[1]	AF160289[2]
F. succisae	*Succisa pratensis*	Germany	NRRL 13613	U34419[1]	AF160291[2]
F. temperatum	*Zea mays*	Belgium	MUCL 52450	HM067695[7]	HM067687[7]
F. thapsinum	*Sorghum bicolor*	South Africa	NRRL 22045	U34418[1]	AF160270[2]
F. tupiense	*Mangifera indica*	Brazil	CML 262	DQ445781[9]	DQ452859[9]
F. udum	Unknown	Germany	NRRL 22949	U34433[1]	AF160275[2]

Species	Host/Substrate	Origin	Culture Collection[a]	GenBank accession[b]	
				β-tubulin	TEF
F. verticillioides	*Zea mays*	Germany	NRRL 22172	U34413[1]	AF160262[2]
F. werrikimbe	*Sorghum leiocladum*	Australia	F19350	EF107133[6]	EF107131[6]
F. xylarioides	*Coffea* sp.	Ivory Coast	NRRL 25486	AY707118[4]	AY707136[4]
Fusarium sp.	*Striga hermonthica*	Madagascar	NRRL 26061	AF160319[2]	AF160303[2]
Fusarium sp.	Unknown	Niger	NRRL 26152	AF160349[2]	AF160306[2]
Fusarium sp.	*Sorghum bicolor* seed	Tanzania	NRRL 26064	AF160346[2]	AF160302[2]
Fusarium sp.	*Zea mays*	Central America	NRRL 25221	U61560[1]	AF160268[1]
Fusarium sp.	*Striga hermonthica*	Africa	NRRL 26793	AF160324[1]	AF160309[1]
Fusarium sp.	*Oryza sativa*	Southeast Asia	NRRL 25615	AF160320[2]	AF160304[2]
Fusarium sp.	Soil	Australia	NRRL 25807	U61542[1]	AF160305[1]
Fusarium sp.	n/a	n/a	NRRL 25195	U61558[1]	AF160298[1]
Fusarium sp.	*Ipomoea batatas*	Central America	NRRL 25346	U61564[1]	AF160296[1]
Fusarium sp.	Ornamental reed	South Africa	NRRL 26756	AF160322[2]	AF160307[2]

Species	Host/Substrate	Origin	Culture Collection[a]	GenBank accession[b]	
				β-tubulin	TEF
Fusarium sp.	Ornamental reed	South Africa	NRRL 26757	AF160323[2]	AF160308[2]
Fusarium sp.	Palm	n/a	NRRL 25204	U61559[1]	AF160299[1]
Fusarium sp.	*Bidens pilosa*	South America	NRRL 29124	AF160326[2]	AF160311[2]
Fusarium sp.	*Zea mays*	Central America	NRRL 25622	DQ448031[2]	AF160301[2]
Fusarium sp.	*Triticum* sp.	South Asia	NRRL 25309	U61563[1]	AF160284[1]
Fusarium sp.	*Oryza sativa*	Southeast Asia	NRRL 25303	U61562[1]	AF160283[1]
Fusarium sp.	Soil	Papua New Guinea	NRRL 26427	AF160313[2]	AF160286[2]

[a] - The abbreviations for the culture collections: CML (Coleção Micológica de Lavras) Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; MUCL (Mycothèque de l'Université Catholique de Louvain), Louvain-la-Neuve, Belgium; MRC (Medical Research Center) Tygerberg, Cape Town, South Africa, F (University of Sydney) Sydney, New South Wales, Australia and NRRL (National Center for Agricultural Utilization Research) Peoria, Illinois. [b] - The references for the studies where these DNA sequences were generated: [1]O'Donnell et al. 1998; [2]O'Donnell et al. 2000; [3]Aoki et al. 2001; [4]Geiser et al. 2005; [5]Otero-Colina et al. 2010; [6]Walsh et al. 2010; [7]Scauflaire et al. 2011; [8]Van Hove et al. 2011; [9]Lima et al., 2012; [10]Kvas et al. 2009, [11]Marasas et al. 1985, [12]Nirenberg and O'Donnell 1998.

Table 3: The results of pathogenicity tests on *P. patula* seedlings with the *Fusarium* species described in this study.

***Fusarium* species**	**Isolate**	**Mean lesion length (mm)[a]**	**Standard Error**
F. fracticaudum	CMW 25237	1.80 (80) a	0.26
F. fracticaudum	CMW 25245	1.92 (79) a	0.23
F. marasasianum	CMW 25246	2.06 (79) a	0.12
F. marasasianum	CMW 25512	2.22 (79) a	0.25
F. pinemorale	CMW 25244	5.21 (80) a	1.09
F. pinemorale	CMW 25247	4.70 (77) a	1.41
F. parvisorum	CMW 25267	8.53 (77) a	2.06
F. parvisorum	CMW 25269	31.22 (75) b	3.18
F. sororula	CMW 25517	2.65 (78) a	0.20
F. sororula	CMWF 1653	2.10 (78) a	0.21
Fusarium sp.	CMW 25516	1.53 (79) a	0.08
Fusarium sp.	Colombia 18	1.83 (78) a	0.16
n/a	Control	0.49 (80) a	0.16

[a] The values in parentheses represent the total number of measurements for each treatment from which the means were calculated. Different numbers of measurements were recorded because readings from dead seedlings were not included. A one way ANOVA (Analysis of Variance) did not indicate much significance between inoculum treatments, besides one isolate (CMW 25269). The observed F-value was 49.59 but the significance probability associated with the F-statistic was <0.0001. Individual means were compared and grouped according to the Duncan Multiple Range Test with a confidence level of 95%. Means that were not significantly different are indicated with the same letter.

Table 4: The results of pathogenicity tests with *F. pinemorale*, *F. parvisorum* and *F. circinatum* on *P. patula* seedlings.

***Fusarium* species**	**Isolate**	**Mean lesion length (mm)[a]**	**Standard Error**
F. pinemorale	CMW 25243	2.07 (27) c	0.05
F. pinemorale	CMW 25247	2.18 (22) c	0.18
F. parvisorum	CMW 25267	24.00 (21) b	3.30
F. parvisorum	CMW 25269	42.63 (30) a	3.62
F. circinatum	FCC 3579	35.69 (26) ab	3.63
n/a	Control	2.07 (29) c	0.08

[a] The values in parentheses represent the total number of measurements for each treatment from which the means were calculated. A one way ANOVA (Analysis of Variance) indicated significance between all the inoculum treatments. The observed F-value was 26.02 and the significance probability associated with the F-statistic was <0.0001. Individual means were compared and grouped according to the Duncan Multiple Range Test with a confidence level of 95%. Means that were not significantly different are indicated with the same letter.

Table 5: The results of the growth studies conducted on *Fusarium fracticaudum*, *F. parvisorum*, *F. marasasianum*, *F. pinemorale* and *F. soroorula*.

Species	Isolate number	Growth (mm) at various Incubation temperatures after 8 days*						
		10℃	15℃	20℃	25℃	30℃	35℃	Growth/day 25℃**
F. fracticaudum	25237	20.83	32.00	51.83	46.33	20.83	0.00	6.89
F. fracticaudum	25245	11.96	36.50	56.65	67.00	13.33	0.00	9.09
F. parvisorum	25267	15.83	41.67	71.83	80.00	52.17	0.00	11.43
F. parvisorum	25268	15.97	43.83	75.50	80.00	44.00	0.00	13.33
F. marasasianum	25253	15.80	45.83	68.34	80.00	52.33	0.00	11.43
F. marasasianum	25261	13.17	46.83	80.00	80.00	48.67	0.00	15.43
F. pinemorale	25243	17.00	33.75	45.60	51.83	41.33	0.00	6.00
F. pinemorale	25244	22.17	40.33	57.13	76.75	44.00	0.00	10.17
F. sororula	25254	14.66	31.33	48.80	62.00	40.66	0.00	7.48
F. sororula	1653	11.33	44.00	66.83	80.00	47.16	0.00	11.43

*- 80.00mm is the diameter of the agar plate and represents the maximum size an isolate can grow to.

**- It was determined that 25℃ is the optimum temperature for these isolates to grow at and the average growth per day was recorded at this temperature.

Figure 1: A maximum likelihood (ML) phylogeny of the *Gibberella fujikuroi* species complex (GFC), including all the *Fusarium* isolates collected from Colombia, inferred from the combined TEF and BT sequence data. The tree is rooted with *F. inflexum* and *F. oxysporum*. A similar phylogenetic topology was generated using Bayesian inference. All of the taxa are grouped into the three so called "American, "African" and "Asian" clades, indicated in green, blue and red, according to O'Donnell et al (1998) biogeographic hypothesis. The yellow blocks indicate the seven major phylogenetic clades identified from this study. Bootstrap support values (>60%) for maximum likelihood and Bayesian posterior probabilities (>0.6) are indicated at the internodes in the order BI/ML. Branches with bootstrap support values less than 60% or posterior probability values less than 0.6 are indicated with a "-". NRRL is the name of the culture collection at the National Regional Research Laboratory, IL, USA.

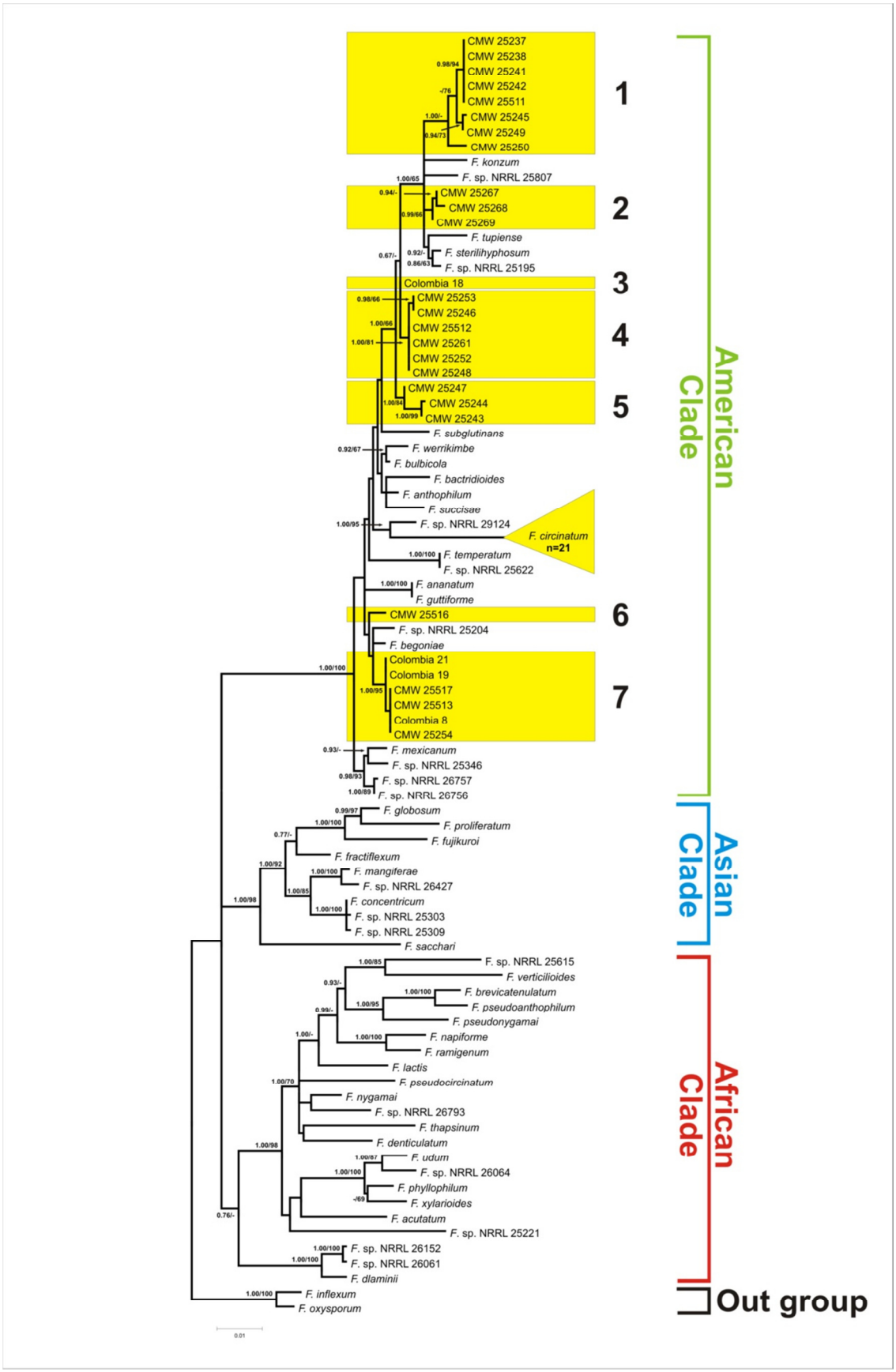


Figure 2: The variation observed in size and shape of the macroconidia produced by *Fusarium fracticaudum* sp. nov. (a-c), *Fusarium parvisorum* sp. nov. (d-f), *Fusarium marasasianum* sp. nov. (g-i), *Fusarium pinemorale* sp. nov. (j-l) and *Fusarium sororula* sp. nov. (m-o). Scale bar = 10 µm.

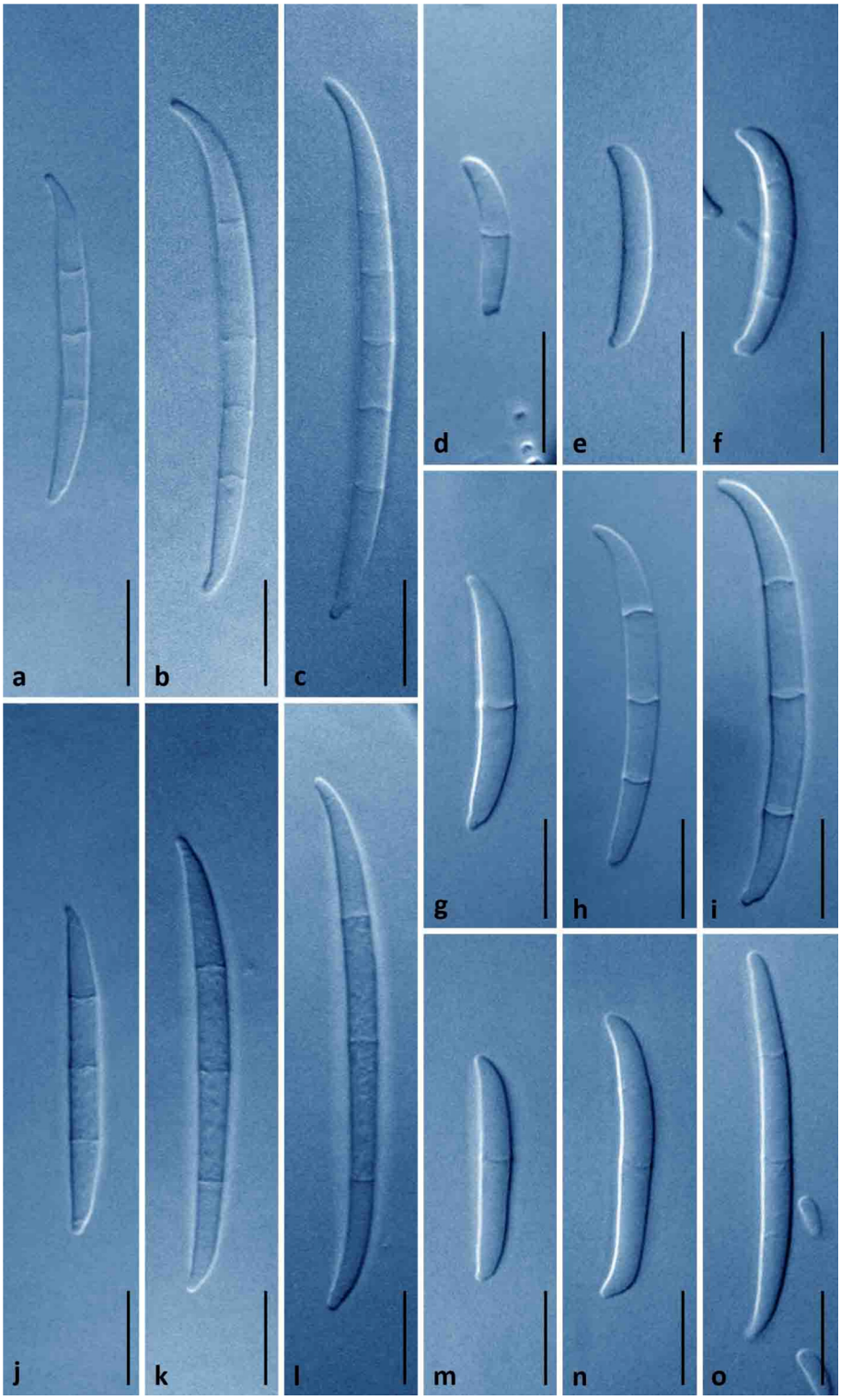


Figure 3: The variation observed in apical and basal foot cells produced by *Fusarium fracticaudum* sp. nov. (a-f), *Fusarium parvisorum* sp. nov. (k-p), *Fusarium marasasianum* sp. nov. (u-z), *Fusarium pinemorale* sp. nov. (af-ak) and *Fusarium sororula* sp. nov. (ap-au) as well as the size and shape of the microconidia produced by *Fusarium fracticaudum* sp. nov. (g-j), *Fusarium parvisorum* sp. nov. (q-t), *Fusarium marasasianum* sp. nov. (aa-ad), *Fusarium pinemorale* sp. nov. (al-ao) and *Fusarium sororula* sp. nov. (av-ay). Scale bar = 5 µm.

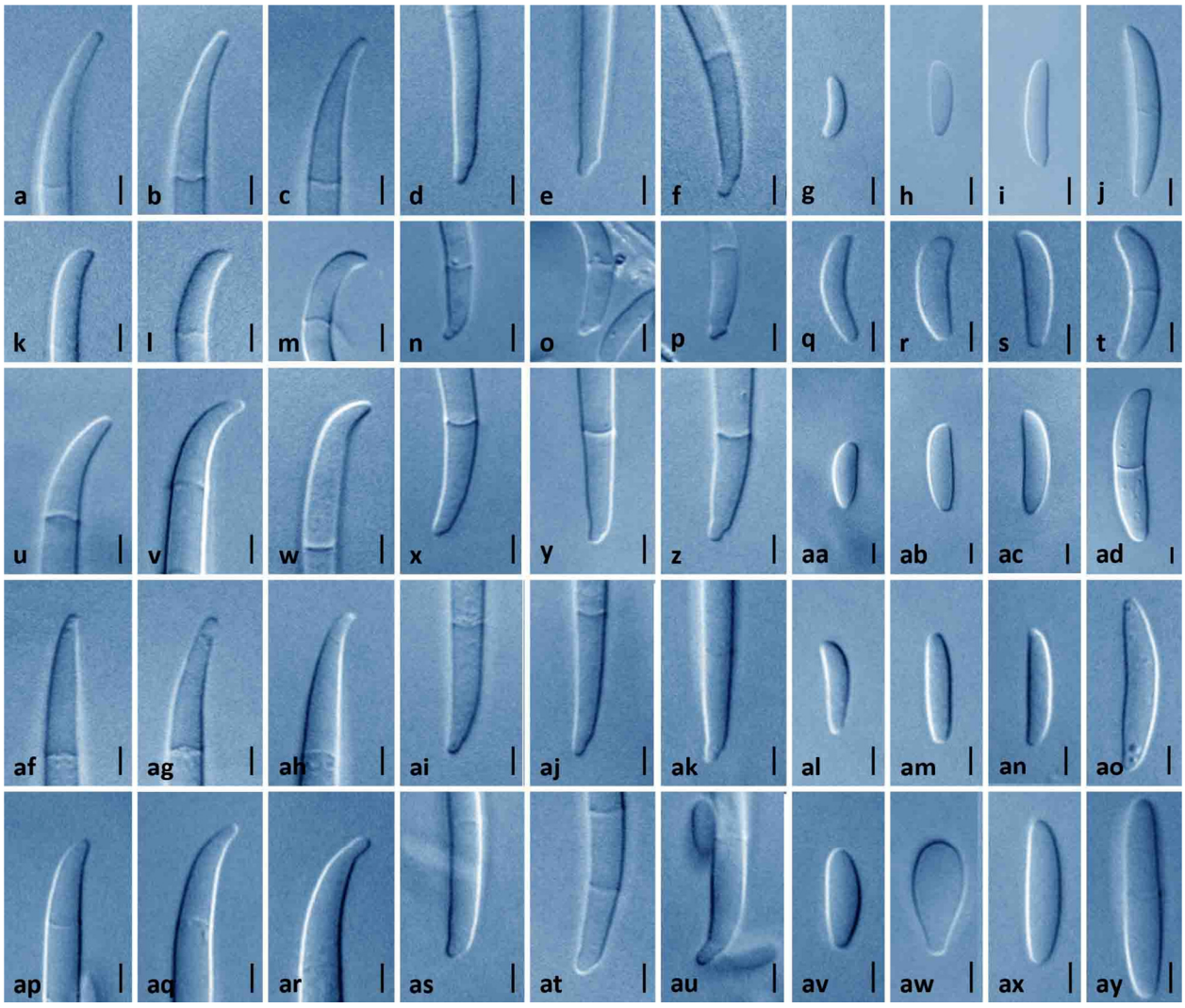


Figure 4: A maximum likelihood (ML) phylogeny of the *Gibberella fujikuroi* species complex (GFC), including all the *Fusarium* isolates collected from Colombia, inferred from translation elongation 1-α (TEF) sequence data. The tree is rooted with *F. inflexum* and *F. oxysporum*. A similar phylogenetic topology was generated using Bayesian inference. All of the taxa are grouped into the three so called "American, "African" and "Asian" clades, indicated in green, blue and red, according to O'Donnell et al (1998) biogeographic hypothesis. Bootstrap support values (>60%) for maximum likelihood and Bayesian posterior probabilities (>0.6) are indicated at the internodes in the order BI/ML. Branches with bootstrap support values less than 60% or posterior probability values less than 0.6 are indicated with a "-". **MP** indicates mating populations of the GFC. NRRL is the name of the culture collection at the National Regional Research Laboratory, IL, USA.

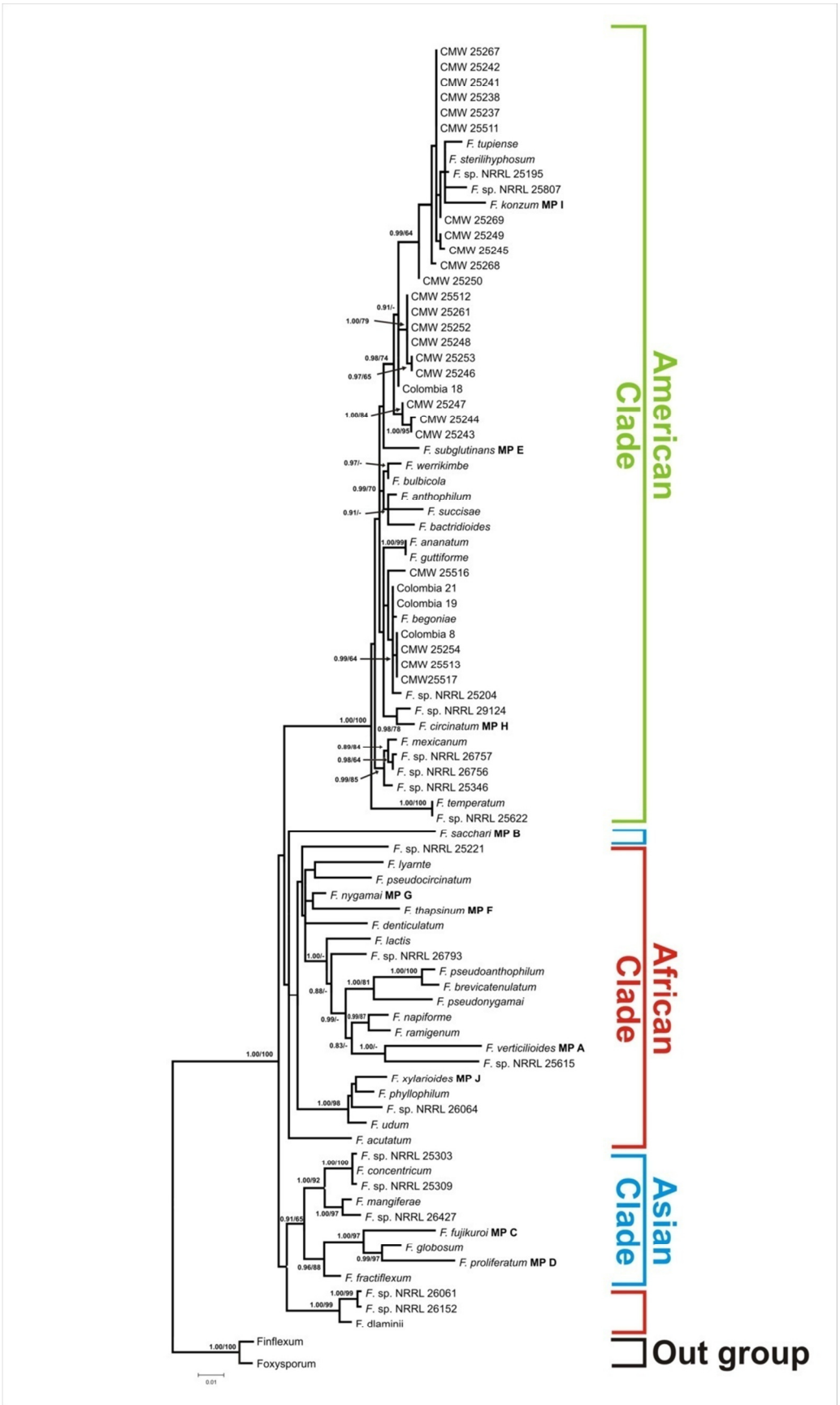
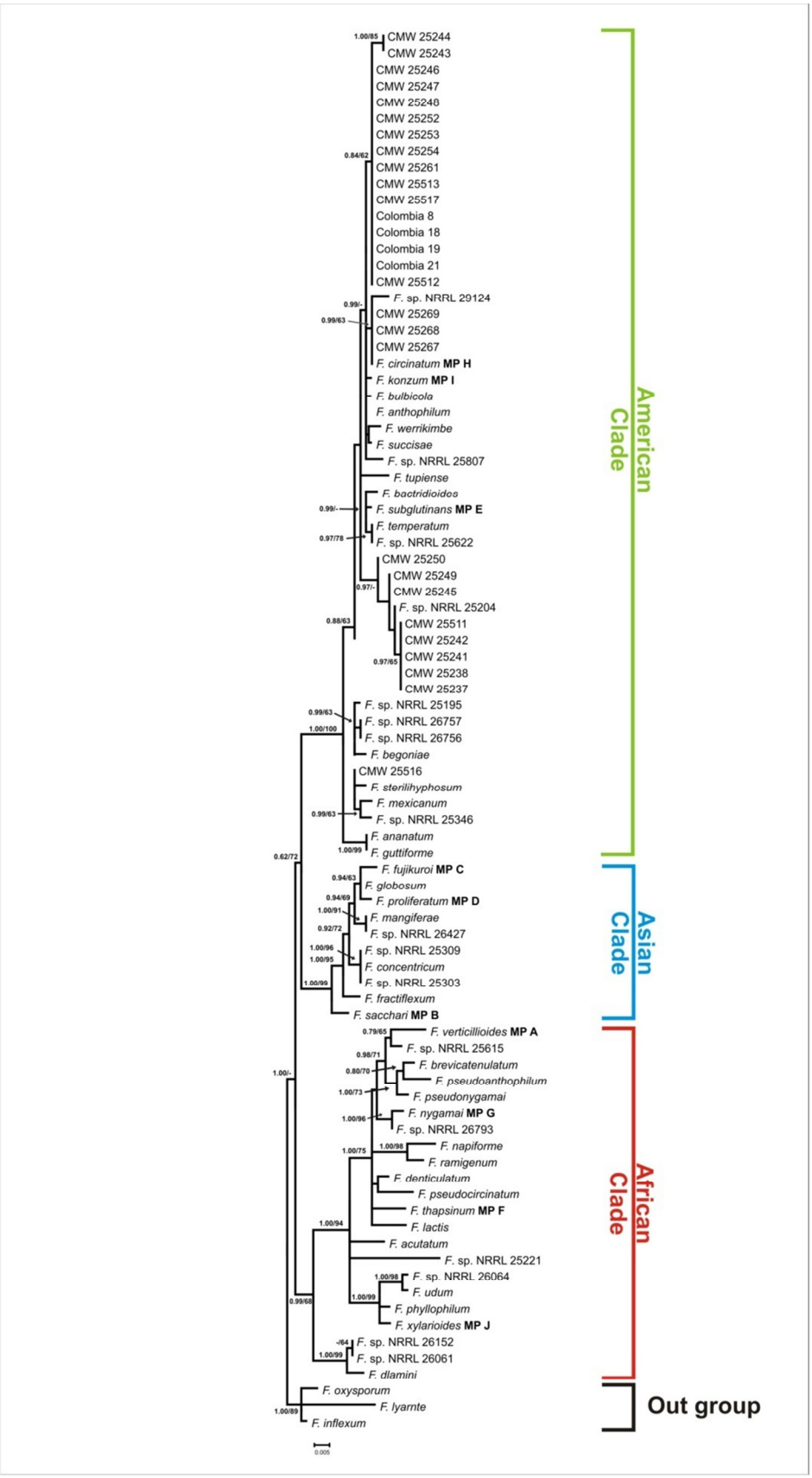


Figure 5: A maximum likelihood (ML) phylogeny of the *Gibberella fujikuroi* species complex (GFC), including all the *Fusarium* isolates collected from Colombia, inferred from β-Tubulin (BT) sequence data. The tree is rooted with *F. inflexum* and *F. oxysporum*. A similar phylogenetic topology was generated using Bayesian inference. All of the taxa are grouped into the three so called "American, "African" and "Asian" clades, indicated in green, blue and red, according to O'Donnell et al (1998) biogeographic hypothesis. Bootstrap support values (>60%) for maximum likelihood and Bayesian posterior probabilities (>0.6) are indicated at the internodes in the order BI/ML. Branches with bootstrap support values less than 60% or posterior probability values less than 0.6 are indicated with a "-". **MP** indicates mating populations of the GFC. NRRL is the name of the culture collection at the National Regional Research Laboratory, IL, USA.



Chapter 3

Fusarium diversity in forest nurseries in South Africa

Abstract

Fusarium is one of the most important fungal genera mainly because it includes a large number of important plant pathogens. In the forestry environment, one of the most important species is the pitch canker fungus, *Fusarium circinatum*. Few studies have, however, considered the occurrence of other *Fusarium* spp. in forestry. The aim of this study was, therefore, to characterize the diversity of *Fusarium* species in commercial *Eucalyptus* and *Pinus* nurseries. For this purpose, seedlings and cuttings of *Pinus* and *Eucalyptus* were collected from five nurseries in KwaZulu-Natal and Mpumalanga. From the plant tissues, a total of 165 isolates of *Fusarium* were isolated and identified using phylogenetic analysis based on portions of the genes encoding translation elongation factor (TEF). The data indicated that the *Fusarium* isolates belonged to one of three species complexes, *i.e.*, the *Fusarium oxysporum* complex, *Fusarium solani* complex and the *Gibberella fujikuroi* complex. The diversity within these complexes was also high, with fifteen distinct lineages identified in the *Fusarium oxysporum* complex, four lineages identified in the *Fusarium solani* complex and three lineages in the *Gibberella fujikuroi* complex. Furthermore, most of this diversity appeared to be made up of novel species. An additional 17 isolates were identified as *F. circinatum* using the species specific primers CIR1A+CIR41 to amplify a portion of the intergenic spacer region (IGS). Further work will seek to characterize these isolates more completely and to determine their relevance in Pinus and *Eucalyptus* nurseries. .

Key words: Commercial nursery, diversity, *Eucalyptus*, *Fusarium oxysporum* species complex, *Fusarium proliferatum*, *Fusarium solani* species complex, *Pinus*, phylogenetic lineages

1. Introduction

Fusarium Link is one of the most diverse fungal genera in the world (Geiser et al. 2013; Leslie and Summerell 2006). Species in this genus can be found in a wide range of environments ranging from the arctic to the tropics. They are commonly distributed in soils and have been isolated from aerial and subterranean plant parts, plant debris and other organic matter (e.g., Burgess 1981; Sangalang et al. 1995; Summerell et al. 1993). Many *Fusarium* species occur as saprophytes but some are important pathogens of plants (e.g., Booth 1971; Bloomberg 1981), produce toxins that affect animals and humans negatively, while others cause diseases in humans and animals (Marasas et al. 1984; Desjardins 2006).

Examples of *Fusarium* diseases in agriculture and horticulture include scab, head blight and crown rot, root rot, cankers and vascular wilts (e.g., Booth 1971; Bloomberg 1981). In fact, *Fusarium* species are associated with seven of the 14 most important planted crop species in the world (Strange and Scott 2005). To further illustrate their importance, at least 79 presentations at the 2013 joint meeting of the American Phytopathological Society and the Mycological Society of America will focus on various species in the genus *Fusarium* (http://www.apsnet.org/meetings/annual/program/Documents/).

A number of *Fusarium* species are associated with diseases of forest trees. From a forestry perspective the most important species are *F. circinatum* Nirenberg & O'Donnell emend. Britz, Coutinho, Wingfield. & Marasas (Viljoen et al. 1994; Wingfield et al. 2008), *F. solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen (James and Burr 2000; Zhang et al. 2006), *F. oxysporum* Schlecht. emend. Snyder & Hansen (Riffle and Strong 1960; Bloomberg 1971; Ebbels and Allen 1979; Bloomberg 1981; Viljoen et al. 1992; Ocamb and Juzwick 1995; Kistler 1997) and *F. verticillioides* (Saccrdo) Nirenberg (Ebbels and Allen 1979; Bloomberg 1981; Lori and Salerno 2003; Salerno and Lori 2007). But of these, *F. circinatum* is the most relevant (Wingfield et al. 2008). Typical of most species in the genus, the ability of *Fusarium* species to grow on a broad range of different substrates and their efficient dispersal mechanisms contribute

to their wide distribution (Burgess 1981). As a result, *Fusarium* species in the forestry environment are usually distributed wherever suitable plant hosts occur (Bloomberg 1981; Ocamb and Juzwick 1995; Viljoen et al. 1992; Zhang et al. 2006; Wingfield et al. 2008), although there are some examples where the ranges of certain species are limited (Saremi et al. 1999).

Despite the efficient dispersal mechanisms of most plant pathogens, including *Fusarium* species, their long-distance dispersal is usually associated with anthropogenic factors (Braiser 2008; Stenlid et al. 2011). Nurseries and nursery practices are often associated with the propagation and spread of both pests and pathogens (Braiser 2008; Dehnen-Schmutz et al. 2010), especially in horticulture, but this is also true for commercial forestry nurseries. In South Africa, seedlings and/or cuttings for species of *Pinus* L., *Eucalyptus* L' Hérit and *Acacia* (Tourn.) L. are produced in nurseries for subsequent cultivation in commercial forestry plantations. The movement of these plants can therefore provide pathogens with an entry into new areas as has been seen for *F. circinatum* (Wingfield et al. 2008; Mitchell et al 2011). This fungus is thought to have been introduced from Mexico to South Africa on infected seed, which likely gave rise to the first outbreaks of the seedling disease caused by *F. circinatum* in the Mpumalanga Province (Viljoen et al. 1994; Wickler and Gordon 2000). Since then the pathogen has spread, probably via infected plant material, to most commercial nurseries around the country where it affects seedlings of various *Pinus* species (Wingfield et al. 2008; Mitchell et al. 2011).

A number of studies have shown that nursery soils are associated with diverse *Fusarium* species. This is particularly true for nurseries that cultivate seedlings of *Pinus* and other conifers (Bloomberg 1981; Viljoen et al. 1992; Axelrood et al. 1995; Ocamb and Juzwik 1995; James and Burr 2000; Lori and Salerno 2003; Stewart et al. 2006; James 2007; Salerno and Lori 2007; Vujanovic et al. 2007). Both pathogenic and non-pathogenic *Fusarium* species have been reported from soil, seed, and specific tissues of seedlings grown in this environment (Morgan 1983; Anderson 1986; Burgess et al. 1988; Dick and Dobbie 2002; Dick and Simpson 2003; Skovgaard et al. 2003; Dumroese and James 2005). For identification purposes, most of these previous studies

relied on morphology, however, research during the last two decades (O'Donnell 2000; O'Donnell et al. 2000; Taylor et al. 2000; Leslie and Summerell 2006; Kvas et al. 2009; O'Donnell et al. 2010) has shown that most *Fusarium* species can be reliably identified using only DNA sequence information for genes such as those encoding translation elongation factor 1 alpha (TEF), β-tubulin (BT) and mitochondrial small subunit ribosomal RNA (mtSSU). As a result, the current information available regarding the distribution of *Fusarium* species in the nursery environment should be viewed with circumspection. This is because many of the species diagnoses made in the absence of DNA-based tools are most likely not correct. .

Apart from the occurrence of the pitch canker pathogen, *F. circinatum* (Wingfield et al. 2008; Mitchell et al. 2011), little is known regarding the *Fusarium* diversity in South African forestry nurseries, especially where *Pinus* and *Eucalyptus* species are propagated. The overall aim of this study was, therefore, to determine the diversity of *Fusarium* species associated with the seedlings and/or cuttings of *Pinus* and *Eucalyptus* grown in commercial nurseries in South Africa. This was accomplished by using standard culture-based approaches for isolation and initial identification, followed by DNA sequence information for TEF. This was done after possible *F. circinatum* isolates had been identified using the PCR-based method developed by Schweigkofler et al. (2004). The data generated in this study should contribute to a reliable inventory of *Fusarium* species in the nurseries, which could be further characterized in terms of pathogenicity to ultimately identify potential risks to nurseries and the forestry industry as a whole.

2. Materials and Methods

2.1 Collection of fungal isolates: The *Fusarium* isolates were obtained from five commercial nurseries in South Africa that produce *Pinus* and/or *Eucalyptus* planting material (Table 1). Two nurseries were located in the Mpumalanga province and three were located in the KwaZulu-Natal province. Seedlings and/or cuttings of *Pinus patula* Schiede: Schltdl. & Cham, *Pinus taeda* L., *Pinus greggii* Engelm.: Parl. (Northern and

Southern provenance), *Pinus elliottii* Engelm., *Eucalyptus nitens* (Deane et Maiden) Maiden, *Eucalyptus grandis* Hill: Maid., *Eucalyptus smithii* R.T. Baker, *Eucalyptus macarthurii* (Deane et Maiden), *Eucalyptus grandis* x *urophylla* (GU), *Eucalyptus nitens* hybrids (NH) and *Eucalyptus nitens* x *grandis* (NG) were collected. All of the samples collected appeared healthy except for a number of *Pinus patula* seedlings from the Richmond nursery, which were showing signs of wilt and root rot.

Plant issue from root meristems, root collars, stems and shoot meristems were plated directly onto half-strength potato dextrose agar (PDA; 20g/L PDA, 15g/L agar; Biolab Diagnostics, Wadeville, South Africa) and *Fusarium* selective medium (FSM; Nash and Snyder 1962). Selected tissue samples were also surface-disinfested by first placing the material in 1.5 % (v/v) sodium hypochlorite for 1 min, then rinsing with sterile distilled water and finally washing for 1 min with 70 % (v/v) ethanol and leaving to air dry. The surface disinfected samples were then plated onto half strength PDA and FSM as described above.

After incubation at 27.5°C for 7 days, fungal cultures were examined microscopically. Those displaying morphological features resembling *Fusarium* species (Leslie and Summerell 2006) were transferred to fresh potato dextrose agar amended with glucose and yeast extract (PDA+; 20g/L PDA, glucose 1g/L, Yeast extract 1g/L; Merck, Wadeville, South Africa) and grown for seven days at 23°C. Conidia were collected by washing the cultures with a 2.5 % (v/v) Tween 60 (Sigma-Aldrich, St Louis, Missouri, USA) solution and spreading 1mL of the spore suspension over water agar medium (WA; 20g/L PDA; Biolab Diagnostics). After 2 days of incubation at 16°C, single germinating conidia were transferred to fresh PDA+ medium and incubated at 23°C for one week. All the cultures collected were maintained and kept in the *Fusarium* culture collection (CMWF) of the Forestry and Agricultural Biotechnology institute (FABI), University of Pretoria, South Africa.

2.2 DNA isolations, PCR amplifications and sequencing: Seven-day-old cultures on PDA+ were used to extract DNA using CTAB (hexadecyltrimethylammonium bromide) (Steenkamp et al. 1999). Fungal tissue was collected by directly scraping mycelium from the surface of the growth media. The collected mycelial tissue was then

homogenised in 500 µL of DNA extraction buffer containing 100mM Tris-HCl (pH 8.0), 10mM ethylenediaminetetra-acetic acid di-sodium salt (EDTA; pH 8.0), 2.0 % w/v sodium dodecyl sulphate (SDS) and 0.2 µg/µL Proteinase K (Sigma-Aldrich, St Louis, Missouri, USA). The mixture was stored at -24°C for 1 hour, followed by incubation at 60°C for another hour. After the incubation step, 180 µL of 5M NaCl and 80 µL 10% (w/v) CTAB solutions were added and further incubated at 65°C for 10 minutes. DNA was purified with 400 µL phenol-chloroform (1:1) (Sambrook et al. 1989). Nucleic acids were precipitated overnight with 0.6 volumes of isopropanol at -20°C, followed by centrifugation at 20 000g at 4°C for 30 minutes. DNA was further purified by washing with 70% (v/v) ethanol and air dried. DNA pellets were re-suspended in 40 µL sterile distilled water. Extracted DNA was visualized using gel electrophoresis (Sambrook et al. 1989) and quantified using a Nanodrop spectrophotometer (NanoDrop, Wilmington, USA) and then stored at -20°C until use.

A BioRad iCycler (BioRad Laboratories, Hercules, California, USA) was used to amplify a portion of the TEF region d using a using EF-1 and EF2 primers (O'Donnell et al. 1998; Geiser et al. 2004). PCR amplification reactions contained 2-4ng/µL of template DNA, 0.25 µM of each primer, 200 µM of each dNTP (Fermentas, Nunningen, Germany), 2.5 mM $MgCl_2$, 0.04 U/µL SuperTherm *Taq* polymerase and *Taq* buffer with KCl (Southern Cross Biotechnology, Cape Town, South Africa). PCR cycling conditions consisted of initial denaturation at 95°C for 3 min, 35 cycles of 94°C for 30s, 56°C (TEF) for 45s, 72°C for 1 min and a final elongation step at 72 °C for 10 min.

The resulting PCR products were precipitated using the polyethylene glycol (PEG) method (Steenkamp et al. 2006) or G50 Sephadex columns (Sigma, Steinheim, Germany). The purified samples were then sequenced using the original PCR primers, an ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and an ABI PRISM 3100™ DNA sequencer (Applied Biosystems). Raw sequence files were examined and manually corrected where necessary using Chromas Lite 2.01 (Technelysium, Australia) and BioEdit version 7.0.5.2 (Hall 1999). The TEF sequences were compared to those in the *Fusarium* identification database (Geiser et al. 2004; http://isolate.fusariumdb.org/index.php) and

GenBank (Benson et al. 2005; http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the BLAST search program (Altschul et al. 1990).

2.3 Phylogenetic analysis: Nucleotide sequences were aligned using MAFFT version 6.0 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) with the L-INS-i option selected (Katoh et al. 2002, 2005; Katoh and Toh 2008), after which the alignments were corrected manually were needed. Three aligned TEF datasets were constructed for sequences belonging to the *Gibberella fujikuroi* complex (GFC) (O'Donnell et al. 1998, 2000; Geiser et al. 2005), *Fusarium oxysporum* complex (FOC) (Baayen et al. 2000) and the *Fusarium solani* complex (FSC) (O'Donnell 2000). These alignments included specific sequences generated in this study as well as those for known members of the GFC, FSC (Tables 2 and 3) and FOC (Fourie et al. 2009), from GenBank.

Phylogenetic relationships for each dataset were inferred from TEF sequence data using maximum likelihood (ML) as implemented in PHYML v2.4.4 (Guindon and Gascuel, 2003). ML analyses utilized the best-fit model parameters as indicated by jModeltest 0.1.1 (Posada 2008). ML analyses of the GFC dataset used the General Time Reversible (GTR; Tavare 1986) and gamma correction (G) to account for among site rate variation. The TEF dataset for the FOC used Kimura's K80 model (Kimura 1980) with G and a proportion of invariable sites (I). The TEF dataset for the FSC used GTR+I+G. Branch support for the ML phylogenies were estimated using bootstrap analysis based on 1000 pseudo replicates. Mega 4 (Tamura et al. 2007) was used to view and edit phylogenetic trees.

2.4 Identification of Fusarium circinatum isolates: Selected isolates were subjected to PCR using the *F. circinatum*-specific primers CIRC1A and CIRC1B (Schweikofler et al. 2004) to allow amplification of a diagnostic portion of the ribosomal RNA intergenic spacer region (IGS) region. These reactions were performed as described previously (Schweikofler et al. 2004) at an annealing temperature of 54℃ with 30 cycles. PCR amplicons analysed and visualized using standard agarose gel electrophoresis (Sambrook et al. 1989).

3. Results

3.1 Fungal isolates: A total of 165 isolates with the distinctive macro- and microcondia associated with *Fusarium* (Leslie and Summerell 2006) were collected from the five nurseries in KwaZulu-Natal and Mpumalanga. From the 165 isolates examined in this study, 48% were recovered from plant roots, 21% from root collars and the remaining 31% from the above ground plant parts. At least one isolate of *Fusarium* could be recovered from each of the host species sampled. From the KwaMbonambi nursery 12 isolates of *Fusarium* were collected, as well as 10 from White River, 34 from Hilton, 47 from Ngodwana, and 54 from Richmond. Of these isolates, 88 were recovered from *Pinus* and 77 from *Eucalyptus*. Furthermore, 34 of the 165 isolates were obtained by first surface disinfecting the plant material before proceeding with isolations. The majority of the isolates were obtained from apparently healthy plant tissue with only 29 isolates collected from diseased *P. patula* from the Richmond nursery. Of the latter isolates, six were obtained from surface disinfected tissue.

3.2 Sequence analyses: Of the 165 isolates collected in this study, 102 were selected for TEF DNA sequence analyses. The 102 isolates selected represent an even diversity of isolates across all five nurseries from both *Pinus* and *Eucalyptus* hosts included in this study. This collection also excluded those isolates that were tentatively identified as *F. circinatum*, using IGS PCR (see below).

Using searches in GenBank and Fusarium-ID databases, all of the 102 isolates were species of *Fusarium*. All of the sequences shared 96-100% sequence similarity with known sequences from one of the three complexes GFC, FOC or FSC (Table 1). Based on the TEF data, most of the isolates (63) examined belong to the FOC, while 11 isolates formed part of the FSC and 28 isolates formed part of the GFC. FOC isolates were collected from all of the five nurseries, Ngodwana (27), Richmond (18), Hilton (8), White River (6) and KwaMbonambi (4) and of these 36 were collected from *Pinus* species and 27 from *Eucalyptus* species. All of the FSC isolates originated from the KwaMbonambi nursery where only *Eucalyptus* species are propagated. All of the GFC

isolates were recovered from *Eucalyptus* tissue and were collected from three nurseries, KwaMbonambi, Hilton and White River.

3.3 Phylogenetic analyses: ML analyses of the aligned data for the species residing in the FOC separated the complex into the four known clades (A-D) (Fourie et al. 2009) (Figure 1). The isolates recovered in the current study were distributed in Clades C and B. Although the bootstrap support for many branches was marginal, 15 lineages/groups were tentatively identified among the isolates examined.

A number of the groups/lineages included isolates only from *Pinus* (Groups 3, 5, 6, 7, 8, 12, 15) or only from *Eucalyptus* (Groups 2, 4, 13, 14). Some isolates also grouped specific to the nursery from which they were obtained. All of the isolates in Groups 4 and 14 (except for isolate MF007A2.1) originated from the same nursery in Hilton and KwaMbonambi where they were isolated from *Eucalyptus* species. The same pattern also occurred for isolates from Richmond (Group 2, Group 3), White River (Group 13) and Ngodwana (Groups 5, 7, 8) which were also all isolated from *Pinus*. The remaining isolates were in groups with little or no support and they were scattered amongst the various *F. oxysporum* strains. Three isolates RN 009 (Group 6); RN 018 (Group 3) and RN 028 residing in Group 6 were collected from diseased *P. patula* in Richmond and grouped closely with known FOC strains.

Phylogenetic analysis of the aligned sequence data for the isolates in the FSC separated the isolates into the three clades (Figure 2) that have previously been recognized by O'Donnell (2000). These included the New Zealand and South American clades that include isolates exclusively from these areas, as well as the "worldwide" clade, previously designated as Clade 3 (O'Donnell 2000). In total, four distinct groups/lineages (Groups 1-4) were identified among the FSC isolates, of which all formed part of the "worldwide" clade. Apart from the isolates identified here, Group 1 also included a known strain that was originally isolated from *Plumeria alba* that is normally grown as an ornamental (Zhang et al. 2006). Group 4 also included a known strain that was isolated from corneal samples (O'Donnell et al. 2007). These two groups represent unique lineages/groups in the FSC.

ML analyses for all of the isolates belonging to the GFC indicated that they all belong to the so-called “Asian” clade (O’Donnell et al. 1998). Within this clade, the isolates examined grouped with *F. proliferatum* (Figure 3). Although all of these isolates shared sequence similarity and grouped with *F. proliferatum*, they formed three distinct groups within the greater F*. proliferatum* clade. Group 1 consisted of isolates collected from Hilton as well as two isolates from White River. Groups 2 and 3 consisted of isolates from KwaMbonambi and Hilton, respectively. The Group 1 isolates were recovered from various *Eucalyptus* hosts, although those in Groups 2 and 3 were isolated from *E. grandis* x *urophylla* and *E. dunnii*, respectively.

3.4 Fusarium circinatum identification: Of the remaining 63 isolates that were subjected to the IGS PCR, 17 were identified as *F. circinatum* (Table 1). All of these isolates were obtained from the Richmond nursery. The isolates were from *P*. *patula* that were showing symptoms of wilting, root and collar rot. The only exception was isolate RN 024, from the root of a healthy *E. nitens* cutting.

4. Discussion

The results of this study revealed a substantial and surprising diversity of *Fusarium* species in South African commercial forestry nurseries. The *Fusarium* species complex that was most frequently sampled in this study was the FOC. Most of the unique groups of isolates also occurred in this complex. These findings emphasize the fact that the members of this complex are widely distributed, genetically diverse and capable of associating with multiple plant hosts (Burgess 1981; Baayen et al. 2000; Salerno et al. 1999; Keane et al. 2000; Salerno et al. 2000; Lori and Salerno 2003; Stewart et al. 2006; Salerno and Lori 2007). FOC has also been shown to be the most common group identified when studies focus on pathogenic *Fusarium* in commercial forest nurseries (reviewed by James and Dumroese 2006) but are extremely variable as they are associated with healthy and diseased plants and seed (Graham and Linderman 1983; Hamm et al. 1990; Axelrood et al. 1995). Although the overall FOC diversity among nurseries and hosts differed, many of the isolates grouped according to host or

locality. However, most FOC isolates examined were obtained from apparently healthy plant tissue, which suggests that the isolate groups observed in the TEF phylogeny likely do not reflect the ecology of pathogens in these environments.

The majority of the FOC isolates examined in this study might represent endophytes, as has been shown previously for other plant hosts (Postma and Rattink 1992; Dhingra et al. 2006; Vu et al. 2006). Accordingly, many FOC strains have been have been employed as biocontrol agents against other fungi, including *Fusarium* species (e.g., Dhingra et al. 2006; Forsyth et al. 2006; Vu et al. 2006). Some FOC strains are also known to contribute to soil suppressiveness by reducing the ability of pathogenic *Fusarium* species to cause disease (e.g., Scher and Baker 1980, 1982; Alabouvette 1986; Mandeel and Baker 1991; Postma and Rattink 1992; Larkin et al. 1996; Alabouvette 1999). Most of the FOC groups recovered in this study were from the Ngodwana and Richmond nurseries that produce *Pinus* seedlings and from which *F. circinatum* is well-known (Britz et al. 2005; Hurley et al. 2007). The distribution of FOC in these two nurseries might, therefore, be responsible for fact that a small number of *F. circinatum* isolates were recovered from plant material collected in the Richmond nursery. Further studies might consider whether these FOC isolates could be used to reduce *F. circinatum* inoculum in nurseries.

Although 15 groups of FOC isolates were identified in this study, additional work is needed to determine whether these represent new or known lineages in this complex. The main reason for this is the fact that most branches in the TEF phylogeny inferred for this complex lacked significant support. Previous studies have shown that the addition of other gene regions such as mtSSU can improve the phylogenetic resolution within this complex dramatically (O'Donnell et al. 2000; Fourie et al. 2009). Nevertheless, most of the tentative FOC groups identified in this study appeared to be unique. Only four of the groups included known strains of the complex, which included known human pathogenic strains (FOC Group 6, FOC Group 11, FOC Group 13) and pathogenic strains of *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *gladioli* (FOC Group 13). Future research should thus seek to infer a robust phylogeny for this complex and to determine whether the various FOC groups identified in this study

represent new taxa or are related to the members of existing *formae speciales* of *F. oxysporum*.

The two species of the GFC that were identified in this study were *F. circinatum* and *F. proliferatum*. The latter species is known as a serious pathogen of many crops (Desjardins et al. 1997; Stankovic et al. 2007) and is often recorded on pine and conifers (James and Burr 2000; Lori and Salerno 2003; reviewed by James and Dumroese 2006; James 2007). Although *F. proliferatum* is not known as a pathogen on *Eucalyptus*, the results presented here suggest an association with various *Eucalyptus* species in the nursery environment. However, *F. proliferatum* was generally not isolated from plants displaying obvious symptoms of disease and was also recovered, in a few instances (*i.e.*, *F. proliferatum* Group 2), from surface disinfected plant material. It is therefore likely that some of these isolates represent entophytes, while others are potentially common soil inhabitants. This is in agreement with what is known for this species as it has been recorded previously as an endophyte of Sorghum (Leslie 2000), mangroves (Cheng et al. 2008), Maize (Saunders and Kohn 2009), *Colobanthus quitensis* (Kunth) Bartl. (Rosa et al. 2010), *Pinus halepensis* Mill. (Botella and Diez 2011), *Dysoxylum binectariferum* Hook.f. ex Bedd (Kumara et al. 2012) and has on occasion been isolated from soil (Jeschke et al. 1990).

Fusarium circinatum is the causal agent of pitch canker on established forest of plantation trees of *Pinus* species (Wingfield et al. 2008; Mitchell et al. 2011). On seedlings, this pathogen causes root and collar rot, wilting and die-back (Wingfield et al. 2008). Despite the fact that this pathogen is widespread and common in commercial seedling nurseries in South Africa (Wingfield et al. 2008; Mitchell et al. 2011), isolates of *F. circinatum* were recovered only from one of the nurseries included in this study. Except for one isolate that was obtained from *E. nitens*, all of these isolates were obtained from *Pinus* seedlings showing symptoms of infection in the Richmond nursery. Because there is no known association between *F. circinatum* and *Eucalyptus* species the single *F. circinatum* isolate likely came from the soil around the roots as it was obtained from *Eucalyptus* roots that were not surface disinfected. Although proper hygiene and nursery practices may explain why *F. circinatum* might occur at low

frequencies, it does not explain why this pathogen was not recovered from Ngodwana, where *Pinus* species, including *P. patula*, are cultivated. This is especially true in light of the fact that *F. circinatum* is frequently isolated from apparently healthy and asymptomatic *Pinus* seedlings (Mitchell et al. 2011). The possibility that the combined effects of reduced inoculum loads due to efficient management practices and the presence of fungi such as FOC may limit disease incidence should, therefore, be explored more closely.

Among the *Fusarium* isolates examined in this study, members of the FSC were identified. Although this complex is well-known for including pathogens of conifers and other plant hosts (James 2000; O'Donnell 2000; Lori and Salerno 2003; Zhang et al. 2006; O'Donnell et al. 2007), FSC isolates are typically less predominant on *Eucalyptus* than *FOC* isolates (Salerno et al. 1999; Keane et al. 2000). However, the FSC isolates are known to form part of a disease complex. For example, the members of the FSC and FOC are generally regarded as less pathogenic, but can form a complex where all species involved contribute to disease (James and Dumroese 2006). Future research should, therefore, attempt to determine the role, if any, of FSC together with FOC strains in the development of disease in *Eucalyptus* plants cultivated in nurseries.

The four FSC groups observed in this study formed part of the so-called "worldwide" clade that is known to contain many *formae speciales* and human pathogens (O'Donnell 2000). Isolates in this clade are usually collected from agricultural substrates and plants (e.g., O'Donnell 2000; Bogale et al. 2009) or from humans (Zhang et al. 2006) and their broad distribution is linked to the movements and distributions of their hosts. Because a number of species have been recognized in this complex (O'Donnell 2000), future studies should also evaluate the taxonomic status of the isolate groups recovered in the present study. Such studies would likely involve pathogenicity assays (Matuo and Snyder 1973), sexual compatibility tests (Matuo and Snyder 1973; VanEtten and Kistler 1988; Covert et al. 2007) and various DNA-based methods (O'Donnell 2000; Zhang et al. 2006) combined with phylogenetics (Aoki et al. 2003, 2005; O'Donnell et al. 2000). However, the findings presented here suggest that novel FSC diversity occurs in the commercial forestry nurseries of South Africa.

Overall the results of this study revealed that diverse *Fusarium* species and isolates are associated with *Pinus* and *Eucalyptus* nursery plants, although their exact roles in these environments remain uncertain. In fact, the diversity in the nursery setting is probably much greater if growth media and soil were also examined. Soil, for example, is known contain many *Fusarium* species (Bloomberg 1981; Axelrood et al. 1995; Ocamb and Juzwik 1995; Stewart et al. 2006; Vujanovic, et al. 2007) and these were most likely overlooked because isolates were obtained from plant tissue. Without the use of DNA-based methods, much of the diversity from this study would have been under-represented and so this study shows the importance of DNA-based techniques in fungal identification. Ideally species inventories generated in studies such as this should be complemented by fully characterizing the species and lineages in terms of pathogenicity and even population biology. In this way it should be possible to build a comprehensive understanding of the ecology and biology of the *Fusarium* spp. in a specific environment. From the perspective of commercial forestry nurseries such information would be valuable as it would allow numerous opportunities for developing strategies to control disease and improve the health of the plants that are propagated.

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Table 1: Geographic origin and host species of the *Fusarium* isolates collected in this study.

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
MF 004 A2	CMWF 1669	*F. oxysporum* species complex NRRL 38591	FOC, 1	Root Collar	*Eucalyptus* NH 70	White River, MP	-25°18'14" 31° 02'43"
MF 004 B	CMWF 1670	*F. oxysporum* species complex NRRL 38591	FOC, 1	Root Collar	*Eucalyptus* NH 70	White River, MP	-25°18'14" 31° 02'43"
NGW 003	CMWF 1704	*F. oxysporum* species complex NRRL 38540	FOC, 1	Root Collar	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 015	CMWF 1714	*F. oxysporum* species complex NRRL 20433	FOC, 1	Root[#]	*P. patula*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 022	n/a	*F. oxysporum* f. sp. *melonis*	FOC, 1	Root	*P. patula*	Ngodwana, MP	-25°34'58" 30°38'29"
RN 031 A1	CMWF 1777	*F. oxysporum* species complex NRRL 38591	FOC, 1	Tip	*E. nitens*	Richmond, KZN	-29°52'9" 30°13'48"
RN 041 B2	CMWF 1790	*F. oxysporum* species complex NRRL 45881	FOC, 1	Root	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 043 B2	CMWF 1794	*F. oxysporum* species complex NRRL 38591	FOC, 1	Root	*E. nitens*	Richmond, KZN	-29°52'9" 30°13'48"
RN 048 B	CMWF 1798	*F. oxysporum* species complex NRRL 45881	FOC, 1	Root[#]	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
Rn 040	n/a	*F. oxysporum* f. sp. *melonis*	FOC, 2	Root Collar	*E. nitens*	Richmond, KZN	29°52'9" 30°13'48"
RN 043 A1	CMWF 1793	*F. oxysporum* species complex NRRL 38591	FOC, 2	Root	*E. nitens*	Richmond, KZN	-29°52'9" 30°13'48"
RN 018	CMWF 1762	*F. oxysporum* species complex NRRL 45881	FOC, 3	Root	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 030 B1	CMWF 1776	*F. oxysporum* species complex NRRL 45881	FOC, 3	Root	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
RN 041 A2	CMWF 1789	*F. oxysporum* species complex NRRL 45881	FOC, 3	Root	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 048 A1	CMWF 1797	*F. oxysporum* species complex NRRL 45881	FOC, 3	Root[#]	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
KW 008	CMWF 1659	*F. oxysporum* f. sp. *ciceris* strain NRRL 32158	FOC, 4	Stem[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 017	CMWF 1661	*F. oxysporum* f. sp. *ciceris* strain NRRL 32158	FOC, 4	Stem[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 017.1	n/a	*F. oxysporum* f. sp. *ciceris* strain NRRL 32158	FOC, 4	Stem[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
NGW 007	CMWF 1707	*F. oxysporum*	FOC, 5	Root	*P. elliottii*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 036	n/a	*Fusarium oxysporum* f. sp. *opuntiarum*	FOC, 5	Root Collar	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 038 B	CMWF 1734	*F. oxysporum*	FOC, 6	Tip	*P. greggii* SN	Ngodwana, MP	25°34'58" 30°38'29"
NGW 045 B	CMWF 1743	*F. oxysporum*	FOC, 6	Root	*P. taeda*	Ngodwana, MP	25°34'58" 30°38'29"
NGW 047 A	CMWF 1746	*F. oxysporum*	FOC, 6	Root[#]	*P. patula*	Ngodwana, MP	25°34'58" 30°38'29"
RN 009	CMWF 1755	*F. oxysporum*	FOC, 6	Root[#]	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 013	n/a	*F. oxysporum*	FOC, 6	Root	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 028	CMWF 1772	*F. oxysporum* species complex NRRL 38515	FOC, 6	Tip	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
NGW 014	CMWF 1713	*F. oxysporum* species complex NRRL 32931	FOC, 7	Root	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
NGW 016	n/a	*F. oxysporum*	FOC, 7	Root	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 018	CMWF 1716	*F. oxysporum* species complex NRRL 32931	FOC, 7	Root	*P. elliottii*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 021	CMWF 1719	*F. oxysporum* species complex NRRL 32931	FOC, 7	Root	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 027	CMWF 1723	*F. oxysporum* species complex NRRL 32931	FOC, 7	Root	*P. greggii* NN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 035	CMWF 1730	*F. oxysporum* species complex NRRL 32931	FOC, 7	Stem	*P. greggii* NN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 036 B2	CMWF 1732	*F. oxysporum* species complex NRRL 32931	FOC, 7	Root Collar	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 001	CMWF 1702	*F. oxysporum* species complex NRRL 38540	FOC, 8	Root Collar	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 023	CMWF 1720	*F. oxysporum* species complex NRRL 38540	FOC, 8	Root	*P. patula*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 008	CMWF 1708	*F. oxysporum* species complex NRRL 38540	FOC, 9	Root[#]	*P. elliottii*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 036 A1	CMWF 1731	*F. oxysporum* species complex NRRL 22548	FOC, 9	Root Collar	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 046 A1	CMWF 1744	*F. oxysporum* species complex NRRL 38540	FOC, 9	Stem	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 046 B1	CMWF 1745	*F. oxysporum* species complex NRRL 38540	FOC, 9	Stem	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
RN 019	CMWF 1763	*F. oxysporum* species complex NRRL 38515	FOC, 9	Root[#]	*E. macarthurii*	Richmond, KZN	-29°52'9" 30°13'48"
RN 036	CMWF 1784	*F. oxysporum* species complex NRRL 32512	FOC, 9	Root	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
MF 006 A2	CMWF 1674	*F. oxysporum* NRRL 38501	FOC, 10	Root Collar	*Eucalyptus* NH 70	White River, MP	-25°18'14" 31° 02'43"
MT 003	CMWF 1677	*F. oxysporum* species complex NRRL 36092	FOC, 10	Root	*Eucalyptus* NH 00	Hilton, KZN	-29°34'09" 30° 16'24"
NGW 040 A1	CMWF 1737	*F. oxysporum* species complex NRRL 32512	FOC, 10	Stem	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 040 B1	CMWF 1738	*F. oxysporum* species complex NRRL 22548	FOC, 10	Stem	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
KW 010	n/a	*F. oxysporum*	FOC, 11	Stem[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
NGW 011	CMWF 1711	*F. oxysporum* species complex NRRL 39464	FOC, 11	Root	*P. patula*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 044	CMWF 1740	*F. oxysporum* species complex NRRL 39464	FOC, 11	Root Collar	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
RN 002	CMWF 1749	*F. oxysporum* species complex NRRL 39464	FOC, 11	Root[#]	*E. macarthurii*	Richmond, KZN	-29°52'9" 30°13'48"
RN 037	CMWF 1785	*F. oxysporum* species complex NRRL 39464	FOC, 11	Stem[#]	*E. smithii*	Richmond, KZN	-29°52'9" 30°13'48"
RN 037	n/a	*F. oxysporum* species complex NRRL 39464	FOC, 11	Stem[#]	*E. smithii*	Richmond, KZN	-29°52'9" 30°13'48"
NGW 039 A	CMWF 1735	*F. oxysporum* species complex NRRL 38595	FOC, 12	Root	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
MF 005 A1	CMWF 1671	*F. oxysporum* species complex NRRL 38600	FOC, 13	Stem	*Eucalyptus* NG 108	White River, MP	-25°18'14" 31° 02'43"
MF 005 B1	CMWF 1673	*F. oxysporum* species complex NRRL 38600	FOC, 13	Stem	*Eucalyptus* NG 108	White River, MP	-25°18'14" 31° 02'43"
MF 007 A2.1	CMWF 1675	*F. oxysporum*	FOC, 14	Root Collar	*Eucalyptus* NG 108	White River, MP	-25°18'14" 31° 02'43"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
MT 007	n/a	*Fusarium oxysporum* NRRL 52690	FOC, 14	Root	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 008 A2	CMWF 1681	*Fusarium oxysporum* NRRL 52690	FOC, 14	Tip	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 008 A2.1	n/a	*Fusarium oxysporum* NRRL 52690	FOC, 14	Tip	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 008 A2.2	n/a	*Fusarium oxysporum* NRRL 52690	FOC, 14	Tip	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 014 A	n/a	*Fusarium oxysporum* NRRL 52690	FOC, 14	Root	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 014 B1	CMWF 1687	*F. oxysporum* species complex NRRL 26449	FOC, 14	Root	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 014 B1.1	n/a	*Fusarium oxysporum* NRRL 52690	FOC, 14	Tip	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
NGW 041	CMWF 1739	*F. oxysporum* species complex NRRL 22548	FOC, 15	Stem	*P. greggii* NN	Ngodwana, MP	-25°34'58" 30°38'29"
KW 012	n/a	*F. solani*	FSC, 1	Root[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 012.1	n/a	*F. solani*	FSC, 1	Root[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 012.2	n/a	*F. solani*	FSC, 1	Root[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW018	n/a	*F. solani*	FSC, 2	Stem[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 004	n/a	*F. solani*	FSC, 3	Root Collar[#]	*Eucalyptus* GU[c]	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 016	n/a	*F. solani*	FSC, 3	Tip#	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
KW 003	n/a	*F. solani*	FSC, 4	Root[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 011	CMWF 1660	*F. solani*	FSC, 5	Root[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 020	CMWF 1662	*F. solani*	FSC, 6	Stem[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 023	CMWF 1663	*F. solani*	FSC, 6	Root Collar[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 025	CMWF 1664	*F. solani*	FSC, 6	Root Collar[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
MT 002 B1	CMWF 1676	*F. proliferatum*	GFC, 1	Root	*Eucalyptus* NH 00	Hilton, KZN	-29°34'09" 30° 16'24"
MT 008 A2	CMWF 1681	*F. proliferatum*	GFC, 1	Tip	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 016 B2	CMWF 1690	*F. proliferatum*	GFC, 1	Root	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 022	CMWF 1694	*F. proliferatum*	GFC, 1	Stem	*Eucalyptus* NH 00	Hilton, KZN	-29°34'09" 30° 16'24"
MT 026 A1	CMWF 1699	*F. proliferatum*	GFC, 1	Root Collar	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 005	CMWF 1678	*F. proliferatum*	GFC, 2	Root Collar	*Eucalyptus* NH 00	Hilton, KZN	-29°34'09" 30° 16'24"
MT 015 A1	CMWF 1688	*F. proliferatum*	GFC, 2	Stem	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 023 A2	CMWF 1695	*F. proliferatum*	GFC, 2	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 024	CMWF 1697	*F. proliferatum*	GFC, 2	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
MF 002 B1	CMWF 1666	*F. proliferatum*	GFC, 3	Tip	*Eucalyptus* NH 70	White River, MP	-25°18'14" 31° 02'43"
MT 009 B1	CMWF 1683	*F. proliferatum*	GFC, 3	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MF 003 B2	CMWF 1668	*F. proliferatum*	GFC, 4	Root Collar	*Eucalyptus* NH 70	White River, MP	-25°18'14" 31° 02'43"
MT 007 A1	CMWF 1679	*F. proliferatum*	GFC, 4	Root	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 007 B1	CMWF 1680	*F. proliferatum*	GFC, 4	Root	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 013 A2	CMWF 1685	*F. proliferatum*	GFC, 4	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 009 A2	CMWF 1682	*F. proliferatum*	GFC, 5	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 013 B	CMWF 1686	*F. proliferatum*	GFC, 5	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 015 B2	CMWF 1689	*F. proliferatum*	GFC, 5	Stem	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 019	CMWF 1691	*F. proliferatum*	GFC, 5	Stem	*Eucalyptus* NH 00	Hilton, KZN	-29°34'09" 30° 16'24"
MT 020	CMWF 1692	*F. proliferatum*	GFC, 5	Root Collar	*Eucalyptus* NH 00	Hilton, KZN	-29°34'09" 30° 16'24"
MT 021	CMWF 1693	*F. proliferatum*	GFC, 5	Root Collar	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 023 B2	CMWF 1696	*F. proliferatum*	GFC, 5	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 025	CMWF 1698	*F. proliferatum*	GFC, 5	Root	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
KW 016.1	n/a	*F. proliferatum*	GFC, 6	Tip[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 022	n/a	*F. proliferatum*	GFC, 6	Stem[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 023.1	n/a	*F. proliferatum*	GFC, 6	Root Collar[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
MT 007 A1.1	n/a	*F. proliferatum*	GFC, 7	Stem	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 012	CMWF 1684	*F. proliferatum*	GFC, 7	Stem	*E. dunnii*	Hilton, KZN	-29°34'09" 30° 16'24"
KW 006	CMWF 1657	*Fusarium* sp.[e]	n/a	Stem[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
KW 007	CMWF 1658	*Fusarium* sp.[e]	n/a	Root[#]	*Eucalyptus* GU	KwaMbonambi KZN	-28°35'51" 32°10'53"
MF 001	CMWF 1665	*Fusarium* sp.[e]	n/a	Stem	*Eucalyptus* NG 108[c]	White River, MP	-25°18'14" 31° 02'43"
MF 003 A1	CMWF 1667	*Fusarium* sp.[e]	n/a	Root Collar	*Eucalyptus* NH 70	White River, MP	-25°18'14" 31° 02'43"
MT 013 A2	CMWF 1685	*Fusarium* sp.[e]	n/a	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 026 B1	CMWF 1700	*Fusarium* sp.[e]	n/a	Root Collar	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
MT 027	CMWF 1701	*Fusarium* sp.[e]	n/a	Root	*E. grandis*	Hilton, KZN	-29°34'09" 30° 16'24"
NGW 002	CMWF 1703	*Fusarium* sp.[e]	n/a	Root	*P. elliottii*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 005	CMWF 1705	*Fusarium* sp.[e]	n/a	Root Collar	*P. elliottii*	Ngodwana, MP	-25°34'58" 30°38'29"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
NGW 006	CMWF 1706	*Fusarium* sp.[e]	n/a	Root	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 009	CMWF 1709	*Fusarium* sp.[e]	n/a	Root[#]	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 010	CMWF 1710	*Fusarium* sp.[e]	n/a	Root	*P. greggii* NN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 012	CMWF 1712	*Fusarium* sp.[e]	n/a	Stem[#]	*P. greggii* NN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 017	CMWF 1715	*Fusarium* sp.[e]	n/a	Root Collar	*P. greggii* NN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 019	CMWF 1717	*Fusarium* sp.[e]	n/a	Root	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 020	CMWF 1718	*Fusarium* sp.[e]	n/a	Tip	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 024	CMWF 1721	*Fusarium* sp.[e]	n/a	Root[#]	*P. elliottii*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 026	CMWF 1722	*Fusarium* sp.[e]	n/a	Stem	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 028	CMWF 1724	*Fusarium* sp.[e]	n/a	Root	*P. patula*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 029	CMWF 1725	*Fusarium* sp.[e]	n/a	Root	*P. greggii* NN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 031	CMWF 1726	*Fusarium* sp.[e]	n/a	Root	*P. taeda*	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 032	CMWF 1727	*Fusarium* sp.[e]	n/a	Root	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 033	CMWF 1728	*Fusarium* sp.[e]	n/a	Root	*P. greggii* NN	Ngodwana, MP	-25°34'58" 30°38'29"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
NGW 034	CMWF 1729	*Fusarium* sp.[e]	n/a	Root Collar	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 038 A2	CMWF 1733	*Fusarium* sp.[e]	n/a	Tip	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 039 B2	CMWF 1736	*Fusarium* sp.[e]	n/a	Root	*P. greggii* SN	Ngodwana, MP	-25°34'58" 30°38'29"
NGW 047 B2	CMWF 1747	*Fusarium* sp.[e]	n/a	Root[#]	*P. patula*	Ngodwana, MP	-25°34'58" 30°38'29"
RN 001	CMWF 1748	*Fusarium* sp.[e]	n/a	Root	*E. macarthurii*	Richmond, KZN	-29°52'9" 30°13'48"
RN 004	CMWF 1750	*Fusarium circinatum*[†]	n/a	Tip	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 005	CMWF 1751	*Fusarium circinatum*[†]	n/a	Stem	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 006	CMWF 1752	*Fusarium circinatum*[†]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 007	CMWF 1753	*Fusarium* sp.[e]	n/a	Root[#]	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 008	CMWF 1754	*Fusarium circinatum*[†]	n/a	Root[#]	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 011	CMWF 1756	*Fusarium* sp.[e]	n/a	Root	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 012	CMWF 1757	*Fusarium* sp.[e]	n/a	Root	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 014	CMWF 1758	*Fusarium* sp.[e]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 015	CMWF 1759	*Fusarium circinatum*[†]	n/a	Tip	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
RN 016	CMWF 1760	*Fusarium* sp.[e]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 017	CMWF 1761	*Fusarium* sp.[e]	n/a	Stem	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 020	CMWF 1764	*Fusarium* sp.[e]	n/a	Root[#]	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 022	CMWF 1765	*Fusarium* sp.[e]	n/a	Root[#]	*E. nitens*	Richmond, KZN	-29°52'9" 30°13'48"
RN 023	CMWF 1766	*Fusarium circinatum*[†]	n/a	Root[#]	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 024	CMWF 1767	*Fusarium circinatum*[†]	n/a	Root	*E. nitens*	Richmond, KZN	-29°52'9" 30°13'48"
RN 026 A2	CMWF 1768	*Fusarium circinatum*[†]	n/a	Tip	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 026 B1	CMWF 1769	*Fusarium circinatum*[†]	n/a	Tip	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 027 A1	CMWF 1770	*Fusarium circinatum*[†]	n/a	Root[#]	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 027 B2	CMWF 1771	*Fusarium circinatum*[†]	n/a	Root[#]	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 028 B1	CMWF 1773	*Fusarium circinatum*[†]	n/a	Tip	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 029 B1	CMWF 1774	*Fusarium* sp.[e]	n/a	Root Collar	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 030 A2	CMWF 1775	*Fusarium* sp.[e]	n/a	Root	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 031 B1	CMWF 1778	*Fusarium* sp.[e]	n/a	Tip	*E. nitens*	Richmond, KZN	-29°52'9" 30°13'48"

Isolate number	CMWF Number[a]	*Fusarium* species[b]		Tissue isolated[c]	Host[d]	Origin	GPS Co-ordinates
		Best Blast Hit	Phylogenetic group membership				
RN 032 A2	CMWF 1779	*Fusarium* sp.[e]	n/a	Root	*P. patula*	Richmond, KZN	-29°52'9" 30°13'48"
RN 033 A1	CMWF 1780	*Fusarium circinatum*[f]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 033 B1	CMWF 1781	*Fusarium circinatum*[f]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 034 A2	CMWF 1782	*Fusarium circinatum*[f]	n/a	Stem	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 035	CMWF 1783	*Fusarium* sp.[e]	n/a	Root	*E. grandis*	Richmond, KZN	-29°52'9" 30°13'48"
RN 038	CMWF 1786	*Fusarium* sp.[e]	n/a	Root	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 039 A2	CMWF 1787	*Fusarium* sp.[e]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 039 B1	CMWF 1788	*Fusarium circinatum*[f]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 042 A1	CMWF 1791	*Fusarium* sp.[e]	n/a	Tip	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 042 B1	CMWF 1792	*Fusarium* sp.[e]	n/a	Tip	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 045 A2	CMWF 1795	*Fusarium* sp.[e]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"
RN 045 B1	CMWF 1796	*Fusarium* sp.[e]	n/a	Root Collar	*P. patula**	Richmond, KZN	-29°52'9" 30°13'48"

[a]- CMWF: Fusarium culture collection at FABI, University of Pretoria, South Africa. [b]- Species were identified using their TEF sequences, which were either compared to those in the Fusarium-ID database (http://isolate.fusariumdb.org/index.php) and reported as the "Best Blast Hit" or based on the phylogenetic groups to which they belong (Figures 1-3). [c]- Samples that were isolated from tissue that was surface disinfected are indicated with [#] and samples that were showing symptoms of wilt and root rot are indicated with *. [d]- GU – *Eucalyptus grandis* x *urophylla* hybrid, NH – *Eucalyptus nitens* hybrid, NG – *Eucalyptus nitens* x *grandis* hybrid, SN – *Pinus greggii* Southern provenance, NN – *Pinus greggii* Northern provenance. [e]- Isolates that were identified as *Fusarium* sp. were identified based on morphology only. [f]- Isolates identified as *F. circinatum* based on the *F. circinatum* diagnostic PCR.

Table 2: Information for the various TEF sequences obtained from GenBank included in this study to represent known species and clades within the FSC.

Species[a]	Host/Substrate[b]	Culture Collection[c]	GenBank number[d]
F. ambrosum	*Xyleborus fornicates* galleries	NRRL 20438	AF178332[4]
F. brasiliense	*Glycine max*	NRRL 31779	AY320150[6]
F. cuneirostrum	*Glycine max*	NRRL 31949	AY320161[6]
F. illudens (*Nectria illudens*)	*Beilschmieda tawa*	NRRL 22090	AF178326[4]
F. martii-phaseoli	*Phaseolus vulgaris*	NRRL 22158	AF178331[4]
F. martii-phaseoli	*Phaseolus vulgaris*	NRRL 22275	AF178335[4]
F. oxysporum f. sp. *lycopersici*	Curcubit sp.	F 1237	JQ965461[1]
F. phaseoli	*Phaseolus vulgaris*	NRRL 31156	AY220187[7]
F. solani	Caladium tuber	FRC S1631	DQ247705[2]
F. solani	Caladium tuber	FRC S1652	DQ247707[2]
F. solani	Caladium tuber	FRC S1674	DQ247699[2]
F. solani	*Pinus elliottii*	FRC S560	DQ247353[2]
F. solani	*Pinus radiata*	FRC S684	DQ247390[2]
F. solani	Guayule root	FRC S725	DQ247335[2]
F. solani	Geranium sp.	FRC S732	DQ247391[2]
F. solani	*Plumeria alba*	NRRL 22353	DQ247571[2]
F. solani	unknown	NRRL 22737	DQ247648[2]
F. solani	*Hoheria populnea*	NRRL 22470	DQ247650[2]
F. solani	*Glycine max*	NRRL 22743	DQ247645[2]
F. solani	Unknown	NRRL 43702	EF453027[3]

Species[a]	Host/Substrate[b]	Culture Collection[c]	GenBank number[d]
F. solani f. sp. *batatas*	*Ipomoea batatas*	NRRL 22400	AF178343[4]
F. solani f. sp. *batatas*	*Ipomoea batatas*	NRRL 22402	AF178344[4]
F. solani f. sp. *cucurbitae* (*Nectria haematococca* MP I)	-	NRRL 22098	AF178327[4]
F. solani f. sp. *cucurbitae* (*Nectria haematococca* MP I)	-	NRRL 22153	AF178346[4]
F. solani f. sp. *cucurbitae* (*Nectria haematococca* MP V)	*Cucurbita* sp.	NRRL 22141	AF178329[4]
F. solani f. sp. *cucurbitae* (*Nectria haematococca* MP V)	*Cucurbita* sp.	NRRL 22142	AF178347[4]
F. solani f. sp. *glycines*	*Glycine max*	NRRL 22823	AF178356[4]
F. solani f. sp. *glycines*	*Glycine max*	NRRL 22825	AF178357[4]
F. solani f. sp. *mori* (*Nectria haematococca* MP III)	*Morus alba*	NRRL 22157	AF178359[4]
F. solani f. sp. *mori* (*Nectria haematococca* MP III)	*Morus alba*	NRRL 22230	AF178358[4]
F. solani f. sp. *piperis* (*Nectria haematococca* f. sp. *piperis*)	*Piper nigrum*	NRRL 22570	AF178360[4]
F. solani f. sp. *pisi* (*Nectria haematococca* MP VI)	*Pisum sativum*	NRRL 22278	AF178337[4]
F. solani f. sp. *pisi*	*Glycine max*	NRRL 22820	AF178355[4]
F. solani f. sp. *robiniae* (*Nectria haematococca* MP VII)	*Robinia pseudoacacia*	NRRL 22161	AF178330[4]
F. solani f. sp. *robiniae* (*Nectria haematococca* MP VII)	*Robinia* sp.	NRRL 22586	AF178353[4]
F. solani f. sp. *xanthoxyli* (*Nectria haematococca* MP IV)	*Xanthoxylum pipertum*	NRRL 22163	AF178328[4]
F. solani f. sp. *xanthoxyli* (*Nectria haematococca* MP IV)	*Xanthoxylum* sp.	NRRL 22277	AF178336[4]
F. solani var *petroliphilum*	Curcubit sp.	NRRL 22735	DQ247646[2]
F. striatum (*Nectria* sp. (Homothallic))	Cotton duck cloth	NRRL 22101	AF178333[4]
F. tucumaniae	*Glycine max*	NRRL 31950	AY320157[6]

Species[a]	Host/Substrate[b]	Culture Collection[c]	GenBank number[d]
Fusarium sp. (*Nectria vasinfecta* (Homothallic))	*Heterodera glycines*	NRRL 22166	AF178350[4]
Fusarium sp. (*Nectria* sp. (Homothallic))	Bark of dicot tree	NRRL 22178	AF178334[4]
Fusarium sp. (*Nectria* sp.)	Bark	NRRL 22354	AF178338[4]
Fusarium sp. (*Nectria* sp.)	Bark	NRRL 22387	AF178339[4]
Fusarium sp. (*Nectria* sp. (Homothallic))	*Liriodendron tulipfera*	NRRL 22389	AF178340[4]
Fusarium sp. (*Nectria* sp.)	Bark	NRRL 22395	AF178341[4]
Fusarium sp. (*Nectria* sp. (Homothallic))	Bark	NRRL 22396	AF178342[4]
Fusarium sp. (*Nectria* sp.)	Bark	NRRL 22412	AF178351[4]
Fusarium sp. (*Nectria vasinfecta* (Homothallic))	Soil	NRRL 22436	AF178348[4]
Fusarium sp. (*Neocosmospora ornamentata* (Homothallic))	*Arachis hypogaea* nut	NRRL 22468	AF178349[4]
Fusarium sp. (*Nectria* sp.)	*Coffea arabica*	NRRL 22574	AF178345[4]
Fusarium sp. (*Nectria borneensis)*	Bark	NRRL 22579	AF178352[4]
Fusarium sp. *(Nectria plagianthi*)	*Hoheria galbrata*	NRRL 22632	AF178354[4]
Geejayessia atrofusca	*Staphylea trifolia*	NRRL 22316	AF178361[5]

[a]- The teleomorph names for applicable species as well as the mode of sexual reproduction (homothallic: refers to the ability of an organism to make a viable cross with itself) are indicated in parentheses. [b]- Host species or substrates were isolates were collected. [c]- The strain numbers according to the studies where the sequences were generated, F (University of Sydney) Sydney, New South Wales, Australia, FRC (*Fusarium* Research Center), Pennsylvania State University, University Park, Pennsylvania, USA and NRRL (National Center for Agricultural Utilization Research) Peoria, Illinois, USA. [d]- The GenBank accession numbers of downloaded TEF sequences from GenBank. The references for the studies where these DNA sequences were generated: [1]Zhang and Gao, unpublished; [2]Zhang et al. 2006; [3]O'Donnell et al. 2007; [4]O'Donnell 2000; [5]Schroers et al. 2011; [6]Aoki et al. 2005 and [7]Aoki et al. 2003.

Table 3: Information for the various TEF sequences obtained from GenBank included in this study to represent known species and clades within the GFC.

Species	Host/Substrate	Origin	Culture Collection[a]	GenBank number[b]
F. acutatum	Unknown	India	NRRL 13308	AF160276[2]
F. ananatum	*Ananas comosus*	England	NRRL 22945	AF160297[2]
F. anthophilum	*Hippeastrum sp.*	Germany	NRRL 13602	AF160292[2]
F. bactridioides	*Cronartium conigenum*	USA	NRRL 20476	AF160290[2]
F. begoniae	*Begonia elatior*	Germany	NRRL 25300	AF160293[2]
F. brevicatenulatum	*Striga asiatica*	Madagascar	NRRL 25446	AF160265[2]
F. bulbicola	*Nerine bowdenii*	Netherlands	NRRL 13618	AF160294[2]
F. circinatum	*Pinus radiata*	USA	NRRL 25331	AF160295[2]
F. concentricum	*Musa sapientum*	Costa Rica	NRRL 25181	AF160282[2]
F. denticulatum	*Ipomoea batatas*	USA	NRRL 25302	AF160269[2]
F. dlaminii	Soil	South Africa	n/a	n/a[12, 13]
F. fractiflexum	*Cymbidium* sp.	Japan	NRRL 28852	AF160288[3]
F. fujikuroi	*Oryza sativa*	Taiwan	NRRL 13566	AF160279[2]
F. globosum	*Zea mays*	Central America	NRRL 26131	AF160285[2]
F. guttiforme	*Ananas comosus*	South America	NRRL 22945	AF160297[2]
F. inflexum	*Vicia faba*	Germany	NRRL 20433	AF8479[2]
F. konzum	*Andropogon gerardii*	North America	MRC 8854	EU220235[13]
F. lactis	*Ficus carica*	USA	NRRL 25200	AF160272[2]
F. lyarnte	Soil	Australia	F19374	EF107118[6]
F. mangiferae	*Mangifera indica*	India	NRRL 25226	AF160281[2]

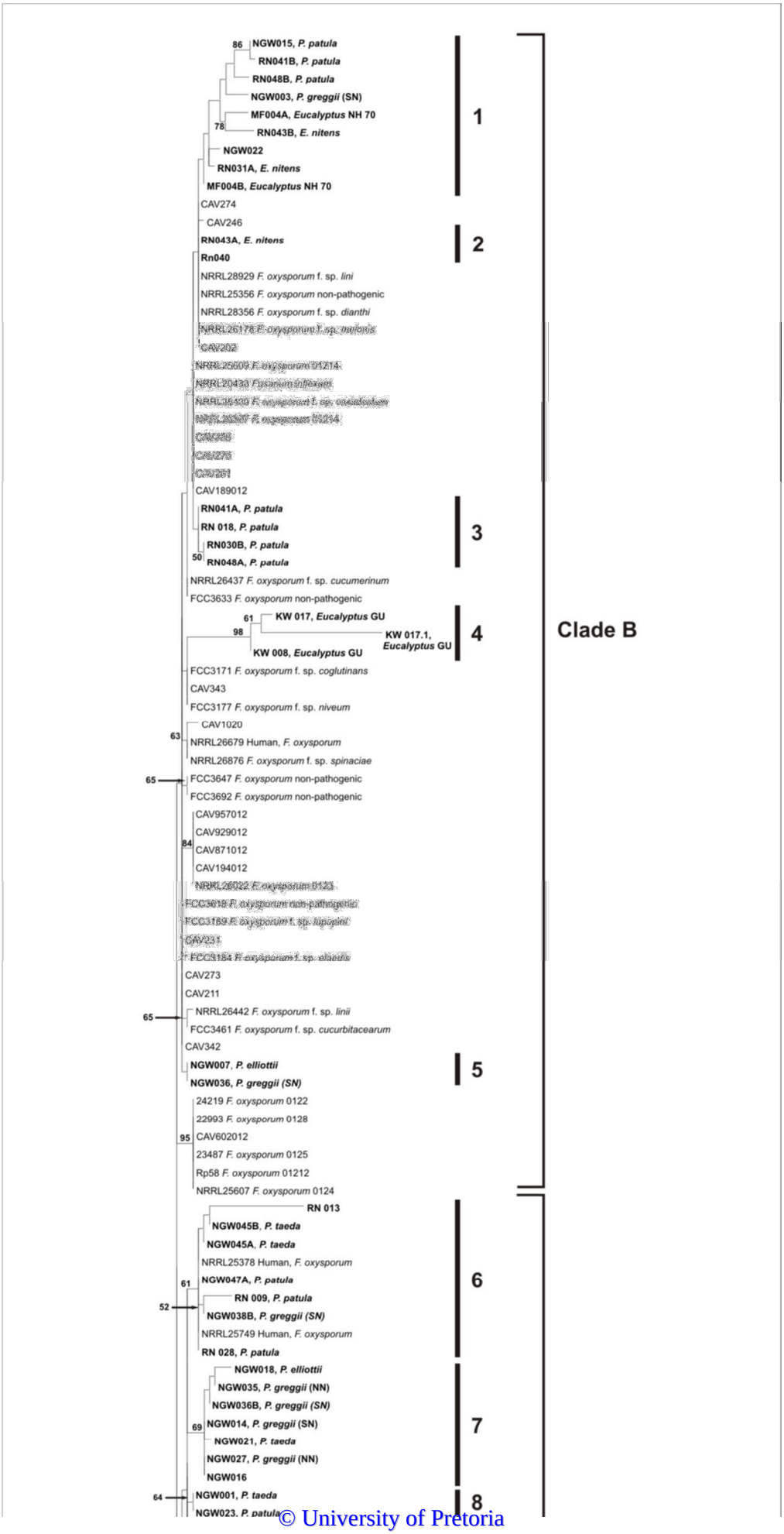
Species	Host/Substrate	Origin	Culture Collection[a]	GenBank number[b]
F. mexicanum	*Mangifera indica*	Mexico	NRRL 53147	GU737282[5]
F. musae	*Musa* sp.	Honduras	MUCL 52574	FN552086[8]
F. napiforme	*Pennisetum typhoides*	South Africa	NRRL 13604	AF160266[2]
F. nygamai	*Sorghum bicolor*	Australia	NRRL 13448	AF160273[2]
F. oxysporum	*Pseudotsuga menziesii*	USA	NRRL 22902	AF160312[2]
F. phyllophilum	*Dracaena deremensis*	Italy	NRRL 13617	AF160274[2]
F. proliferatum	*Cattleya* sp.	Germany	NRRL 22944	AF160280[2]
F. proliferatum	*Triticum aestivum*	USA	NRRL 31071	AF291058[10]
F. proliferatum	Unknown	Unknown	NRRL 43667	EF 452998[11]
F. proliferatum	Unknown	Unknown	NRRL 43666	EF452997[11]
G. intermedia	*Allium sativum*	Italy	E 1163	JQ762604[12]
F. pseudoanthophilum	*Zea mays*	Zimbabwe	NRRL 25206	AF160264[2]
F. pseudocircinatum	*Solanum* sp.	Zimbabwe	NRRL 22946	AF160271[2]
F. pseudonygamai	*Pennisetum typhoides*	Ghana	NRRL 13592	AF160263[2]
F. ramigenum	*Ficus carica*	Nigeria	NRRL 25208	AF160267[2]
F. sacchari	*Saccharum officinarum*	USA	NRRL 13999	AF160278[2]
F. sterilihyphosum	*Mangifera indica*	India	MRC 2802	AF160300[2]
F. subglutinans	*Zea mays*	USA	NRRL 22016	AF160289[2]
F. succisae	*Succisa pratensis*	Germany	NRRL 13613	AF160291[2]
F. temperatum	*Zea mays*	Belgium	MUCL 52450	HM067687[7]
F. thapsinum	*Sorghum bicolor*	South Africa	NRRL 22045	AF160270[2]

Species	Host/Substrate	Origin	Culture Collection[a]	GenBank number[b]
F. tupiense	*Mangifera indica*	Brazil	CML 262	DQ452859[9]
F. udum	Unknown	Germany	NRRL 22949	AF160275[2]
F. verticillioides	*Zea mays*	Germany	NRRL 22172	AF160262[2]
F. werrikimbe	*Sorghum leiocladum*	Australia	F19350	EF107131[6]
F. xylarioides	*Coffea* sp.	Ivory Coast	NRRL 25486	AY707136[4]
Fusarium sp.	*Striga hermonthica*	Madagascar	NRRL 26061	AF160303[2]
Fusarium sp.	Unknown	Niger	NRRL 26152	AF160306[2]
Fusarium sp.	*Sorghum bicolor* seed	Tanzania	NRRL 26064	AF160302[2]
Fusarium sp.	*Zea mays*	Central America	NRRL 25221	AF160268[1]
Fusarium sp.	*Striga hermonthica*	Africa	NRRL 26793	AF160309[1]
Fusarium sp.	*Oryza sativa*	Southeast Asia	NRRL 25615	AF160304[2]
Fusarium sp.	Soil	Australia	NRRL 25807	AF160305[1]
Fusarium sp.	n/a	n/a	NRRL 25195	AF160298[1]
Fusarium sp.	*Ipomoea batatas*	Central America	NRRL 25346	AF160296[1]
Fusarium sp.	Ornamental reed	South Africa	NRRL 26756	AF160307[2]
Fusarium sp.	Ornamental reed	South Africa	NRRL 26757	AF160308[2]
Fusarium sp.	Palm	n/a	NRRL 25204	AF160299[1]
Fusarium sp.	*Bidens pilosa*	South America	NRRL 29124	AF160311[2]
Fusarium sp.	*Zea mays*	Central America	NRRL 25622	AF160301[2]
Fusarium sp.	*Triticum* sp.	South Asia	NRRL 25309	AF160284[1]
Fusarium sp.	*Oryza sativa*	Southeast Asia	NRRL 25303	AF160283[1]

Fusarium sp.	Soil	Papua New Guinea	NRRL 26427	AF160286[2]

[a]- The abbreviations for the culture collections: CML (Coleção Micológica de Lavras) Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; MUCL (Mycothèque de l'Université Catholique de Louvain), Louvain-la-Neuve, Belgium; MRC (Medical research council) Tygerberg, South Africa; F (University of Sydney) Sydney, New South Wales, Australia and NRRL (National Center for Agricultural Utilization Research) Peoria, Illinois. [b]- The references for the studies where these DNA sequences were generated: [12]Marasas et al. 1985; [1]O'Donnell *et al*., 1998; [2]O'Donnell et al., 2000; [3]Aoki et al. 2001; [10]Kwon et al. 2001; [4]Geiser et al. 2005; [11]O'Donnell et al. 2007; [13]Kvas et al. 2009; [5]Otero-Colina et al. 2010; [6]Walsh et al. 2010; [7]Scauflaire et al. 2011; [8]Van Hove et al. 2011; [9]Lima et al. 2012 and Tonti et al. unpublished.

Figure 1: A maximum likelihood (ML) phylogeny of the TEF sequences for the *Fusarium oxysporum* species complex (FOC), including all the *Fusarium oxysporum* isolates collected from this study, as well other sequences from the *F. oxysporum* species complex across four clades (Fourie et al. 2009). In this study this is the largest complex and includes 64 of the 102 isolates examined. The tree is rooted with *F. commune* strain NRRL 22903. Again the complex was split into four clades A-D, as seen previously (Fourie et al. 2009). Bootstrap support values (>50%) for maximum likelihood are indicated at the internodes. NRRL: Culture collection at the National Regional Research Laboratory, IL, USA; FCC (Fusarium culture collection) and CAV culture collections of the Tree Protection Co-Operative programme (TPCP), Forestry and Agricultural Forestry Institute (FABI), University of Pretoria, South Africa.



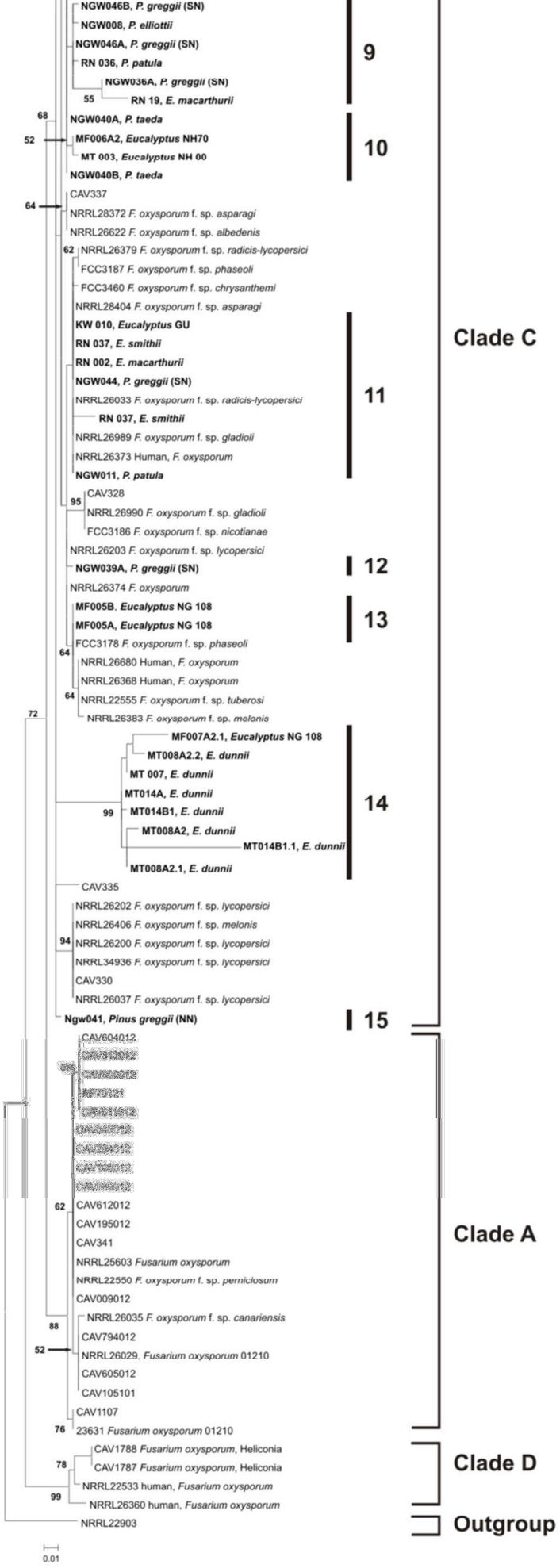


Figure 2: A maximum likelihood (ML) phylogeny of the *Fusarium solani* species complex (FSC), including all the *Fusarium solani* isolates (11) collected from this study and known *Fusarium solani* strains from various hosts and locations, inferred from TEF sequence data. The FOC was split into three well supported clades, New Zealand, South America and Worldwide, or 1-3 as seen previously (O'Donnell et al. 1998). The tree is rooted with *F. oxysporum* and isolate NRRL 22316. Bootstrap support values (>50%) for maximum likelihood are indicated at the internodes. F (University of Sydney), Sydney, New South Wales, Australia; FRC (*Fusarium* Research Center), Pennsylvania State University, University Park, Pennsylvania, USA and NRRL, Culture collection at the National Regional Research Laboratory, IL, USA.

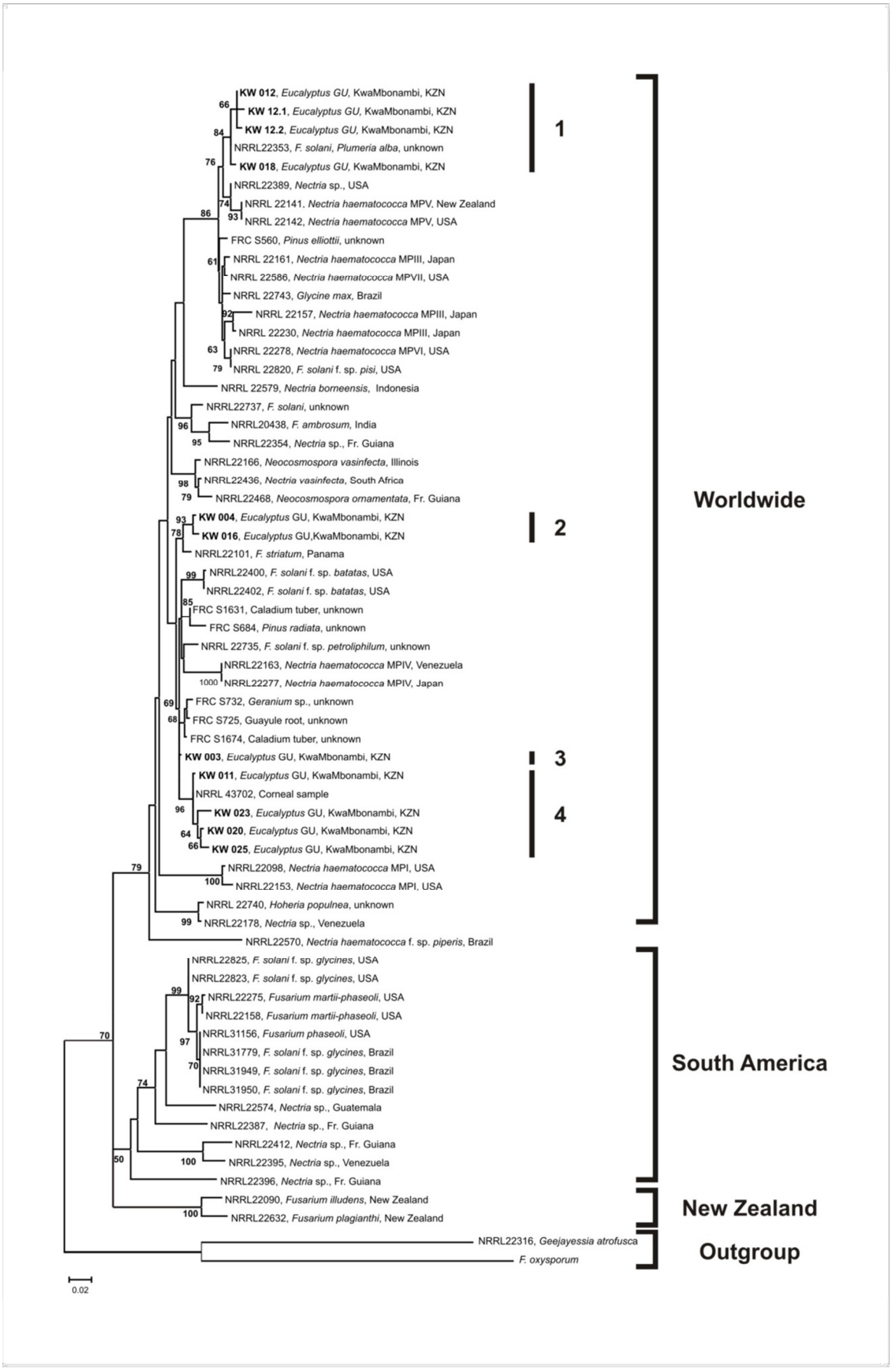
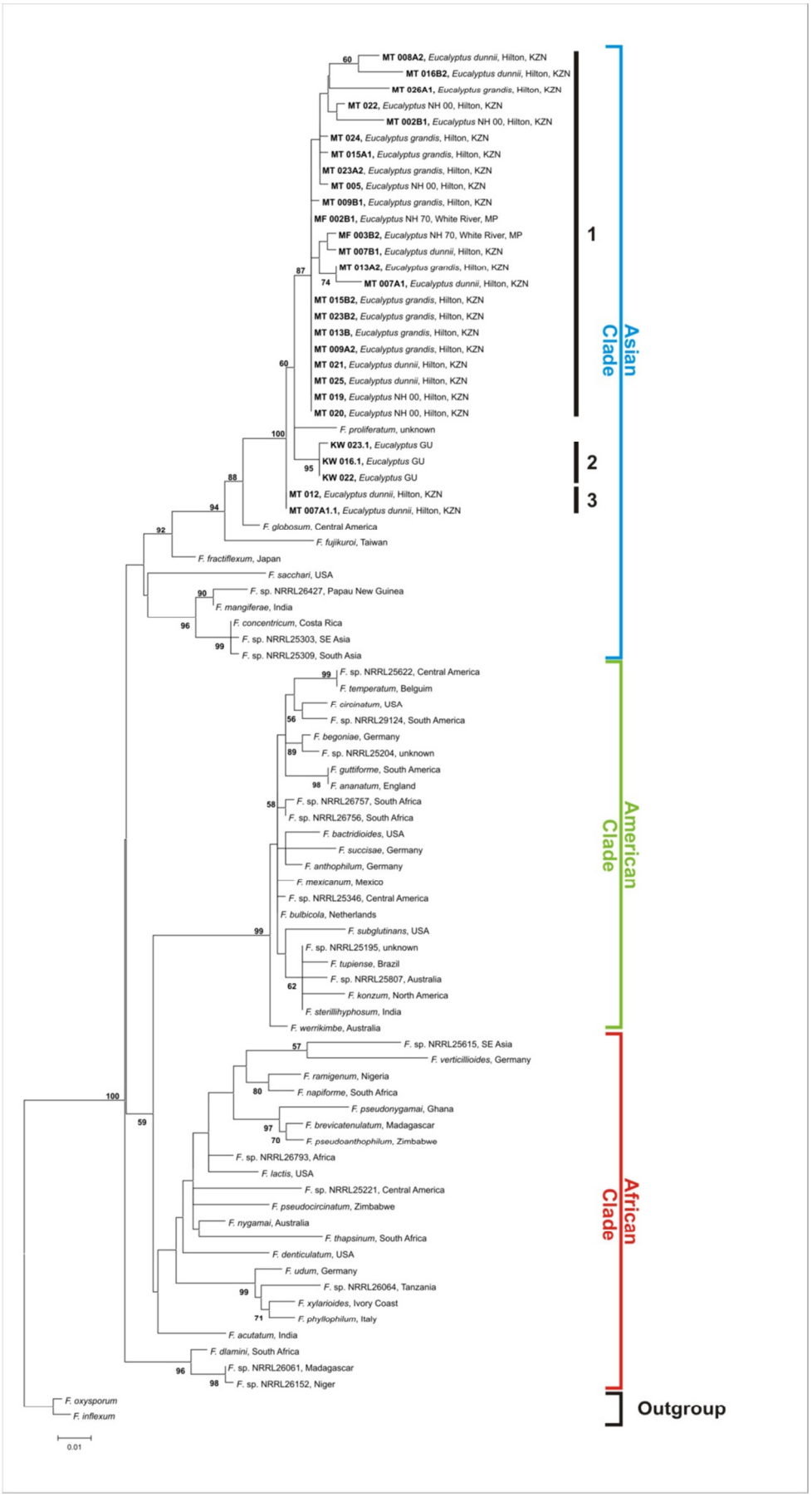


Figure 3: A maximum likelihood (ML) phylogeny of the *Gibberella fujikuroi* species complex (GFC), including all the *Fusarium proliferatum* isolates collected from this study (28), known *F. proliferatum* strains and other *Fusarium* species and strains in the GFC inferred from TEF sequence data. The GFC was split into three clades that correspond to the clades designated by (O'Donnell et al. 1998; O'Donnell et al. 2000). The tree is rooted with *Fusarium oxysporum* and *Fusarium inflexum*. Bootstrap support values (>50%) for maximum likelihood are indicated at the internodes. NRRL, Culture collection at the National Regional Research Laboratory, IL, USA.



Summary

The genus *Fusarium* is responsible for disease on a large number of plant hosts. Although a number of *Fusarium* species are associated with disease of forestry tree species, the true diversity of these fungi in the forestry environment is unknown because they either cause no disease or the symptoms they induce are relatively mild and easily overlooked. The first research chapter of this dissertation aimed to use DNA-based procedures to characterize the *Fusarium* isolates that were recovered from diseased *Pinus* trees and nursery seedlings in Colombia that were exhibiting symptoms typical of *F. circinatum* infection. Among these isolates ten *Fusarium* species were identified of which one was *F. circinatum* and the remaining seven, new to Science. Descriptions were provided for five of these new species (*i.e.*, *F. marasasianum*, *F. fracticaudum*, *F pinemorale*, *F. sororula* and *F. parvisorum*). The results of pathogenicity assays on *Pinus patula* seedlings further showed that one of the new species, *F. parvisorum*, poses a significant threat to the forestry industry in Colombia and globally. The work presented in the second research chapter of this dissertation explored the diversity of *Fusarium* in the forestry nursery setting. The results of DNA-based identifications showed that the seedlings/cuttings of *Pinus* and *Eucalyptus* species in South African nurseries are associated with diverse fusaria. More than 20 distinct members of the *Fusarium oxysporum*, *Fusarium solani* and *Gibberella fujikuroi* species complexes were identified. The majority of these *Fusarium* isolates were obtained from apparently healthy tissue, although a number of isolates were also recovered from diseased plant tissue. Overall the results of this MSc study showed that commercial forestry environments, particularly nurseries, are home to a large diversity of *Fusarium* species. Future research should therefore seek to determine the ecological role of these *Fusarium* species and isolates. In doing so it should be possible to understand whether these fusaria pose risks to the forestry sector and/or whether some could potentially be exploited for purposes such as plant growth promotion and biocontrol.