Woodrot fungi in the Garden Route National Park and surrounds of South Africa: Identification and Taxonomy

By

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Declaration

I, the undersigned, declare that the thesis, which I hereby submit for the degree of *Philosophiae* Doctor at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

James Michel Tchotet Tchoumi

SIGNATURE: ……

DATE: ……

To my wife,
Gwladys TANKEU MEIKEU
&
our daughter,
Ann-kyra Nirina TCHOTET

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PREFACE

Addressing the concerns of microbial diseases affecting natural forests, particularly those caused by wood-rotting Basidiomycetes, is of paramount importance for the protection of these woody ecosystems. This is of particular importance in Africa, where the threat of these fungi to the health of trees and woody plants have received less attention compared to human-induced threats such as deforestation, mining and agriculture. Basidiomycete wood-rotting fungi are essential components of forest ecosystems, which, as primary decomposers of dead plant materials, play an important role in nutrient recycling. However, some are a threat to the well-being and sustainability of natural forest ecosystems and the leading causes of wood-rot diseases of living trees. They can, depending on the fungus involved and other factors, induce a complete and permanent change in the structure and composition of forests. Although their ecological importance and epidemiology has been extensively documented, particularly in the northern hemisphere, aspects such as their diversity and taxonomy in natural ecosystems are still under-explored in the southern hemisphere regions.

This thesis contributes to the knowledge of the diversity, ecology and taxonomy of wood-rotting macro-fungi in natural ecosystems in Africa, focusing on indigenous forests in the Garden Route National Park (GRNP) (South Africa). In these forests, native trees are selectively harvested for timber based on signs and symptoms resembling those induced by wood-rotting Basidiomycetes. Material collected from infected trees from surrounding areas was also included in the studies presented in this thesis. The research conducted for this thesis have led to the recognition of several previously unknown fungal species as well as first reports of previousely described fungi for South Africa. The results that emerged from these studies are documented in five chapters in this thesis.

The first chapter of the thesis is a synthesis of the scientific literature, which provides a general context for the studies presented in the research chapters. It includes a review of the importance of wood-rotting Basidiomycetes in forest ecosystems, with an emphasis on their ecological role and epidemiology. It also provides information regarding the various categories of wood-rotting macro-fungi, and discusses the principal methods used for their characterisation. The chapter concludes by presenting some of the management strategies applied to control their dissemination and impact.

The four research chapters focused on the identification, ecology and taxonomy of wood-rotting macro-fungi associated with declining trees in the GRNP and surroundings. Chapter Two, provides base-line information pertaining to the identity and species richness of wood-rotting Basidiomycetes occurring on trees showing wood-rot symptoms in timber-harvesting compartments of the GRNP indigenous forests. It also discusses the effects of timber harvesting on the spread of these macro-fungi and their distribution across the investigated forest compartments.

Chapter Three focuses on the characterisation of *Ganoderma* species associated with *Acacia cyclops* trees, which are dying in the areas adjacent to the GRNP. This was done based on morphology and phylogenetic analyses. Results from these analyses led to the discovery of two new species and a previously described species.

In Chapters Four and Five, the taxonomy and phylogeny of members residing in the two main groups of macro-fungi associated with wood-rot symptoms reported in Chapter Two and Three, namely *Ganoderma* and Hymenochaetaceae, are considered. Hence, Chapter Four deals with the taxonomy and phylogeny of *Ganoderma* species recovered from the GRNP, supplemented with

material from other localities in the country as well as those of non-native *A. cyclops* identified in Chapter Three. Chapter Five addresses the same two aspects, but with a focus on species in the Hymenochaetaceae. Morphological and phylogenetic studies presented in both chapters revealed eight species of *Ganoderma*, of which two are new to science. Ten Hymenochaetaceae species were identified, of which four were described as new species in the study.

This thesis makes a significant contribution to the knowledge of the diversity, ecology and taxonomy of wood-rotting macro-fungi in natural forests in general, and more particularly in those in southern Africa. Although studies in the different research chapters focused mainly on the GRNP of South Africa, it laid the foundation for future studies on other indigenous forests. This is particularly important in southern Africa, where very little information is available on the presence of these macro-fungi in natural ecosystems.

CHAPTER 1

LITERATURE REVIEW: WOOD-ROT BASIDIOMYCETES AS PATHOGENS AND DRIVERS OF FOREST ECOSYSTEMS

1. GENERAL INTRODUCTION

The protection of forest ecosystems is an important concern globally, and especially in Africa where deforestation, mining and farming represent important threats (Houghton 1999; Corvalan et al. 2005; Anonymous 2010; Norris et al. 2010). While significant attention has been given to these anthropogenic activities, very little has been rendered to microbial diseases that may also be affecting these ecosystems (Castello et al. 1995; Gilbert 2002). Among the microbial diseases that affect natural forests, wood-rot diseases caused by Basidiomycetes are of particular importance.

Wood-rotting Basidiomycetes are the main cause of wood-rot diseases in natural forests ecosystems and, thus, play a key role in their dynamics (Ryvarden 1991; Shaw and Kile 1991; Castello et al. 1995; Hansen and Goheen 2000; Gilbert 2002; Worrall et al. 2005; Bendel et al. 2006; Heilmann-Clausen and Boddy 2008; Stenlid et al. 2008; Boddy et al. 2008; Garbelotto and Gonthier 2013; Singh et al. 2013). The large majority of wood-rotting Basidiomycetes are saprotrophic on dying or dead plant materials (Ryvarden 1991; Boddy et al. 2008), however, some represent important pathogens of living trees (Ryvarden 1991; Shaw and Kile 1991; Worrall et al. 2005; Bendel et al. 2006; Schmidt 2006; Boddy et al. 2008). These include various root-rotting species within the genera *Armillaria*, *Ganoderma*, *Heterobasidium* and *Phellinus* (Ryvarden 1991; Shaw and Kile 1991; Schmidt 2006; Schwarze 2007; Boddy et al. 2008; Garbelotto and Gonthier 2013; Singh et al. 2013).

Pathogenic wood-rotting Basidiomycetes usually infect and colonize trees via their root systems (Shaw and Kile 1991; Boddy et al. 2008). Consequently, tree vigour decreases and they are more vulnerable to other pathogens and/or adverse weather conditions (Hansen and Goheen 2000).

The most serious consequences however, are those encountered in the timber production sector where drastic losses can be recorded due to an overall drop in the quality, quantity and value of merchantable wood (Gilbertson, 1980; Hennon 1995; Bendz-Hellgren and Stenlid 1997; Cruickshank et al. 2009; Warren et al. 2013).

Despite the harmful effect they may have on forests, wood-rotting Basidiomycetes remain essential components of these ecosystems. Some species, including edible mushrooms, are important sources of income to rural forest populations, e.g. in Africa (Adekunle and Ajao 2005; Egbe et al. 2013), while others such as *Ganoderma* are believed to have spiritual and pharmaceutical values in e.g. some Asian countries including China (Jong and Birmingham 1992; Paterson 2006). In natural ecosystems, wood-rotting Basidiomycetes play an important role in forest regeneration as they participate in nutrient recycling and in creating habitats for other organisms (Castello et al. 1995; Holah et al. 1997; Stubblefield et al. 2005; Worrall et al. 2005; Bendel et al. 2006; Weir et al. 2012; Stokland et al. 2012; Runkle 2013; Fukasawa et al. 2015; Jusino et al. 2015). Although wood-rotting Basidiomycetes include well-established pathogens, wood colonization and decay by these fungi are generally slow processes that only become noticeable after onset of certain signs and symptoms, death, or during tree harvesting (Garbelotto 2004; Garbelotto and Gonthier 2013).

Substantial research from the northern hemisphere have documented the diversity and ecological role of wood-rotting Basidiomycetes in natural ecosystems in those regions (Holah *et al.* 1993; Castello et al. 1995; Hansen and Goheen 2000; Garbelotto 2004; Hoff et al. 2004; Gonthier et al. 2005; Worrall *et al*. 2005; Bendel *et al*. 2006; Heilmann-Clausen and Boddy 2008; Lonsdale et al. 2008; Stenlid et al. 2008; Boddy et al. 2008; Garbelotto and Gonthier 2013; Weir et al. 2012; Stokland et al. 2012; Runkle 2013; Fukasawa et al. 2015; Jusino et al. 2015). However, in native

African forests, despite the strong pressure of silvicultural practices that may favour the proliferation of wood-rotting Basidiomycetes including the debarking of trees, timber harvesting and deforestation for agricultural purposes (Seydack et al. 1995; Sonwa et al. 2007; Gockowski et al. 2010; Gockowski and Sonwa 2011; Anonymous 2012), limited studies have focused on the diversity of these fungi (Douanla-Meli et al.2007; Yombiyeni et al. 2011), while their ecological role is generally overlooked.

In order to give context to the significance of the research presented in the rest of this thesis, an overview of the literature pertaining to the importance of wood-rotting Basidiomycetes in forest ecosystems is presented, with a particular focus on their epidemiology and ecological role. Furthermore, the principal methods used for their identification as well as certain management strategies applied for controlling their dissemination are discussed.

2. ECOLOGICAL ROLE OF WOOD-ROTTING BASIDIOMYCETES IN FOREST ECOSYSTEMS

The ecological role of wood-rotting Basidiomycetes in natural forest ecosystems can be both beneficial and detrimental (Castello et al. 1995; Hoff et al. 2004; Worrall et al. 2005; Bendel et al. 2006). Under natural conditions, wood-rotting Basidiomycetes mostly play a positive role in that they are essential in natural ecosystem functioning (Hoff et al. 2004). However, they can also act as disturbance forces that create gaps within forests and which can result in a complete change of their structure and composition (Hoff et al. 2004; Hansen and Goheen 2000; Worrall et al. 2005; Bendel et al. 2006).

2.1. NEGATIVE IMPACT OF WOOD-ROTTING BASIDIOMYCETES IN FOREST ECOSYSTEMS

2.1.1. Effects on canopy gap formation

Gap formation in forest canopies represent key structural elements that significantly influence the structure (Lang and Knight 1983; Spies and Franklin 1991; Shaw and Kile 1991; Castello et al. 1995; Arsenault and Bradfield 1995; Holah et al. 1933; 1997), species composition (Arsenault and Bradfield 1995; Holah et al. 1933; 1997; Runkle 1998; 2000 and 2013) as well as the functioning of forests (Mladenoff 1987; Entry and Emmingham 1995; Lertzman et al. 1996; Runkle 1981; 2000). Canopy gaps generally result from combined actions of several interdependent factors that determine the frequency at which tree death usually occur in forests. Some of these factors include the topographic position of the forest, its soil characteristics and the presence of disturbance factors such as adverse weather conditions, human activities, pests and diseases (Hunter and Parker 1993; Turner and Romme 1994; Lundquist 1995; Lertzman et al. 1996; Hansen and Goheen 2000; Worrall et al. 2005; Stenlid et al. 2008).

Many studies have highlighted the important role played by some native and non-native wood-rotting Basidiomycetes in the creation and expansion of disease centres which resulted in gap formation in the canopies of various forest ecosystems (Shaw and Kile 1991; Castello et al. 1995; Hennon 1995; Holah et al. 1997; Hansen and Goheen 2000; Worrall et al. 2005; Bendel et al. 2006; Stenlid et al. 2008). Due to their capacity to infect and kill living root systems and decay functional tissues of major roots, butts and stems of living trees, pathogenic wood-rotting Basidiomycetes predispose affected trees to mechanical failure such as wind-throws, which subsequently lead to gap formation. Gaps thus created can expand with time, due to continuous death and falling of trees at their margins (Hennon 1995; Hansen and Goheen 2000; Worrall et al. 2005; Bendel et al. 2006). Worrall et al. (2005) argued, for example, that root diseases combined with spruce beetle and dwarf mistletoe activities were the main agents of gap initiation

in *Picea abies* (L.) Karst. forest stands in Crawford Notch in the United State of America (USA), while Armillaria root disease associated with wind-throw were agents of gap expansion. Similarly, Bendel *et al.* (2006) also highlighted that *Armillaria ostoyae* (Romagnesi) Herink and *Heterobasidion annosum* s.str. ((Fr.) Bref), two well-known root-rotting macro-fungi in the Northern hemisphere, were responsible for the large-scale mortality and canopy gap creation in unmanaged mountain pine forests in the Swiss National Park. They found that, 74% of gap formations in the canopy were ascribed to *H. annosum*, 14% to *A. ostoyae* and 7% to the combined effect of both pathogens.

Death of trees at the vicinities of mortality centres and their expansions into gaps in forest canopies may result in drastic structural and compositional shifts in forests. Generally, these gaps are randomly dispersed within affected forest stands, vary in size and shape, increase with time and often persist for years (Lundquist 1993; Runkle 1998; Hansen and Goheen 2000; Worrall et al. 2005; Bendel et al. 2006). This was illustrated by Lundquist (1993) who used a roadside observations and aerial photo survey approach to assess the frequency and size of canopy gaps caused by Armillaria root disease on *Pinus* stands in the Eastern Transvaal (Mpumalanga Province), South Africa. He indicated that of the 1763 *Pinus* stands monitored, 290 were affected by the disease and its severity was shown to increase rapidly with age.

2.1.2. Effects on forests structure and composition

Pathogenic wood-rotting Basidiomycetes are among the driving forces that can induce changes in the structure and composition of natural forests (Castello et al. 1995). They have the ability to attack, kill and decompose trees of all sizes and ages with as result, a compositional and structural shift of the forest community (Holah et al. 1993; Castello et al. 1995; Hansen and

Goheen 2000; Gilbert 2002). In British Colombian forests, for instance, one of the pioneer tree species, *Pinus contorta* Dougl. ex. Loud. (Douglas-fir), was attacked and destroyed by the pathogenic root-rotting fungus *A. ostoyae* resulting in gap formation within the forests. Gaps thus created became colonized with less susceptible species and shade tolerant trees such as *Thuja plicata* Donn ex D. Don (Western red cedar), *Abies lasiocarpa* (Hook.) Nutt. (Subalpine fir) or *Tsuga heterophylla* (Raf.) Sarg. (Western hemlock) (Shaw and Kile 1991). In another study, Holah et al. (1993) also reported that species composition including shrub, herbs and tree strata had a higher species diversity within *Phellinus weirii* (Murrill) Gilb. infection centres compared to adjacent non-infected areas. These observations are consistent, as it is generally at the vicinity of these infection centres that young seedlings sprout, and thus, contribute to forests regeneration (Runkle 1998; 2000 and 2013).

2.2. POSITIVE ROLE OF WOOD-ROTTING BASIDIOMYCETES IN FOREST ECOSYSTEMS

2.2.1. Maintenance of forest health and stability

Wood-rotting Basidiomycetes play a key role in maintaining the balance and health of forests. By their ability to attack, kill and decay all size of trees, wood-rotting Basidiomycetes can selectively remove less vigorous trees, dying or already dead, for the benefit of younger trees that would be more robust and healthy. This important ecological function is facilitated by their aptitude to convert organic substrates into humus and/or by recycling nutrients through the breakage of complex plant cell wall molecules, including cellulose, hemicellulose and lignin from living trees, fallen logs or dead wood (Gilbertson 1980; Jill et al. 2004; Schmidt 2006; Boddy et al. 2008; Boddy et al. 2009). In doing so, they thus affect plant and animal species richness and diversity by contributing to the creation of new habitats as well as to the occurrence

of new plant communities (Gilbertson 1980; Castello et al. 1995; Hansen and Goheen 2000; Hoff et al. 2004, Runkle 2013).

2.2.2. New habitat formation

Although tree death usually occurs around infection centres, it is also at these levels that new biological communities generally emerge (Gilbertson 1980; Castello et al. 1995; Holah et al. 1997; Stubblefield et al. 2005; Bendel et al. 2006; Runkle 1998; 2000; Stokland et al. 2012; Weir et al. 2012; Fukasawa, 2012; Fukasawa et al. 2015). Forest soil in gaps usually receive more light, which progressively changes it micro-climatic conditions and thus lead to habitat modification as well as changes in species (plants and animals) composition (Boone et al. 1988; Hennon 1995 ; Castello et al. 1995; Hansen and Goheen 2000; Stubblefield et al. 2005). Hence, by colonizing and degrading wood, wood-rotting Basidiomycetes indirectly create suitable habitat conditions for the establishment of many other wildlife species (Stubblefield et al. 2005; Weir et al. 2012; Stokland et al. 2012; Jusino et al. 2015), plants (Holah et al. 1997; Worrall et al. 2005; Bendel et al. 2006; Runkle, 2013) as well as other fungi (Fukasawa et al. 2015). Cavity nesting birds for example, would preferentially excavate trees that are already softened by wood-rotting fungi in order to minimize the energy they would have spent to excavate healthy trees (Gilbertson 1980; Stubblefield et al. 2005; Stokland et al. 2012; Jusino et al. 2015). Moreover, when the weakened trees fall due to defect root systems, they can easily be colonized and decomposed by other fungi for food and energy (Lindner et al. 2011; Fukasawa et al. 2015) or serve as shelter for mice and other small rodents (Stubblefield *et al*., 2005; Stokland et al. 2012). More light reaching the forest floor result in seedlings of plant species that are tolerant or resistant to root-rot infections to gradually colonize gaps (Boone et al. 1988; Shaw and Kile 1991; Castello et al. 1995; Fukasawa 2012). Hence, although wood-rotting Basidiomycetes have

shown the ability to parasitize and destroy living trees, they also significantly contribute to forest stability (Lonsdale et al. 2008).

3. CATEGORIES OF WOOD-ROTTING BASIDIOMYCETES

Wood-rotting Basidiomycetes include both pathogenic and saprophytic fungi (Gilbertson 1980; Ryvarden, 1991; Shaw and Kile, 1991; Schmidt, 2006; Boddy et al. 2008). Pathogenic species usually attack and kill vigorous or stressed trees, allowing the secondary invaders, usually saprophytes to establish and decompose dead woody matter. Wood-rotting Basidiomycetes have evolved complex enzymatic and non-enzymatic mechanisms, which enable them to penetrate and grow inside wood cell walls and to hydrolyse their major structural components which they then utilize as food (Schwarze et al. 2000; Schwarze 2007; Boddy and Heilmann-Clausen 2008; Eastwood et al. 2011; Floudas et al. 2012).

Plant cell walls are composed of three major components namely celluloses, hemicelluloses and lignin (Cooke and Rayner 1984; Ryvarden 1991; Schwarze 2007; Boddy and Heilmann-Clausen 2008; Eastwood et al. 2011; Floudas et al. 2012). Based on which of these compounds is preferably degraded, and also on the appearance of the wood left behind, wood-rotting Basidiomycetes are classified into two main groups: brown rot fungi and white rot fungi (Figure 1A and B) (Ryvarden 1991; Schmidt 2006).

3.1. BROWN ROT FUNGI

Brown rot fungi belong to the Basidiomycetes (Gilbertson 1980; Ryvarden 1991; Yoon and Kim 2005; Schmidt 2006; Schwarze 2007). They are only able to degrade, through enzymatic and non-enzymatic processes, hemicelluloses and celluloses of wood cell walls. Their degradation

processes usually result in the affected wood becoming brown, with pockets, cracking into cubical patterns and resulting in crumbly wood. Hence the term brown rot or dry rot as previously named (Figure 1A) (Gilbertson 1980; Ryvarden 1991; Schmidt 2006; Schwarze 2007; Boddy and Heilmann-Clausen 2008; Eastwood et al. 2011). Brown rot fungi, preferentially degrade softwood trees, such as conifers, fallen logs, as well as timber in storage (Gilbertson 1980; 1981; Highley 1987; Ryvarden 1991; Ferraz et al. 2001; Schmidt 2006). In North America where this group has been extensively studied, Gilbertson (1980; 1981) reported that, out of the 1669 wood-rotting Basidiomycetes recorded in that region, about 113 (approximately 7%) cause brown rots in confiner forests, of which 71 belong to the Polyporaceae and the others distributed within different families such as the Agaricales, Aphyllophorales and Tremellales. Some important genera of brown rot fungi include *Antrodia, Coniophora, Daedalea, Fomitopsis, Gloeophyllum, Oligoporus, Laetiporus, Pycnoporellus* and *Wrightoporia* (Ryvarden 1991; Schimdt 2006).

3.2. WHITE ROT FUNGI

White rot fungi include both Basidiomycete and some Ascomycete fungi (Worrall et al. 1997; Schmidt 2006; Schwarze 2007; Boddy and Heilmann-Clausen 2008; Floudas et al. 2012; Stokland et al. 2012). White rot fungi differ from the brown rot fungi by their ability to use all wood cell wall components, including celluloses, hemicelluloses and lignin. The wood left behind after their degradation activity is usually spongy and soft in consistency and generally presents a bleached appearance (Figure 1B) (Cooke and Rayner 1984; Ryvarden 1991; Schmidt 2006; Schwarze 2007; Boddy and Heilmann-Clausen 2008). This attribute makes them one of the most important groups of wood degrading fungi, which significantly contribute to the regulation and functioning of forest ecosystems (Hoff et al. 2004; Lee et al. 2014). Although

their ability to decompose wood usually rely on the prevalent conditions within the wood, most white rot fungi frequently occur on hardwoods that are resistant to brown rot fungi (Schmidt 2006; Schwarze 2007; Boddy and Heilmann-Clausen 2008). However, they can also degrade softwood trees (Schwarze 2007; Boddy and Heilmann-Clausen 2008). Some of the most important white rot fungal genera include *Armillaria*, *Ganoderma*, *Heterobasidion*, *Phellinus* and *Trichaptum* (Schmidt 2006).

4. EPIDEMIOLOGY OF WOOD-ROTTING BASIDIOMYCETES

In forest ecosystems, wood-rot diseases are mostly caused by wood-rotting Basidiomycetes (Ryvarden 1991; Shaw and Kile 1991; Castello et al. 1995; Garbelotto 2004; Worrall et al. 2005; Bendel et al. 2006; Boddy et al. 2008; Garbelotto and Gonthier 2013). These fungi rely on basidiospores and mycelium networks to infect and colonize their host trees (Garbelotto 2004; Luley 2005; Fricker et al. 2008; Boddy et al. 2009; Shortle and Dudzik 2012). Insects and anthropologic activities can also enhance their dissemination (Hãgvar 1999; Coetzee et al. (2001a); Persson et al. 2011; Warren et al. 2013). Host trees usually respond to wood-rot infections by displaying a cascade of signs and symptoms, which in extreme cases can eventually result in death and decay of trees (Garbelotto 2004; Shortle and Dudzik 2012).

4.1. INFECTION AND COLONIZATION

The infection cycle of pathogenic wood-rotting Basidiomycetes, usually commence with the germination and penetration of basidiospores into the tree tissues, followed by the colonization and digestion of tree tissues through growth and establishment of the fungal hyphal network systems (Figure 2) (Garbelotto 2004; Luley 2005; Fricker et al. 2008; Boddy et al. 2009; Shortle and Dudzik 2012). In general, tree infection occurs through fresh open wounds, which expose

the sapwood and/or heartwood of roots, butts or stems to air-borne basiodiospores or soil-borne mycelium (Garbelotto 2004; Gonthier et al. 2005; Luley, 2005; Hickman et al. 2011; Shortle and Dudzik 2012). After germination of basidiospores, the resulting mycelium penetrates into the cell walls of the infected wood, and by enzymatic processes begins to digest their main components (Schwarze and Baum 2000; Luley 2005; Schimdt 2006; Schwarze 2007). Affected wood tissues usually react by the formation of discoloured zones or compartments as the tree tries to contain the infection (Shigo 1991; Pearce 1996).

The potential of infection and colonization of wood-rotting Basidiomycetes are influenced by several factors among which the prevailing environmental conditions within the host environment (Singh 1983; Pearce and Malajczuk 1990; Whiting and Rizzo 1999; Mswaka and Magan 1999; Gonthier et al. 2005; La Porta et al. 2008; Fricker et al. 2008; Boddy et al. 2009; Shortle and Dudzik 2012). Singh (1983) investigated the influence of nutrients and pH on the susceptibility of four conifer species, including Norway spruce [*Picea abies* (L.) Karst.], Black spruce [*P. mariana* (Mill.) B.S.P.], Sitka spruce [*P. sitchensis* (Bong.) Carr.] and Scots pine (*Pinus sylvestris* L.) to *Armillaria* root rot infection. He argued that seedlings of the four conifer species grown on poor nutrient soil with low pH showed less fitness and were more susceptible to *Armillaria* root rot disease. Moreover, these seedlings presented an early stage of infection and had more mycelial growth in their root systems as compared to their counterparts that were grown in enriched soil conditions with a higher pH.

In Australia, soil temperature and moisture content was shown to have a significant effect on rhizomorph formation, growth and subsequently the potential for infection of eucalypt species by *A. luteobubalina* Watling & Kile. The study revealed that at 4°C with low water content rhizomorph production and growth of *A. luteobubalina* was completely inhibited. Rhizomorph

production and growth only started to occur at 10 °C and increased significantly between 16° to 20 °C with water increase (Pearce and Malajczuk 1990). Whiting and Rizzo (1999) corroborated these observations. They indicated that incidence of *Armillaria* root disease on orchard crops and hardwood forest trees in northern California caused by *A. mellea* (Vahl) P.Kumm. and *A. gallica* Marxm. could be exacerbated by fluctuations of water potential in the soil. They observed that all isolates from both species, tested for radial colony growth, grew well under high moisture conditions, but decreased abruptly as the water potential declined. Thus, abiotic factors such as soil temperature, water, nutrients and pH may increase plant sensitivity to wood-rot infections, and may also exacerbate the aggressiveness of the wood-rotting fungal species involved.

4.2. SIGNS AND SYMPTOMS

Trees colonized by wood-rotting Basidiomycetes progressively lose their consistency and strength and may become more vulnerable to unfavourable weather conditions (Shortle and Dudzik 2012). Onset of above ground symptoms only becomes noticeable when tree physiology is seriously altered. Hence, the appearance of symptoms may differ according to the host infected, its age, the fungus involved and the ambient conditions prevailing in the host environment. Some wood-rotting Basidiomycetes mainly infect and decay top parts of trees and seldom reach the root system (Garbelotto 2004; Hickman et al. 2011; Shortle and Dudzik 2012). Other wood-rotting Basidiomycetes distinguish themselves by their ability to cause root, butt and heart rots and are regarded as among the most devastating pathogens (Garbelotto 2004). They commonly attack and destroy root systems and/or basal stem sections of living trees resulting in rapid death of young trees, while mature ones can still withstand for several years (Riffle and Walla 1986; Garbelotto 2004).

When the tree physiology is altered to a certain threshold, visible symptoms such as loss of crown and falling leaves begin to appear. In advanced stages, wood decay starts to establish and might be associated to other signs such as stem/branch bleeding, resinosis or dieback (Garbelotto 2004; Shortle and Dudzik 2012). Depending on the fungi associated with the rot, affected wood may become brown or white, and progressively becoming stringy, spongy or crumbly (Schimdt 2006; Schwarze 2007). Basidiomes may be produced either in clusters of mushrooms such as with *Armillaria* species, or as woody shelf like conks such as with *Ganoderma* and *Phellinus* species. Other indicators include the presence of white mycelial mats and/or rhizomorphs on and/or beneath the bark of invaded trees (Garbelotto 2004; Fricker et al. 2008; Shortle and Dudzik 2012).

4.3. MEANS OF DISPERSAL

Like most living organisms in terrestrial forest ecosystems, wood-rotting Basidiomycetes, in order to survive, move constantly in search of new nutrient resources when those on which they were previously established start running out (Thompson and Boddy 1983; Thompson and Rayner 1983; Nordén and Larsson 2000; Hallenberg and Kuffer 2001; Fricker et al. 2008; Garbelotto and Gonthier 2013). Their dissemination within a forest can be done either by aerial spores and/or mycelial growth from previously infected substrata, or it can be facilitated in some instances by insects and human activities (Figure 2) (Hãgvar 1999; Nordén and Larsson 2000; Hallenberg and Kuffer 2001; Vasaitis et al. 2008; Fricker et al. 2008; Persson et al. 2011).

In forest ecosystems, organic resources are heterogeneously distributed. Air-borne basidiospores are one of the most important means by which wood-rotting Basidiomycetes reach their new resources. Basidiospores are usually released from the basidiomes in great numbers (Nordén and

Larsson 2000; Gonthier et al. 2005; Sanderson 2005; Stenlid 2008). Due to their small size and low weight, they are easily transported by air currents over short (within the vicinity of the basidiome) and/or long distances (several kilometres away from the source) to infect new wounds and stumps (Nordén and Larsson 2000; Hallenberg and Kuffer 2001; Sanderson 2005; Stenlid. 2008). In an old-growth forest stand in southern Sweden, Nordén and Larsson (2000) reported that the Basidiomycete fungus *Phlebia centrifuga* P. Karst was producing about 1.21 x 10^7 basidiospores during 24 hours. The spores were settling within 100 m from the basidiome source and their concentration was decreasing gradually as the distance from the basidiome increased. In another study, a single basidiome of the fungus *Ganoderma boninense* Pat., causal agent of basal stem rot of oil palm (*Elaies guinensis*) trees was reported to release about 2 million infecting basidiospores per minute (Sanderson 2005). Despite their effectiveness for dissemination, most basidiospores have short life-spans due to their limited reserves and high sensitivity to dehydration as well as to UV radiation and thus, many die in transit (Kallio 1973; Rotem and Aust 1991; Stenlid 2008). In addition to basidiospores, some wood-rotting Basidiomycetes also use asexual spores as agents of dispersal, although their role seems to be less important in comparison to basidiospores (Schmidt 2006).

Wood-rotting Basidiomycetes have evolved the ability of growing vegetatively to infect and colonize new substrata (Fricker et al. 2008; Boddy et al. 2009). Vegetative propagation can occur through root to root contacts or grafts, or via mycelium growing directly on the soil and infecting stumps or freshly wounded trees (Thompson and Boddy 1983; Thompson and Rayner, 1983; Fricker et al. 2008; Boddy et al. 2009; Garbelotto and Gonthier 2013). Wood-rotting Basidiomycetes mostly persist in forests as mycelium (Fricker et al. 2008; Boddy et al. 2009). Because of its unlimited reserves, mycelium can spread over long distances; invade lager

surfaces and uptake nutrients from large organic sources (Boddy et al. 2009). Mycelium is consisted of interlinked hyphae which in some species (*Armilaria* spp. for instance) can aggregate into thick melanised linear mycelia with apical growth known as rhizomorphs (Fricker et al. 2008). Rhizomorphs are able to cover sizeable forest areas and persist for decades thus, ensuring a permanent availability of inoculum for infection of new plant generations (Thompson and Boddy 1983; Thompson and Rayner 1983; Smith et al. 1992; Ferguson et al. 2003). Well-known examples are that of *A. gallica* and *A. ostoyae*, which actually may represent the largest and oldest living organisms ever recorded. A single clone of *A. gallica* was reported weighing about 10000 kg, extending over 15 ha and about 1500 years old (Smith et al. 1992); while for *A. ostoyae*, the largest sized was about 965 ha and about 8650 years (Ferguson et al. 2003).

While processes of dispersion of wood-rotting Basidiomycetes by aerial basidiospores and mycelial growth are well documented, less, is known regarding other potential means by which these fungi might be disseminating. Insects are one of the alternative means by which fungi are widely dispersed. They can transport spores or mycelium of various fungi to targeted hosts, bore into the wood and thus facilitate access to air-borne spores, or weaken trees by drilling and chewing their wood and thus making them more vulnerable to other pathogens (Hãgvar 1999; Müller et al. 2002, Persson et al. 2009; Persson et al. 2011, Strid et al. 2014).

Although interactions between insects and Basidiomycetes have been a somewhat overlooked field, studies by Hãgvar (1999) and Persson et al. (2011) have revealed the significant role that such interactions play in the dissemination of these fungi. Hâgvar (1999) reported that 61 beetle species from 16 families were regularly and preferentially visiting living basidiomes of *Fomitopsis pinicola* (Sw.) P. Karst. and *Fomes fomentarius* (L.) Fr. for feeding and/or to breed in the host tree on which they were attached. When leaving the basidiomes, the beetles were

generally covered with spores that they propagated onto new hosts. Similarly, from their investigations, Persson et al. (2011) came to the conclusion that beetles could play a major role in the establishment of wood-rotting fungi as they noted that mycelia of wood-rotting Basidiomycetes, including *F. pinicola*, *Phlebiopsis gigantea* (Fr.) Jülich, *Stereum sanguinolentum* (Alb. & Schwein.) Fr. and *Trichaptum abietinum* (Pers. ex J.F. Gmel.) Ryvarden were both present in the insect galleries as well as on beetles associated with these galleries.

Human activities can favour the dispersal of wood-rotting Basidiomycetes. Forest management activities can create suitable conditions for the propagation of wood-rotting Basidiomycetes. In forests where logging and thinning practices are regularly performed, wounds created during these operations can serve as entrance points for primary infections by wood-rot pathogens and leftover stumps may also be colonized and serve as inoculum reservoirs for subsequent infections. In dense and old stands in contrast, secondary spread by root contacts would be more favourable due to the proximity and high density of roots in the forest soil (Morrison *et al*. 2001, Garbelotto 2004; Gregory et al. 2010; Warren et al. 2013). Movement of infected plant material can also facilitate the dissemination of wood-rotting Basidiomycetes. This was for example the case in South Africa, where Coetzee et al. (2001a) showed that the *Armillaria* root rot of oak (*Quercus*) and other woody ornamental trees and shrubs in the Company Gardens, Cape Town, South Africa was most likely established in the mid-1600s as a result of early Dutch settlers introducing this pathogen on potted plants such as grapes or citrus. Timber transportation by flotation may also be another source of contamination in the sense that if driftwood is infected, it can end up in places where the pathogen was still unknown (Stenlid 2008).

5. IDENTIFICATION OF WOOD-ROTTING BASIDIOMYCETES

The accurate identification of a pathogen is an essential step towards the studying of diseases and the establishment of effective control strategies (Rossman and Palm-hernández 2008). Identification of wood-rotting Basidiomycetes, like in most other groups of fungi, has applied different methods including morphological, biological and phylogenetic approaches to recognize species.

The use of morphological species recognition (MSR) to identify Basidiomycete fungi is an old practise, but, still widely used (Ryvarden and Johansen 1980; Ryverden 1991; Hawksworth et al. 1996; Taylor et al. 200). Basically, MSR methods rely on the identification of species based on the diagnosis of their macro- and microscopic characters (Taylor et al. 2000). Macroscopic characteristics used to discriminate among Basidiomycetes generally revolve around the basidiome. It includes the basidiome structure, either perennial or annual; the way it is attached to the substrate (stipitate, sessile or resupinate) and the consistency and colour of its pileus and pore faces (Figure 3; 4). Ryverden (2004) pointed out, for example, that species in the genus *Ganoderma* could be distinguished based on their basidiomes, which are either annual or perennial, with or without a stipe and exhibiting a pileus surface ranging from shiny and laccate to dull, and a pore surface almost creamy. *Phellinus* species have been reported to display almost the same basidiome characteristics with the exceptions that the pileus surface here can be pubescent or glabrous and radially cracked, while pore surface appears brownish or dull brown (Larsen and Cobb-Poulle 1990). Microscopic features involve basidiospores sizes and shapes (globose, ellipsoid or cylindrical) as well as their ornamentations (Figure 5); hyphal systems, which can be monomitic (generative hyphae only), dimitic (generative and skeletal/binding hyphae) or trimitic (presence of the three categories of hyphae), clamp connections of the hyphae (Figure 6A, B, C, D) as well as the shapes of basidia and cystidia (Figure 7A, B, C, D)

(Ryvarden and Johansen 1980; Ryverden 1991). Other studies have considered colony growth (Nobles 1965; Stalpers 1978) as well as the characterization of some biochemical compounds (Fukasawa et al. 2015).

Although the use of the MSR method to delineate species of Basidiomycete fungi seems to be rapid and pragmatic, particularly for identifications in the field, it has many drawbacks. One of them is the lack of uniformity in morphological characters to be used (Moncalvo and Ryverden 1997, Taylor et al. 2000; Cai et al. 2011). Additionally, some morphological features such as the occurrence of basidiomes are seasonal and they are thus not always present when collecting, and pileus appearances have been shown to vary significantly with the geographical distribution and micro-climatic conditions (Ryvarden 1995; Kim et al. 2002). Moreover, when subjected to other methods of identification such as biological (BSC) or phylogenetic species (PSC) recognition methods, species identified based on morphology often fail to distinguish morphologically similar species (Anderson and Stasovski 1992; Hibbett et al. 1995; Smith and Sivasithamparam 2000; Taylor et al. 2000).

The biological species recognition (BSR) approach is an alternative method used to demarcate fungi. It relies on mating compatibility tests to differentiate between two or more fungal species. According to this approach, a species will be regarded as a group of individuals from the same population, capable of interbreeding and produce fertile offspring (Mayr 1940). However, this method has certain weaknesses negating its applicability to all groups of fungi. For instance, meiospores are required before mating tests can be carried out. Yet, it is acknowledged that about 20% of fungi can reproduce asexually and thus do not produce meiospores (Reynolds 1993). This restriction, therefore, makes the BSR criterion non-applicable for this category of fungi. Additionally, some fungi are homothallic and able to self-produce their meiospores and

thus making it difficult to infer mating. For some heterothallic fungi, although producing meiospores, mating may not occur in culture and other fungi are just not cultivable (Taylor et al. 2000). Nevertheless, in spite all these limitations, mating behavioural tests have been employed to discriminate species across different genera of wood-rotting Basidiomycetes including *Armillaria* (Korhonen and Hintikka 1974; Ullrich and Anderson 1978; Anderson and Ullrich 1982; Abomo-Ndongo et al. 1997), *Ganoderma* (Adaskaveg and Gilbertson 1986, 1989; Pilotti et al. 2002) and *Phellinus* (Fiasson and Niemelä 1984; Fischer 1987, 1996; Rajchenberg 2011).

The introduction of molecular techniques in the field of mycology has contributed significantly to the discovery and identification of new fungal species at various taxonomic levels (Anderson and Stasovski 1992; Moncalvo et al. 1995a; Hong and Jung 2004). These diagnostic methods appear to be relatively fast and reliable for the identification of fungi because they rely on the processing of objective data (molecules) of the target organisms, unlike other conventional methods (Schmidt 2006). Molecular techniques, including DNA comparison methods and phylogenetic analyses, applying the phylogenetic species recognition (PSR) concept, have been widely applied to delineate and establish evolutionary relationships between similar and/or closely related fungal species across different genera of wood-rotting Basidiomycetes (Moncalvo et al. 1994; Johannesson and Stenlid 1998; Jeong et al. 2005; Garcia-Sandoval et al. 2011). DNA-based techniques that have been applied to identify wood-rotting Basidiomycetes include, Restriction fragment length polymorphisms (RFLPs) of mitochondrial and ribosomal DNA regions such as the internal transcribed spacer (ITS) and intergenic spacer (IGS) regions (Smith and Anderson 1989; Fischer and Wagner 1999; Adair et al. 2002); Randomly amplified polymorphic DNA (RAPD)-analysis (Hseu et al. 1996; Ito et al. 1998); Amplified fragment length polymorphisms (AFLPs) (Lind et al. 2005; Kauserud et al. 2006; Kim et al. 2006); PCR-

RFLP (Harrington and Wingfield 1995; Mwenje et al. 2003; Matsushita and Suzuki 2005) and multiplex PCR method using taxon-specific primers (Guglielmo et al. 2007; Nicolotti et al. 2009). Other DNA based strategies used to discriminate between Basidiomycetes from environmental samples i.e. fungal DNA extracted directly from infected woody material include Terminal-RFLP (T-RFLP) (Johannesson and Stenlid 1999; Allmér et al. 2006), denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) (Muyzer and Smalla 1998; Rajala et al. 2010). Studies of Ovaskainen et al. (2010) and Ovaskainen et al. (2013) also indicated that high-throughput sequencing (pyrosequencing) could efficiently demarcate between wood-rotting Basidiomycetes.

With DNA sequencing, PSR method, based on phylogenetic analyses, has become one of the most powerful tools to infer phylogeny of fungi both at intra-specific and inter-specific levels (White et al. 1990). DNA sequence information of certain regions of the nuclear, ribosomal and mitochondrial genomes have been extensively used in the phylogeny and taxonomy of Basidiomycetes as they have shown to give clear resolutions in the demarcation of fungi even at different taxonomic levels (White et al. 1990; Moncalvo et al. 1994; Parmasto et al. 2014). Some of these regions include the ITS gene region (ITS1-5.8S-ITS2) (Singh et al. 2013), IGS (IGS1-5S-GS2) (Coetzee et al. 2003b), the mitochondrial small subunit (mtSSU) and nuclear large subunit (LSU) (Parmasto et al. 2014). Nucleotide sequence data analyses of ITS and IGS gene regions of the rDNA, efficiently discriminate species within the genus *Armillaria* and confirm previous morphological and biological identifications of certain species (Anderson and Stasovski 1992; Coetzee et al. 2001b; Coetzee et al. 2003b). Furthermore, this approach also contributed significantly to the identification of new species where no basidiomes could be found for morphological characterization or where mating could not be done due to absence of tester

strains (Coetzee et al. 2003a; Coetzee et al. 2005; Mwenje et al. 2003). Moreover, Moncalvo (1995a, b) used this same approach to establish the phylogenetic relationships between species of *Ganoderma*. Other studies, including those of Hong and Jung (2004), Douanla-Meli and Langer (2009), Wang et al. (2012) and Cao et al. (2012) used additional makers such as mtSSU, RPB1, RPB2 and the translation elongation factor 1-α (TEF1-α) to bring more insight in the classification between *Ganoderma* species. Furthermore, studies of Wagner and Fischer (2001), Dai (2010) and Parmasto et al. (2014) suggested that, the nLSU was a suitable marker to differentiate genera and species within the Hymenochaetaceae.

In the light of the foregoing, it is clear that DNA sequencing techniques have offered alternative methods to solve the issues of identification and classification of fungi in general, including of Basidiomycete fungi. Phylogenetic analyses, have contributed significantly in the discovery of new species and to the proper identification of misidentified ones (Smith and Sivasithamparam 2000). They, therefore, constitute reliable tools for a rapid and accurate diagnostic of wood-rotting Basidiomycetes as compared to other conventional methods.

6. MANAGEMENT OF WOOD-ROTTING BASIDIOMYCETES

Wood diseases, particularly those related to rot are almost impossible to eliminate once they have established in a stand (Garbelotto and Gonthier 2013). However, certain precautions can be taken in order to limit or slow their expansion. One of the strategies involves the immediate treatment of stumps surfaces with chemicals or biological control agents after logging, as they usually constitute one of the major sources of primary infection (Garbelotto 2004; Garbelotto and Gonthier 2013). A diverse range of chemicals including borate, urea and methyl bromide, as well as biological control fungi such as *Phlebiopsis gigantea* (Fr.) Jülich and *Trichoderma*

species have been widely used in control strategies against pathogenic wood-rotting Basidiomycetes (Rishbeth 1976, Thies and Sturrock 1995; Varese et al. 1999; Johansson et al. 2002; Vasiliauskas et al. 2004; Oliva et al. 2008). Chemical treatments can interfere directly on the metabolism of fungal pathogens, inhibit the germination of basidiospores or be used as fumigants to reduce the inoculum concentration in the soil and woody substrates, as it was the case with *A.mellea, H. annosum* and *P. weirii* respectively (Rishbeth 1976, Thies and Sturrock 1995; Johansson et al. 2002; Vasiliauskas et al. 2004; Oliva et al. 2008). While, the biological agents, *P. gigantea* and species of *Trichoderma* have been applied with some success on stumps to control *H. annosum* and *P. weirii* infections (Thies and Sturrock 1995; Varese et al. 1999; Vasiliauskas et al. 2004).

Stump removal, including with their root systems or simply the complete uprooting of all diseased trees in an infection centre and one or two rows of asymptomatic trees beyond; combined with trenching, can significantly reduce the inoculum in a stand. Indeed, trees uprooting and/or complete removal of stumps will lower stand density and thus interrupt secondary spread by root contacts and rhizomorphs that are growing on/or in the soil (Morrison et al. 1991; Thies and Sturrock 1995; Sturrock 2000; Garbelotto 2004; Vasaitis et al. 2008; Shaw III et al. 2012; Cleary et al. 2013; Gonthier et al. 2014). However, this approach is time-consuming, costly because it requires adequate equipment and sometimes unfriendly to the environment as it can lead to soil compaction and/or aggravate its erosion (Thies and Sturrock 1995).

A climatic control approach can also be considered to limit the dissemination of wood-rotting Basidiomycetes and thus their potential of infection. Silvicultural operations such as thinning, pruning or logging often lead to wounds on living and healthy trees, which with leftover stumps

are most likely to primary infections by air-borne basidiospores. Taking into account the biology of the wood-rotting fungi involved, these operations should rather be carried out either in winter when low temperatures can hamper the production of basidiospores or during summer when high temperatures can seriously compromise their germination (Garbelotto 2004; Gonthier et al. 2005; Garbelotto and Gonthier 2013).

If the pathogenic wood-rotting fungi target specific host trees, rotations can be envisaged to reduce the incidence of the disease (Morrison et al. 1991). Planting of resistant trees species without, however completely removing those that are sensitive may also contribute to decrease the incidence of the disease (Morrison et al. 1991; Sturrock, 2000; Garbelotto, 2004; Vasaitis et al. 2008; Hickman et al. 2011; Gonthier et al. 2014).

Prescribed burning is an alternative way of controlling diseases caused by wood-rotting Basidiomycetes. It was successfully applied to control the viability of *A. ostoyae* in Mixed-Conifer Forest Soils in the Blue Mountains of Oregon (Filip and Yang-Erve 1997). Prescribed burn strategy involves the use of fire in an infected stand under well-defined conditions. Fire will reduce the inoculum reservoirs by burning leftover stumps and roots with basidiomes as well as rhizomorphs that spread vegetatively in and/or on the soil (Filip and Yang-Erve 1997; Lygis et al. 2010).

Quarantine measures, combined with the above-cited strategies would significantly help enhance the level of protection against wood-rot diseases. Quarantine measures aimed to prevent introduction of new pathogenic species or strains from infected plants, soil or other materials susceptible of carrying them to areas where they were not known before. It is an upstream

control strategy that is particularly effective in the fight against exotic introductions that can represent serious threats to indigenous communities (Maloy 1993).

7. CONCLUSIONS

Wood-rotting Basidiomycetes are essential to the sustainability of forests as they contribute to the emergence of new biological communities. However, pathogenic species can induce irreversible changes in the structure and composition of forests. Their presence and potential impact on African natural forests suggest the need for more detailed studies of their diversity, taxonomy and ecology on the continent. The present thesis aims at contributing to the knowledge of the diversity and taxonomy of wood-rotting macro-fungi, particularly those with pathogenic potential such as *Ganoderma* and Hymenochaetaceae species in African native forests. It focuses on the indigenous forests of the Garden Route National Park (GRNP) in the southern Cape region of South Africa, where signs and symptoms of wood-rot diseases are commonly reported on native trees, as well as on *Acacia cyclops*, a non-native tree growing along the coast in this area (Roux et al. 2013).

Native forests in the GRNP are selectively harvested, based on the occurrence of signs of wood-rot and crown death of trees (Seydack et al. 1995). In most cases very little is known regarding the cause of this decay and death. Based on some preliminary studies (Roux et al. 2011; Roux et al. 2013), it is hypothesised that it may be due to attacks by wood-rotting fungi, as fruit bodies resembling species of the pathogenic wood-rot genera *Phellinus* sensu lato (s.l.) and *Ganoderma* s.l. were observed on the dying trees and stumps. However, further research is needed to validate this hypothesis. In doing so, it is imperative to establish beforehand the identity and host range of

the Basidiomycetous wood-rotting fungi associated with wood-rot symptoms in this natural ecosystem. This thesis is dedicated to this preliminary work through three specific objectives:

- access the diversity and host affinities of wood-rotting Basidiomycetes associated with declining native trees in the GRNP in the southern Cape region of the country;
- characterise the Basidiomycete species associated with dying *Acacia cyclops* trees in coastal areas of the southern Cape region of the country;
- establish the phylogenetic relationships and taxonomic status of the two main groups of wood-rotting Basidiomycetes encountered in the GRNP.

8. REFERENCES

Abomo-Ndongo S, Mohammed C, Guillaumin JJ, 1997. Sexual behavior of *Armillaria heimii* and *A. mellea* isolates from Africa. *European journal of forest pathology* 27, 207–224.

Adair S, Kim SH, Breuil C, 2002. A molecular approach for early monitoring of decay basidiomycetes in wood chips. *FEMS Microbiology Letters* 211, 117–122.

Adaskaveg JE, Gilbertson RL, 1986. Culture studies and genetics of sexuality of *Ganoderma lucidum* and *G. tsugae* in relation to the taxonomy of the *G. lucidum* complex. *Mycologia* 78, 694–705.

Adaskaveg JE, Gilbertson RL, 1989. Cultural studies of four North American species in the *Ganoderma lucidum* complex with comparisons to *G. lucidum* and *G. tsugae*. *Mycological Research* 92, 182–191.

Adekunle VAJ, Ajao K, 2005. Contributions of edible mushrooms (A non-timber forest product of tropical ecosystem) to rural livelihood in Oyo State, Nigeria. *Pakistan Journal of Social Sciences*, 3, 809–812.

Allmér J, Vasiliauskas R, Ihrmark K, Stenlid J, Dahlberg A, 2006. Wood-inhabiting fungal communities in woody debris of Norway spruce (Picea abies (L.) Karst.), as reflected by sporocarps, mycelial isolations and T-RFLP identification. *FEMS Microbiology Ecology*, 55, 57–67.

Anderson JB, Stasovski E, 1992. Molecular phylogeny of northern hemisphere species of Armillaria. *Mycologia* 84, 505–516.

Anderson JB, Ullrich RC, 1982. Diploids of *Armillaria mellea*: synthesis, stability, and mating behavior. *Canadian Journal of Botany* 60, 432–439.

Anonymous, 2010. Global forest resources assessment-main report. FAO forestry paper 163. Food and Agricultural Organization of the United Nations, Rome.

Anonymous, 2012. Monitoring for sustainable indigenous forest management in the Garden Route National Park. South African National Parks.

Arsenault A, Bradfield GE, 1995. Structural-compositional variation in three age-classes of temperate rain- forests in southern coastal British Columbia. *Canadian Journal of Botany* 73, 54–64.

Bendel M, Kienast F, Bugmann H, Rigling D, 2006. Incidence and distribution of *Heterobasidion* and *Armillaria* and their influence on canopy gap formation in unmanaged mountain pine forests in the Swiss Alps. *European Journal of Plant Pathology* 116, 85–93.

Bendz-Hellgren M, Stenlid J, 1997. Decreased volume growth of *Picea abies* in response to *Heterobasidion annosum* infection. *Canadian Journal of Forest Research* 27, 1519–1524.

Boddy L, Frankland JC, van West P, 2008. Ecology of Saprotrophic Basidiomycetes. London: Academic Press/Elsevier.

Boddy L, Hynes J, Bebber DP, Fricker MD, 2009. Saprotrophic cord systems : dispersal mechanisms in space and time. *Mycoscience* 50, 9–19.

Boldt CE, Alexander RR, Larson MJ, 1983. Interior ponderosa pine in the Black Hills. In: R.M. Bums (Technical Compiler), Silvicultural Systems for the Major Forest Types of the United States. Agricultural Handbook No. 445. USDA Forest Service, Washington, DC, pp. 80–83.

Boone RD, Sollins P, Cromack KJ, 1988. Stand and Soil Changes along a Mountain Hemlock Death and Regrowth Sequence. *Ecology* 69, 714–722.

Cai L, Giraud T, Zhang N, Begerow D, Cai G, Shivas RG, 2011. The evolution of species concepts and species recognition criteria in plant pathogenic fungi. *Fungal Diversity* 50, 121–133.

Cao Y,Wu S-H, Dai YC, 2012. Species clarification of the prize medicinal Ganoderma mushroom “Lingzhi.”. *Fungal Dversity* 56, 49–62.

Castello JD, Leopold DJ, Smallidge PJ, 1995. Pathogens, Patterns, and Processes in Forest Ecosystems. *BioScience* 45, 16–24.

Cleary MR, Arhipova N, Morrison DJ, Thomsen IM, Sturrock RN, Vasaitis R, Gaitnieks T, Stenlid J, 2013. Stump removal to control root disease in Canada and Scandinavia: A synthesis of results from long-term trials. *Forest Ecology and Management*, 290, 5–14.

Clough Y, Faust H, Tscharntke T, 2009. Cacao boom and bust: sustainability of agroforests and opportunities for biodiversity conservation. *Conservation Letters* 2, 197–205.

Coetzee MPA, Wingfield BD, Bloomer P, Ridley GS, Kile GA, Wingfield M.J, 2001b. Phylogenetic relationships of Australian and New Zealand *Armillaria* species. *Mycologia* 93, 887–896.

Coetzee MPA, Wingfield BD, Bloomer P, Ridley GS, Wingfield MJ, 2003a. Molecular identification and phylogeny of *Armillaria* isolates from South America and Indo-Malaysia. *Mycologia* 95, 285–293.

Coetzee MPA, Wingfield BD, Coutinho TA, Wingfield MJ, 2000. Identification of the causal agent of *Armillaria* root rot of *Pinus* species in South Africa. *Mycologia* 92, 777–785.

Coetzee MPA, Wingfield BD, Harrington TC, Steimel J, Coutinho TA, Wingfield MJ, 2001a. The root-rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* 10, 387–396.

Coetzee MPA, Wingfield BD, Kirisits T, Chhetri DB, Bloomer P, Wingfield MJ, 2005. Identification of *Armillaria* isolates from Bhutan based on DNA sequence comparisons. *Plant Pathology* 54, 36–45.

Coetzee MPA, Wingfield BD, Roux J, Crous PW, Denman S, Wingfield MJ, 2003b. Discovery of two northern hemisphere *Armillaria* species on Proteaceae in South Africa. *Plant Pathology* 52, 604–612.

Coetzee, MPA, Wingfield BD, Bloomer P, Wingfield MJ, 2005. Phylogenetic analyses of DNA sequences reveal species partitions amongst isolates of *Armillaria* from Africa. *Mycological Research* 109, 1223–1234.

Cooke RC, Rayner ADM, 1984. Ecology of saprotrophic fungi. *Longman and New York.*, 415p.

Corvalan C, Hales S, McMichael A, 2005. Ecosystems and human well-being - health synthesis: a report of the millennium ecosystem assessment, World Health Organization, Geneva.

Cruickshank MG, Morrison DJ, Lalumière A, 2009. The interaction between competition in interior Douglas-fir plantations and disease caused by *Armillaria ostoyae* in British Columbia. *Forest Ecology and Management* 257, 443–452.

Dai YC, 2010. Hymenochaetaceae (Basidiomycota) in China. *Fungal Diversity* 45, 131–343.

Douanla-Meli C, Langer E, 2009. *Ganoderma carocalcareus* sp. nov., with crumbly-friable context parasite to saprobe on *Anthocleista nobilis* and its phylogenetic relationship in *G. resinaceum* group. *Mycological Progress* 8, 145–155.

Douanla-Meli C, Ryvarden L, Langer E, 2007. 'Studies of tropical African pore fungi (Basidiomycota, Aphyllophorales): three new species from Cameroon'. *Nova Hedwigia* 84, 409–420.

Durrheim GP, 2006. Regional indigenous forest management plan for the Southern Cape and Tsitsikamma: A summary. South African National Parks, Knysna. 1–17.

Eastwood DC, Floudas D, Binder M, Majcherczyk A, Schneider P, Aerts A, … Watkinson S C, 2011. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. *Science* 333, 762–765.

Egbe EA, Kinge TR, Maureen TE, Nji T, Afui MM, 2013. Diversity and distribution of macrofungi (mushrooms) in the Mount Cameroon Region. *Journal of Ecology and the Natural Environment* 5, 318–334.

Entry JA, Emmingham WH, 1995. Influence of forest age on nutrient availability and storage in coniferous soils of the Oregon Coast Range. *Canadian Journal of Forest Research* 25, 114–120.

Ferguson BA, Dreisbach TA, Parks CG, Filip GM, Schmitt CL, 2003. Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. *Canadian Journal of Forest Research* 33, 612–623.

Fiasson JL, Niemelä T, 1984. The Hymenochaetales: a revision of the European poroid taxa. *Karstenia* 24, 14–28.

Filip GM and Yang-Erve L, 1997. Effects of Prescribed Burning on the Viability of *Armillaria ostoyae* in Mixed-Conifer Forest Soils in the Blue Mountains of Oregon. *Northwest Science*, 7, 137–144.

Fischer M, 1987. Biosystematische Untersuchungen an den Porlingsgattungen *Phellinus* Quél. und *Inonotus* Karst. *Bibliotheca Mycologica* 107, 1–139.

Fischer M, Wagner T, 1999. RFLP analysis as a tool for identification of lignicolous basidiomycetes: European polypores. *European journal of forest pathology* 29, 295-304.

Fischer M. 1996. Molecular and microscopial studies in the Phellinus pini group. *Mycologia* 88, 230–238.

Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, … Hibbett DS, 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science*, *336*, 1715–1719.

Franzen M, Mulder MB, 2007. Ecological, economic and social perspectives on cocoa production worldwide. *Biodiversity Conservation* 16, 3835–3849.

Fricker MD, Bebber D, Boddy L, 2008. Mycelia networks: Structure and dynamics. In: Boddy L, Frankland JC, van West P, (eds) Ecology of Saprotrophic Basidiomycetes. London: Academic Press/Elsevier.

Fukasawa Y, 2012. Effects of wood decomposer fungi on tree seedling establishment on coarse woody debris. *Forest Ecology and Management* 266, 232–238.

Fukasawa Y, Takahashi K, Arikawa T, Hattori T, Maekawa N, 2015. Fungal wood decomposer activities influence community structures of myxomycetes and bryophytes on coarse woody debris. *Fungal Ecology* 14, 44–52.

Garbelotto M, 2004. Root and butt rot diseases. In Encyclopedia of Forest Sciences: Volume 2, ed. J Burley, J Evans, JA Youngquist, pp. 750–58. Oxford: Elsevier

Garbelotto M, Gonthier P, 2013. Biology, epidemiology, and control of *Heterobasidion* species worldwide. *Annual Review of Phytopathology* 51, 39–59.

Garcia-Sandoval R, Wang Z, Binder M, Hibbett D, 2011. Molecular phylogenetics of the Gloeophyllales and relative ages of clades of Agaricomycotina producing a brown rot. *Mycologia* 103, 510–524.

Gilbert GS, 2002. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* 40, 13–43.

Gilbertson RL, 1980. Wood-Rotting Fungi of North America. *Mycologia*, 72, 1–49.

Gilbertson RL, 1981. "North American wood-rotting fungi that cause brown rots." *Mycotaxon* 12, 372–416.

Gockowski J, Sonwa D, 2011. Cocoa Intensification Scenarios and Their Predicted Impact on CO_2 Emissions, Biodiversity Conservation, and Rural Livelihoods in the Guinea Rain Forest of West Africa. *Environmental Management* 48, 307–321.

Gockowski J, Tchatat M, Dondjang JP, Hietet G, Fouda T, 2010. An empirical analysis of the biodiversity and economic returns to cocoa agroforests in southern Cameroon. *Journal of Sustainable Forestry* 29, 638–670.

Gonthier P, Anselmi N, Capretti P, Bussotti F, Feducci M, Giordano L, … Garbelotto M, 2014. An integrated approach to control the introduced forest pathogen Heterobasidion irregulare in Europe. *Forestry* 87, 471–481.

Gonthier P, Garbelotto MM, Nicolotti G, 2005. Seasonal Patterns of Spore Deposition of Heterobasidion Species in Four Forests of the Western Alps. *Phytopathology* 95, 759–767.

Gregory M, Maffei HM, Chadwick K, Max TA, 2010. Armillaria Root Disease-Caused Tree Mortality following Silvicultural Treatments (Shelterwood or Group Selection) in an Oregon Mixed-Conifer Forest: Insights from a 1O-Year Case Study. *Western Journal of Applied Forestry* 25, 136–143.

Guglielmo F, Bergemann SE, Gonthier P, Nicolotti G, Garbelotto M, 2007. A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees. *Journal of Applied Microbiology* 103, 1490–1507.

Haack RA, Byler JW, 1993. Insects and pathogens. Regulators of forest ecosystems. *Journal Forestry* 91, 32–37.

Hãgvar S, 1999. Saproxylic beetles visiting living sporocarps of *Fomitopsis pinicola* and *Fomes fomentarius*. *Norwegian Journal of Entomology* 46, 25–32.

Hallenberg N, Kuffer N, 2001. Long-distance spore dispersal in wood-inhabiting Basidiomycetes. *Nordic Journal of Botany* 21, 431–436.

Hansen EM; Goheen EM, 2000. *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in western North America. *Annual Review of Phytopathology*, 38, 515–539.

Harrington TC, Wingfield BD, 1995. A PCR-based identification method for species of Armillaria. *Mycologia* 87, 280–288

Hawksworth D, Kirk P, Sutton B and Pegler D, 1996. Ainsworth's and Bisby's Dictionary of the Fungi, 8th ed. CABI, Wallingford, UK.

Heilmann-Clausen J, Boddy L, 2008. Distribution patterns of wood-decay Basidiomycetes at the landscape to global scale. In: Boddy L, Frankland JC, van West P, (eds) Ecology of Saprotrophic Basidiomycetes. London: Academic Press/Elsevier.

Hennon P, 1995. Are Heart Rot Fungi Major Factors of Disturbance in Gap-Dynamic Forests ? *Northwest Science* 69, 284–293.

Hibbett DS, Fukumasa-Nakai Y, Tsuneda A, Donoghue MJ, 1995. Phylogenetic diversity in shiitake inferred from nuclear ribo- somal DNA sequences. *Mycologia* 87, 618–638.

Hickman GW, Perry EJ, Davis RM, 2011. Wood decay Fungi in Landscape Trees. *PEST NOTES* Publication 74109, 4 p.

Hoff JA; Klopfenstein NB; Tonn JR; McDonald GI; Zambino PJ; Rogers JD; Peever TL; Carris, LM, 2004. Roles of woody root-associated fungi in forest ecosystem processes: recent advances in fungal identification. Res. Pap. RMRS-RP-47. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 6 p.

Holah JC, Wilson MV, Hansen EM, 1993. Effects of a native forest pathogen, *Phellinus weirii*, on Douglas-fir forest composition in western Oregon. *Canadian Journal of Forest Research*, 23, 2473–2480.

Holah JC, Wilson MV, Hansen EM. 1997. Impacts of a native root-rotting pathogen on successional development of old-growth Douglas fir forests. *Oecologia* 111, 429–33.

Hong SG, Jung HS, 2004. Phylogenetic analysis of Ganoderma based on nearly complete mitochondrial small-subunit ribosomal DNA sequences. *Mycologia* 96,742–755.

Hseu RS, Wang HH, Wang HF, Moncalvo JM, 1996. Differentiation and grouping of isolates of the *Ganoderma lucidum* complex by random amplified polymorphic DNA-PCR compared with grouping on the basis of internal transcribed spacer sequences. *Applied and Environmental Microbiology* 62, 1354–1363.

Hunter JC, Parker VT, 1993. The disturbance regime of an old-growth forest in coastal California. *Journal of Vegetation Science* 4, 19–24.

Ito Y, Fushimi T, Yanagi SO, 1998. Discrimination of species and strains of basidiomycete genus Coprinus by random amplified polymorphic DNA (RAPD) analysis. *Mycoscience* 39, 361–365.

Jeong WJ, Lim YW, Lee JS, Jung HS, 2005. Phylogeny of *Phellinus* and related genera inferred from combined data of ITS and mitochondrial SSU rDNA sequences. *Journal of Microbiology and Biotechnology* 15, 1028–1038.

Johannesson H, Stenlid J, 1998. Molecular identification of wood-inhabiting fungi in an unmanaged *Picea abies* forest in Sweden. *Forest Ecology and Management* 4525, 1–9

Johansson SM, Pratt JE, Asiegbu FO, 2002. Treatment of Norway spruce and Scots pine stumps with urea against the root and butt rot fungus *Heterobasidion annosum* - Possible modes of action. *Forest Ecology and Management* 157, 87–100.

Jong SC, Birmingham JM, 1992. Medicinal benefits of the mushroom *Ganoderma*. *Advances in Applied Microbiology*, 37, 101–34.

Jusino MA, Lindner DL, Banik MT, Walters JR, 2015. Heart rot hotel: fungal communities in red-cockaded woodpecker excavations. *Fungal Ecology* 14, 33–43.

Kallio T, 1973. Influence of ultraviolet radiation on the colony forma- tion of *Fomes annosus* diaspores suspended in water. *Karstenia* 14, 5–8.

Keca N, 2005. Characteristics of *Armillaria* species development and their growth at different temperatures. *Glasnik Sumarskog Fakulteta, Univerzitet u Beogradu* 91, 149–162.

Kim HK, Shim MY, Seo GS and Kim HG, 2002. Comparison of characteristics of *Ganoderma lucidum* according to geographical Origins (III): Classification between species of genus Ganoderma using dikaryon- monokaryon mating. *Mycobiology* 30, 61–64.

Korhonen K, Hintikka V, 1974. Cytological evidence for somatic diploidization in dikaryotic cells of *Armillariella mellea*. *Archives of Microbiology* 95,187–192.

La Porta N, Capretti P, Thomsen IM, Kasanen R, Hietala AM, Von Weissenberg K, 2008. Forest pathogens with higher damage potential due to climate change in Europe. *Canadian Journal of Plant Pathology* 30, 177–195.

Lang GE, Knight DH, 1983. Tree growth, mortality, recruitment, and canopy gap formation during a 10-year period in a tropical moist forest. *Ecology* 64, 1075–1080.

Larsen MJ, Cobb-Poulle LA, 1990. *Phellinus* (Hymenochaetaceae) a survey of the world taxa. *Synopsis Fungorum* 3, 1–206.

Lee H, Jang Y, Choi YS, Kim MJ, Lee J, Lee H, … Kim JJ, 2014. Biotechnological procedures to select white rot fungi for the degradation of PAHs. *Journal of Microbiological Methods* 97, 56–62.

Lertzman KP, Sutherland GD, Inselberg A and Saunders SC, 1996. Canopy Gaps and the Landscape Mosaic in a Coastal Temperate Rain Forest. *Ecology* 77, 1254–1270.

Lindner DL, Vasaitis R, Kubartová A, Allmér J, Johannesson H, Banik MT, Stenlid J, 2011. Initial fungal colonizer affects mass loss and fungal community development in *Picea abies* logs 6yr after inoculation. *Fungal Ecology* 4, 449–460.

Lonsdale D, Pautasso M, Holdenrieder O, 2008. Wood-decaying fungi in the forest: conservation needs and management options. *European Journal of Forest Research*, 127, 1–22.

Luley CJ, 2005. Basidiomycetes wood decay life cycle. http://www.mwcog.org/uploads/committee-documents/a15bX1dZ20090608122014.pdf.

Lundquist JE, 1991. Distribution of Armillaria root disease in the Black Hills. Tech. Rep. R2–49, *Forest Pest Management*, Lakewood, co, 11 pp.

Lundquist JE, 1993. Spatial and temporal characteristics of canopy gaps caused by Armillaria root disease and their management implications in lowveld forests of South Africa. *European Journal of Plant Pathology* 23, 362–371.

Lundquist JE, 1995. Characterizing disturbance in managed ponderosa pine stands in the Black Hills. *Forest Ecology and Management* 74, 61–74.

Lygis V, Vasiliauskaite I, Stenlid J, Vasaitis R, 2010. Impact of forest fire on occurrence of *Heterobasidion annosum* s.s. root rot and other wood-inhabiting fungi in roots of *Pinus mugo*. *Forestry* 83, 83–92.

Maloy OC, 1993. Plant Disease Control: Principles and Practice. Wiley, New York, NY, USA, 346 pp.

Maphosa L, Wingfield B, Coetzee M, Mwenje E, Wingfield MJ, 2006. Phylogenetic relationships among *Armillaria* species inferred from partial elongation factor 1-alpha DNA sequence data. *Australasian Plant Pathology* 35, 513–520.

Mayr E, 1940. Speciation phenomena in birds. *American Naturalist* 74, 249–278.

Mladenoff DJ, 1987. Dynamics of nitrogen mineralization and nitrification in hemlock and hardwood tree fall gaps. *Ecology* 68, 1171–1180.

Moncalvo JM, Ryvarden L, 1997. A nomenclatural study of the Ganodermataceae Donk. *Fungiflora* 10, 1–114.

Moncalvo JM, Wang HF, Hseu RS, 1995a. Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters. *Mycological Research* 99, 1489–1499.

Moncalvo JM, Wang HF, Wang HH, Hseu RS, 1994. The use of ribosomal DNA sequence data for species identification and phy- logeny in the Ganodermataceae. In: Buchanan PK, Hseu R-S, Moncalvo J-M (eds) *Ganoderma* Syst. Phytopathol. Pharmacol. National Taiwan University, Taipei, Proceedings of contributed symposium59A,B,5th International Mycological Congress, Vancouver, August 14–21, pp 31–44.

Moncalvo JM,Wang HH, Hseu RS, 1995b. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. *Mycologia* 87, 223–238.

Morrison DJ, Merler H, Norris DJ, 1991. Detection, recognition and management of *Armillaria* and *Phellinus* root diseases in the southern interior of British Columbia. FRDA Report 179. 25 p.

Morrison DJ, Pellow KW, Nemec AFL, Norris DJ, Semenoff P, 2001. Effects of selective cutting on the epidemiology of armillaria root disease in the southern interior of British Columbia. *Canadian Journal of Forest Research*, 31, 50–70.

Mswaka AY, Magan N, 1999. Temperature and water potential relations of tropical Trametes and other wood-decay fungi from the indigenous forests of Zimbabwe. *Mycological Research* 103, 1309–1317.

Müller MM, Varama M, Heinonen J, Hallaksela AM, 2002. Influence of insects on the diversity of fungi in decaying spruce wood in managed and natural forests. *Forest Ecology and Management* 166, 165–181.

Muyzer G, Smalla K, 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek* 73, 127–141.

Mwenje E, Wingfield BD, Coetzee MPA, Wingfield MJ, 2003. Molecular characterisation of Armillaria species from Zimbabwe. *Mycological Research* 107, 291–296.

Nicolotti G, Gonthier P, Guglielmo F, Garbelotto MM, 2009. A Biomolecular Method for the Detection of Wood Decay Fungi : A Focus on Tree Stability Assessment. *Arboriculture and Urban Forestry* 35, 14–19.

Nobles MK, 1965. Identification of cultures of wood-inhabiting Hymenomycetes. *Canadian Journal of Botany* 43, 1097–1139.

Nordén B, Larsson KH, 2000. Basidiospore dispersal in the old-growth forest fungus *Phlebia centrifuga* (Basidiomycetes). *Nordic Journal of Botany* 20, 215–219.

Norris K, Asase A, Collen B, Gockowski J, Mason J, Phalan B, Wade A, 2010. Biodiversity in a forest agricultural mosaic—the changing face of West African rainforests. *Biological Conservation* 143, 2341–2350.

Oliva J, Samils N, Johansson U, Bendz-Hellgren M, Stenlid J, 2008. Urea treatment reduced *Heterobasidion annosum* s.l. root rot in *Picea abies* after 15 years. *Forest Ecology and Management* 255, 2876–2882.

Ovaskainen O, Nokso-Koivisto J, Hottola J, Rajala T, Pennanen T, Ali-KoveroH, Miettinen O, Oinonen P, Auvinen P, Paulin L, Larsson Karl-Henrik, Mäkipää R, 2010. Identifying wood-inhabiting fungi with 454 sequencing - what is the probability that BLAST gives the correct species? *Fungal Ecology* 3, 274–283.

Ovaskainen O, Schigel D, Ali-Kovero H, Auvinen P, Paulin L, Nordén B, Nordén J, 2013. Combining high-throughput sequencing with fruit body surveys reveals contrasting life-history strategies in fungi. *International Society for Microbial Ecology* 7, 1696–709.

Parmasto E, Saar I, Larsson E, Rummo S, 2014. Phylogenetic taxonomy of Hymenochaete and related genera (Hymenochaetales). *Mycological Progress* 13, 55–64.

Paterson RRM, 2006. *Ganoderma* a therapeutic fungal biofactory. *Phytochemistry* 67, 1985–2001.

Pautasso M, Holdenrieder O, Stenlid J, 2005. Susceptibility to Fungal Pathogens of Forests Differing in Tree Diversity. *Ecological Studies* 176, 263–289.

Pearce MH, Malajczuk N, 1990. Factors affecting growth of *Armillaria luteobubalina* rhizomorphs in soil. *Mycological Research* 94, 38–48.

Pearce RB, 1996. Antimicrobial defences in the wood of living trees Tansley Review No. 87. *New Phytologist* 132, 203–233.

Persson Y, Ihrmark K, Stenlid J, 2011. Do bark beetles facilitate the establishment of rot fungi in Norway spruce? *Fungal Ecology* 4, 262–269.

Persson Y, Vasaitis R, Långström B, Öhrn P, Ihrmark K, Stenlid J, 2009. Fungi vectored by the bark beetle ips typographus following hibernation under the bark of standing trees and in the forest litter. *Microbial Ecology* 58, 651–659.

Pilotti CA, Sanderson FR, Aitken EAB, 2002. Sexuality and interactions of monokaryotic and dikaryotic mycelia of *Ganoderma boninense*. *Mycological Research* 106, 1315–1322.

Rajala T, Peltoniemi M, Pennanen T, Mäkipää R, 2010. Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs. *Canadian Journal of Forest Research* 40, 2384–2397.

Rajchenberg M, 2011. Nuclear behavior of the mycelium and the phylogeny of Polypores (Basidiomycota). *Mycologia* 103, 677–702.

Reynolds DR, 1993. The fungal holomorph: An overview. In The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics (D. R. Reynolds and J. W. Taylor, Eds.), pp. 15–25. CAB International, Wallingford, UK.

Rice R, Greenberg R, 2000. Cacao cultivation and the conservation of biological diversity. *Ambio* 29, 167–173.

Riffle JW, Walla JA, 1986. Perennial wood-rotting fungi that cause stem decays of hardwoods. In: Riffle, J.W.; Peterson, G.W., Tech. Coords. Diseases of Trees in the Great Plains. Gen. Tech. Rep. RM-129. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station., 149p.

Rind B, Losel DM, 2005. Effect of nutrients and temperature on the growth of *Armillaria mellea* and other fungi. *Journal of Biological Sciences* 2, 326–331.

Rishbeth J, 1976. Chemical Treatment and Inoculation of Hardwood Stumps for Control of *Armillaria Mellea*. *Annals of Applied Biology* 82, 57–70.

Rishbeth J, 1978. Effects of soil temperature and atmosphere on growth of *Armillaria* rhizomorphs. *Transactions of the British Mycological Society* 70, 213–220.

Rossman AY, Palm-hernández ME, 2008. Systematics of Plant Pathogenic Fungi : Why It Matters. *Plant Disease* 92, 1376–1386.

Rosso P and Hansen E, 1998. Tree vigour and the susceptibility of Douglas fir to Armillaria root disease. *European Journal of Forest Pathology* 28, 43–52.

Rotem J, Aust HJ, 1991. The Effect of Ultraviolet and Solar Radiation and Temperature on Survival of Fungal Propagules. *Journal of Phytopathology* 133, 76–84.

Roux J, Chapman D, Coetzee MPA, 2011. *Phellinus* sensu lato species associated with wood rot of native tree species in South Africa and Namibia. IUFRO Forest Protection Joint Meeting. "Pathogens, insects and their associations affecting forestry worldwide", 8-11 November 2011, Colonia del Sacramento, Uruguay.

Roux J, Mbenoun M, Misse AC, Coetzee MPA, Wingfield MJ, 2012. Molecular identification of *Armillaria* isolates associated with root-rot disease of cacao in Cameroon. 17th International Cocoa Research Congress (COPAL), Yaounde, Cameroon.

Roux J, Wingfield MJ, Coetzee MPA, 2013. Exploratory studies of *Ganoderma* species in the Garden Route of South Africa. Proceedings of the 10th International Congress of Plant Pathology; 25–30 Aug 2013, Beijing, China.

Runkle JR, 1981. Gap Regeneration in Some Old-growth Forests of the Eastern United States. *Ecology* 62, 1041–1051.

Runkle JR, 1998. Changes in Southern Appalachian canopy tree gaps sampled Thrice. *Ecology* 79, 1768–1780.

Runkle JR, 2000. Canopy tree turnover in old-growth mesic forests of eastern North America. *Ecology* 81, 554–567.

Runkle JR, 2013. Thirty-two years of change in an old-growth Ohio beech-maple forest. *Ecology* 94, 1165–1175.

Ryvarden L and Johansen I, 1980. A preliminary polypores flora of East Africa. *Fungiflora* 1–636.

Ryvarden L, 1991. Genera of Polypore, nomenclature and taxonomy. *Synopsis Fungorum* 5, 1–363.

Ryvarden L, 1995. Can we trust morphology in Ganoderma?In: Buchanan PK, Hseu R-S, Moncalvo J-M (eds) Proc. Contrib. Symp. 59A, B 5th Int.Mycol. Congr. Taipei, pp 19–24

Ryvarden L, 2004. Neotropical polypores Part 1. *Synopsis Fungorum* 19, 1–229.

Sanderson FR, 2005. An Iinsight into sspore dispersal of *Ganoderma boninense* on Oil Palm. *Mycopathologia* 159, 139–141.

Schmidt O, 2006. Wood and Tree Fungi Biology, Damage, Protection, and Use. (D. D. Czeschlik & A. Schlitzberger, Eds.) (Springer.). Heidelberg.

Schwarze F, Engels J and Mattheck C, 2000. Fungal Strategies of Wood Decay in Trees. *Springer, Berlin, Heidelberg.*, 183p.

Schwarze FWMR, 2007. Wood decay under the microscope. *Fungal Biology Reviews* 21, 133–170.

Seydack AHW, Vermeulen WJ, Heyns HE et al., 1995. An unconventional approach to timber yield regulation for multi-aged, multispecies forests. II. Application to a South African forest. *Forest Ecology and Management* 77, 155–68.

Seydack AHW, Vermeulen WJ, Heyns HE, Durrheim GP, Vermeulen C, Willems D, Ferguson MA, Huisamen J, Roth J, 1995. An unconventional approach to timber yield regulation for multi-aged, multispecies forests. II. Application to a South African forest. *Forest Ecology and Management* 77, 155–168.

Shaw CG, Kile GA, 1991: *Armillaria* Root Disease. Agriculture Handbook No. 691. Washington, DC: Forest Service, United States Department of Agriculture.

Shaw III CG, Omdal DW, Ramsey-Kroll A and Roth LF, 2012. Inoculum Reduction Measures to Manage Armillaria Root Disease in a Severely Infected Stand of Ponderosa Pine in South-Central Washington: 35-Year Results. *Western Journal of Applied Forestry* 27, 25–29.

Shigo AL, 1991. Modern arboriculture: a systems approach to the care of trees and their associates. Durham, NH: Shigo and Trees, Associates. 424 p.

Shortle WC, Dudzik KR, 2012. Wood Decay in Living and Dead Trees : A Pictorial Overview. Gen. Tech Rep. NRS-97. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 26p.

Singh P, 1983. Armillaria root rot: Influence of soil nutrients and pH on the susceptibility of conifer species to the disease. *European Journal of Forest Pathology* 13, 92–101.

Singh SK, Doshi A, Pancholy A, Pathak R, 2013. Biodiversity in wood-decay macro-fungi associated with declining arid zone trees of India as revealed by nuclear rDNA analysis. *European Journal of Plant Pathology* 136, 373–382.

Smith BJ, Sivasithamparam K, 2000. Internal transcribed spacer ribosomal DNAsequence of five species of Ganoderma fromAustralia. *Mycological Research* 104, 943–951.

Smith ML, Anderson JB, 1989. Restriction fragment length polymorphisms in mitochondrial DNAs of Armillaria: identification of North American biological species. *Mycological Research* 93, 247–256.

Smith ML, Bruhn JN, Anderson JB, 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* 356, 428–431.

Sonwa D, Ngongmeneck BA, Weise SW, Tchatat M, Adesina AA, Janssens MJJ, 2007. Diversity of plants in cocoa agroforests in the humid forest zone of Southern Cameroon. *Biodiversity and Conservation* 16, 2385–2400.

Spies TA, Franklin JF, 1991. The structure of natural young, mature, and old-growth Douglas-fir forests in Or- egon and Washington. Pages 91-109 in L. F Ruggerio, K. B. Aubry, A. B. Carey, and M. H. Huff, editors. Wildlife and vegetation of unmanaged Douglas-fir forests. United States Department of Agriculture Forest Service, General Technical Report PNW-GTR-285.

Stalpers JA, 1978. Identification of wood-inhabiting fungi in pure culture. *Studies in Mycology* 16, 1–248.

Stanosz GR, Patton RF, 1990. Stump colonization by Armillaria in Wisconsin aspen stands following clear cutting. *European Journal of Plant Pathology* 20, 339–346.

Stenlid J, 2008. Population biology of forest decomposer basidiomycetes. *In:* Boddy L, Frankland JC, van West P, (eds) Ecology of Saprotrophic Basidiomycetes. London: Academic Press/Elsevier.

Stenlid J, Penttilä R, Dahlberg A, 2008. Wood-decay Basidiomycetes in boreal forests: distribution and community development. In: Boddy L, Frankland JC, van West P, (eds) Ecology of Saprotrophic Basidiomycetes. London: Academic Press/Elsevier.

Stokland JN, Siitonen J, Jonsson BG, 2012. Biodiversity in DeadWood. Ecology, Biodiversity and Conservation. *Cambridge University Press, Cambridge.*, 495p.

Strid Y, Schroeder M, Lindahl B, Ihrmark K, Stenlid J, 2014. Bark beetles have a decisive impact on fungal communities in norway spruce stem sections. *Fungal Ecology* 7, 47–58.

Sturrock RN, 2000. Management of Root Diseases by Stumping and Push-falling. Technology Transfer Note Forestry Research Applications Pacific Forestry Centre, Number 16, 8p.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC, 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* 31, 21–32.

Thies WG, Sturrock RN, 1995. Laminated root rot in western North America. USDA Forest Service Resource Bulletin PNW-GTR-349, Portland, Oreg.

Thompson W, Boddy L, 1983. Decomposition of Suppressed Oak Trees in Even-Aged Plantations.. Ii. Colonization of Tree Roots By Cord- and Rhizomorph-Producing Basidiomycetes. *New Phytologist* 93, 277–291.

Thompson W, Rayner ADM, 1983. Extent, development and function of mycelial cord systems in soil. *Transactions of the British Mycological Society* 81, 333–345.

Turner MJ, Romme WH. 1994. Landscape dynamics in crown-fire ecosystems. Landscape *Ecology* 9, 59–77.

Ullrich RC, Anderson JB, 1978. Sex and diploidy in *Armillaria mellea*. *Experimental Mycology* 2, 119–129.

Van der Kamp BJ, 1991. Pathogens as agents of diversity in forested landscapes. *Forestry Chronicle* 67, 353–354.

Varese GC, Buffa G, Luppi AM, Gonthier P, Nicolotti G, Cellerino GP, 1999. Effects of biological and chemical treatments against *Heterobasidion annosum* on the microfungal communities of Picea abies stumps. *Mycologia* 91, 747–755.

Vasaitis R, Stenlid J, Thomsen IM, Barklund P, Dahlberg A, 2008. Stump Removal to Control Root Rot in Forest Stands . *A Literature Study. Silva Fennica* 42, 457–483.

Vasiliauskas R, Lygis V, Thor M, Stenlid J, 2004. Impact of biological (Rotstop) and chemical (urea) treatments on fungal community structure in freshly cut *Picea abies* stumps. *Biological Control* 31, 405–413.

Wagner T, Fischer M, 2001. Natural groups and a revised system for the European poroid Hymenochaetales (Basidiomycota) supported by nLSU rDNA sequence data. *Mycological Research* 105,773–782.

Wang X-C, Xi R-J, Li Y, Wang D-M, Yao Y-J, 2012. The species identity of the widely cultivated Ganoderma, "*G. lucidum*" (Ling-zhi), in China. *PLoS One* 7:e40857.

Warren G, Baines P, Plamondon J, Pitt DG, 2013. Effects of precommercial thinning on the forest value chain in northwestern New Brunswick: Part 3 – Incidence of root and butt decay. *The Forestry Chronicle*, 464–473.

Weir RD, Phinney M, Lofroth EC, 2012. Big, sick, and rotting: Why tree size, damage, and decay are important to fisher reproductive habitat. *Forest Ecology and Management* 265, 230–240.

White TJ, Bruns T, Lee S, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protoc. A Guid. toMethods Appl. Academic Press, Inc., New York, pp 315-322.

Whiting EC, Rizzo DM, 1999. Effect of water potential on radial colony growth of *Armillaria mellea* and *A. gallica* isolates in culture. *Mycologia* 91, 627–635.

Worrall JJ, Lee TD, Harrington TC, 2005. Forest dynamics and agents that initiate and expand canopy gaps in *Picea abies* forests of Crawford Notch , New Hampshire , USA. *Journal of Ecology* 93, 178–190.

Yombiyeni P, Douanla-meli C, Amalfi M, Decock C, 2011. Poroid *Hymenochaetaceae* from Guineo – Congolian rainforest : *Phellinus gabonensis* sp . nov . from Gabon – taxonomy and phylogenetic relationships. *Mycological Progress* 10, 351–362.

Yoon J-J, Kim Y-K, 2005. Degradation of crystalline cellulose by the brown-rot basidiomycete *Fomitopsis palustris*. *Journal of Microbiology* 43, 487–492.

Figure 1. Types of wood-rots. A. Brown rot. B. White rot (source: https://en.wikipedia.org/wiki/Wood-decay_fungus).

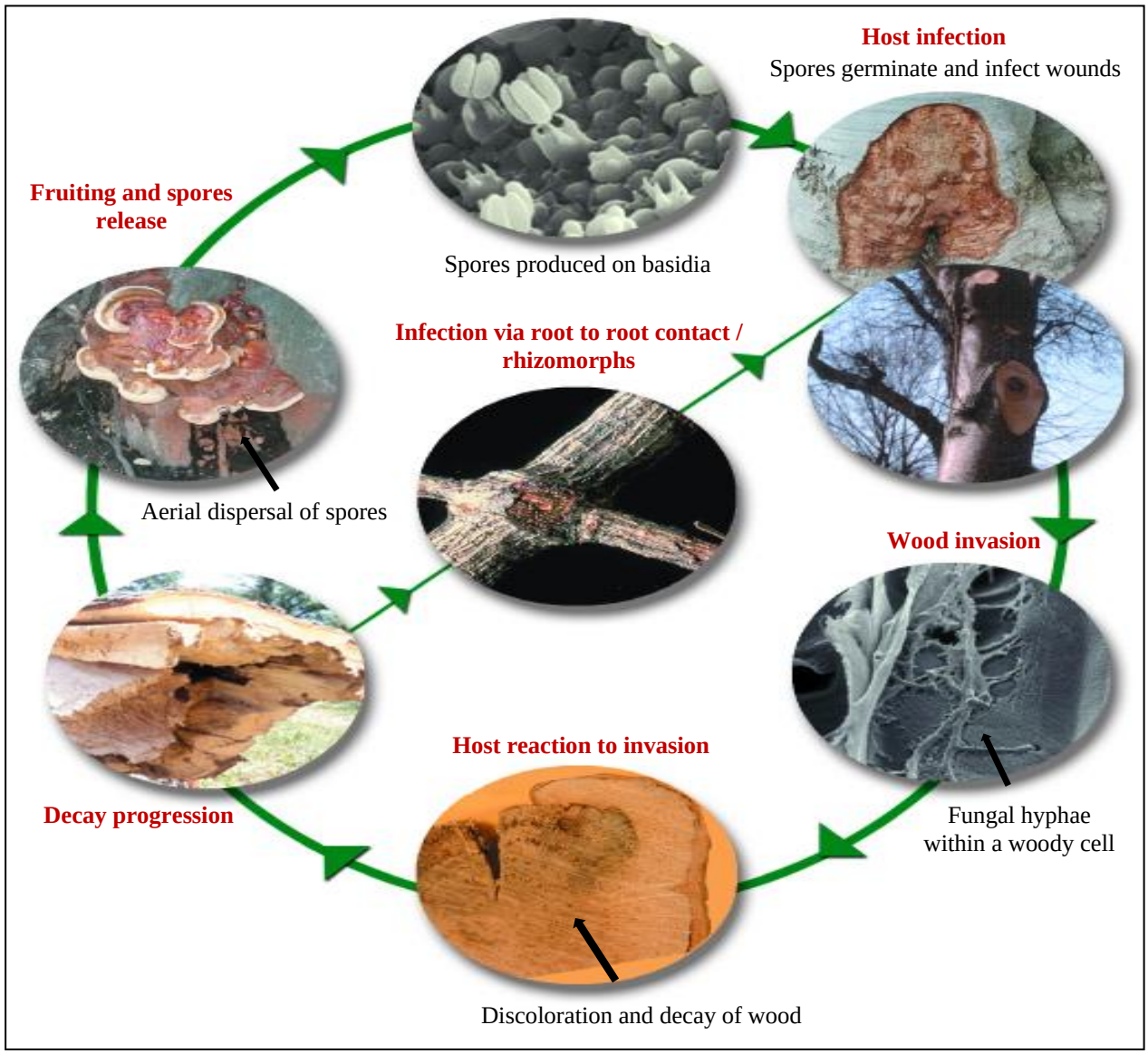

Figure 2. Wood-rotting Basidiomycetes life cycle (Luley 2005 modified).

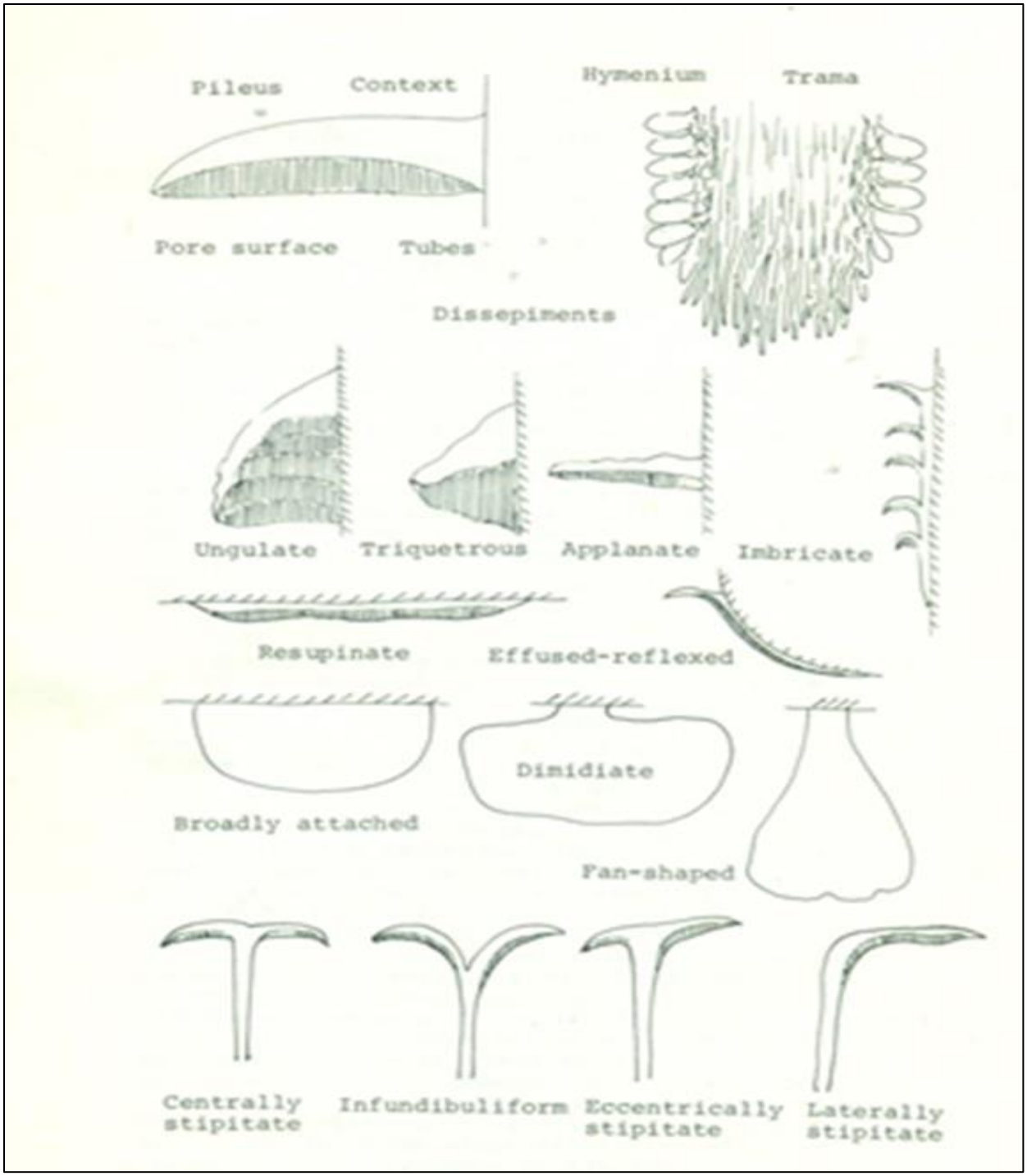

Figure 3. Types of basidiomes, their attachments and technical terms of their sections (Ryvarden and Johansen 1980).

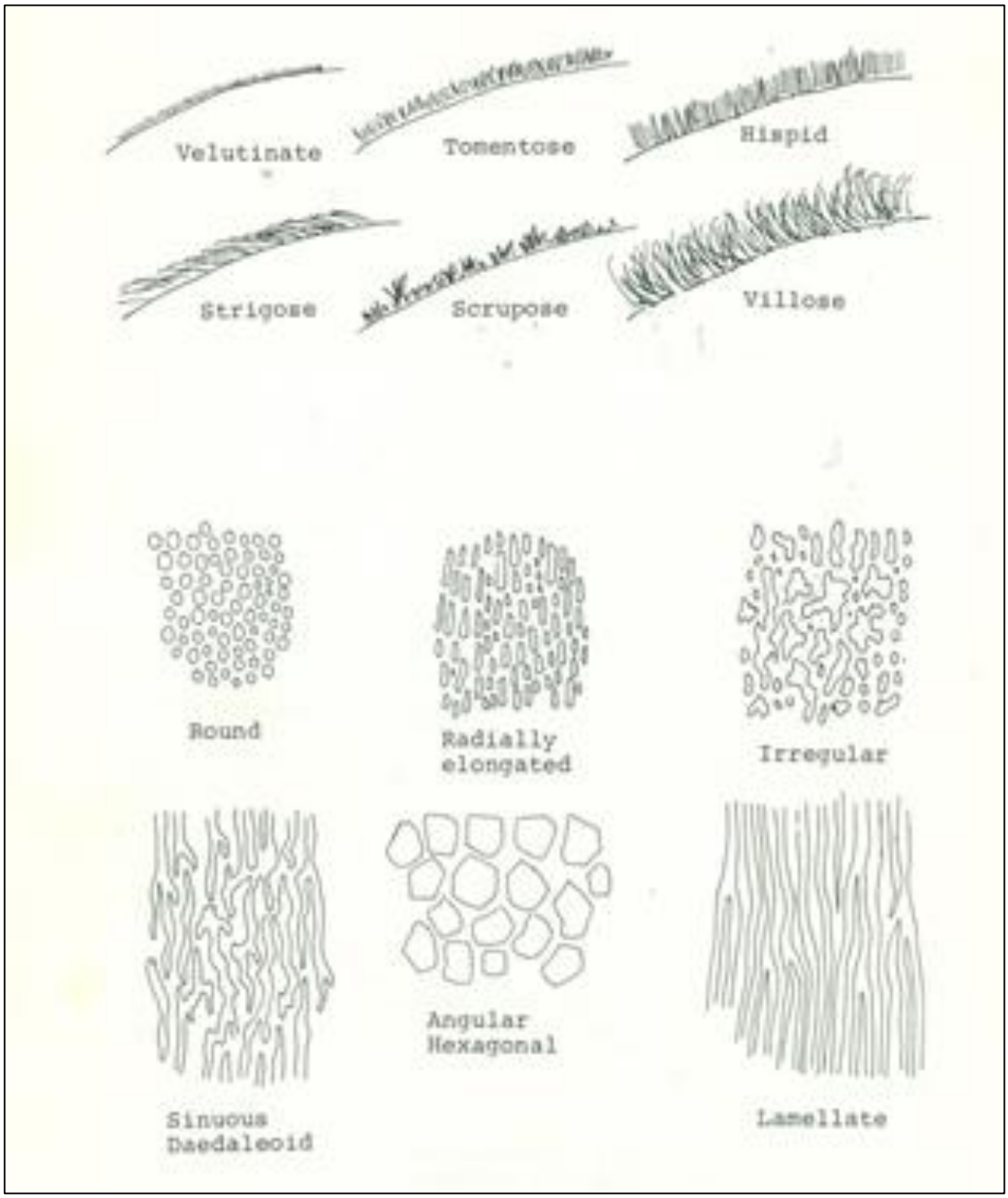

Figure 4. Types of pileus and hymenophore faces in the Polyporaceae (Ryvarden and Johansen, 1980).

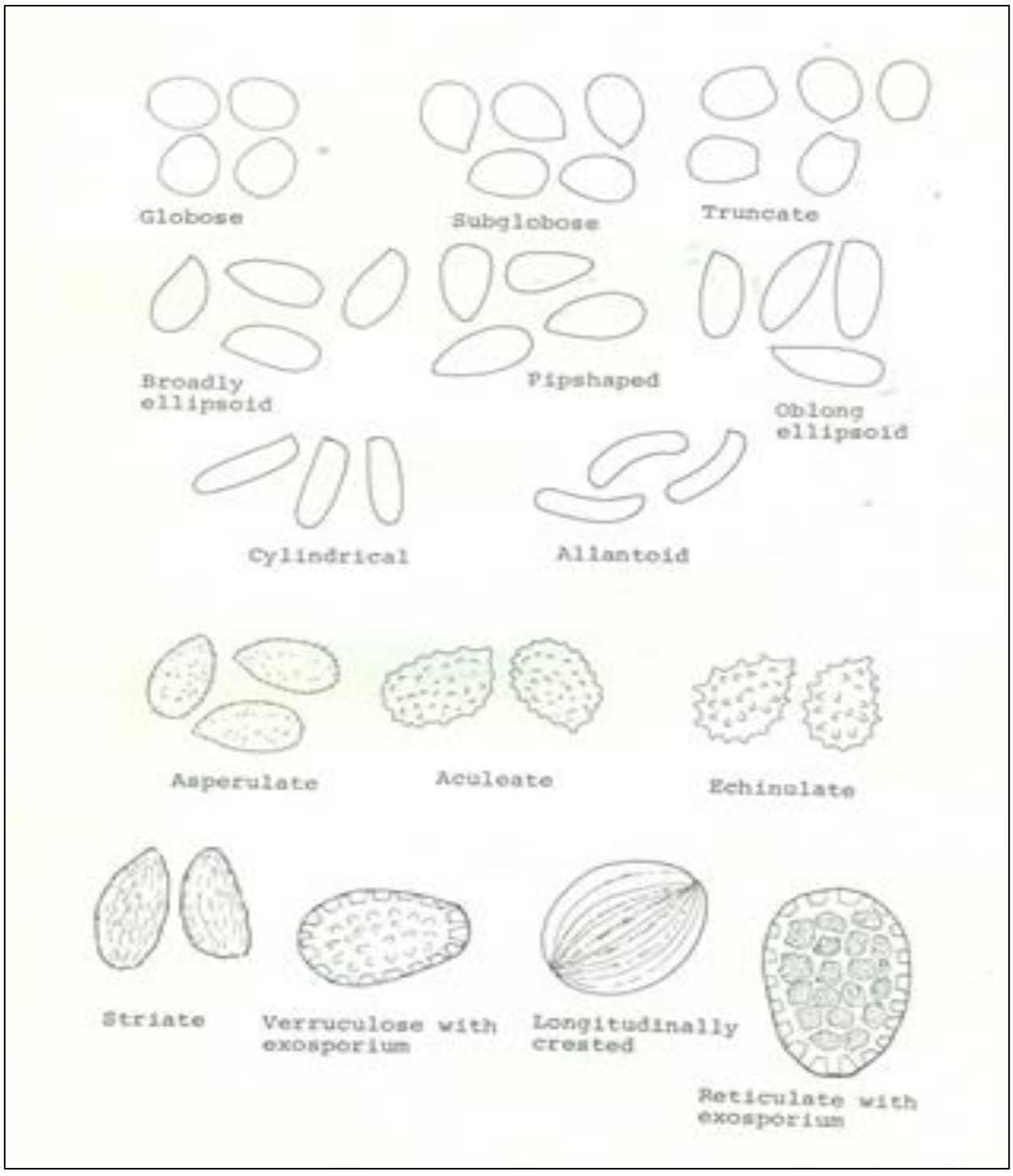

Figure 5. Types of basidiospores and their ornamentations in the Polyporaceae (Ryvarden and Johansen 1980).

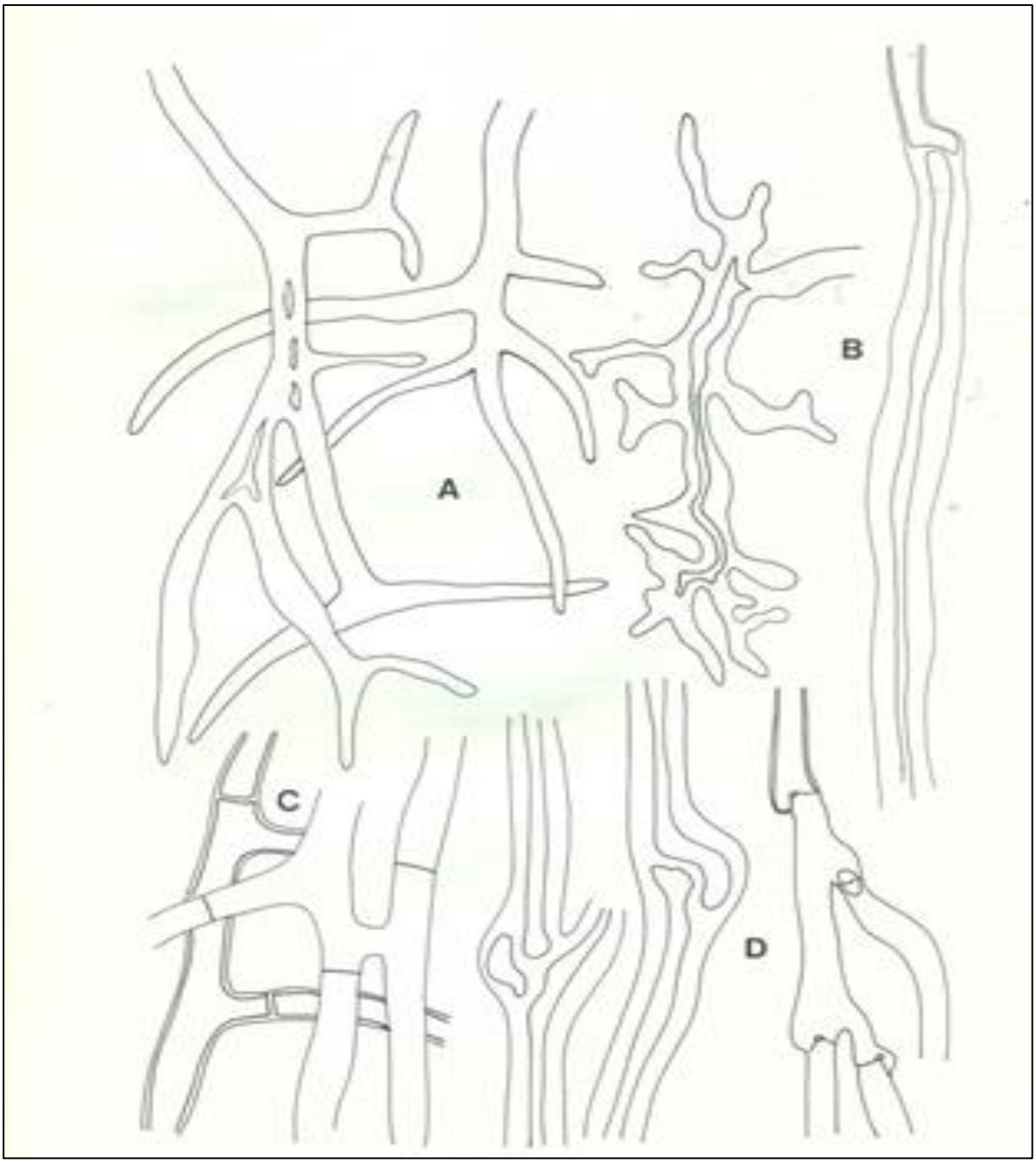

Figure 6. Hyphal system. A. biding hyphae. B. Skeletal hyphae. C. Simple septate generative hyphae. D. Clamped generative hyphae (Ryvarden and Johansen 1980).

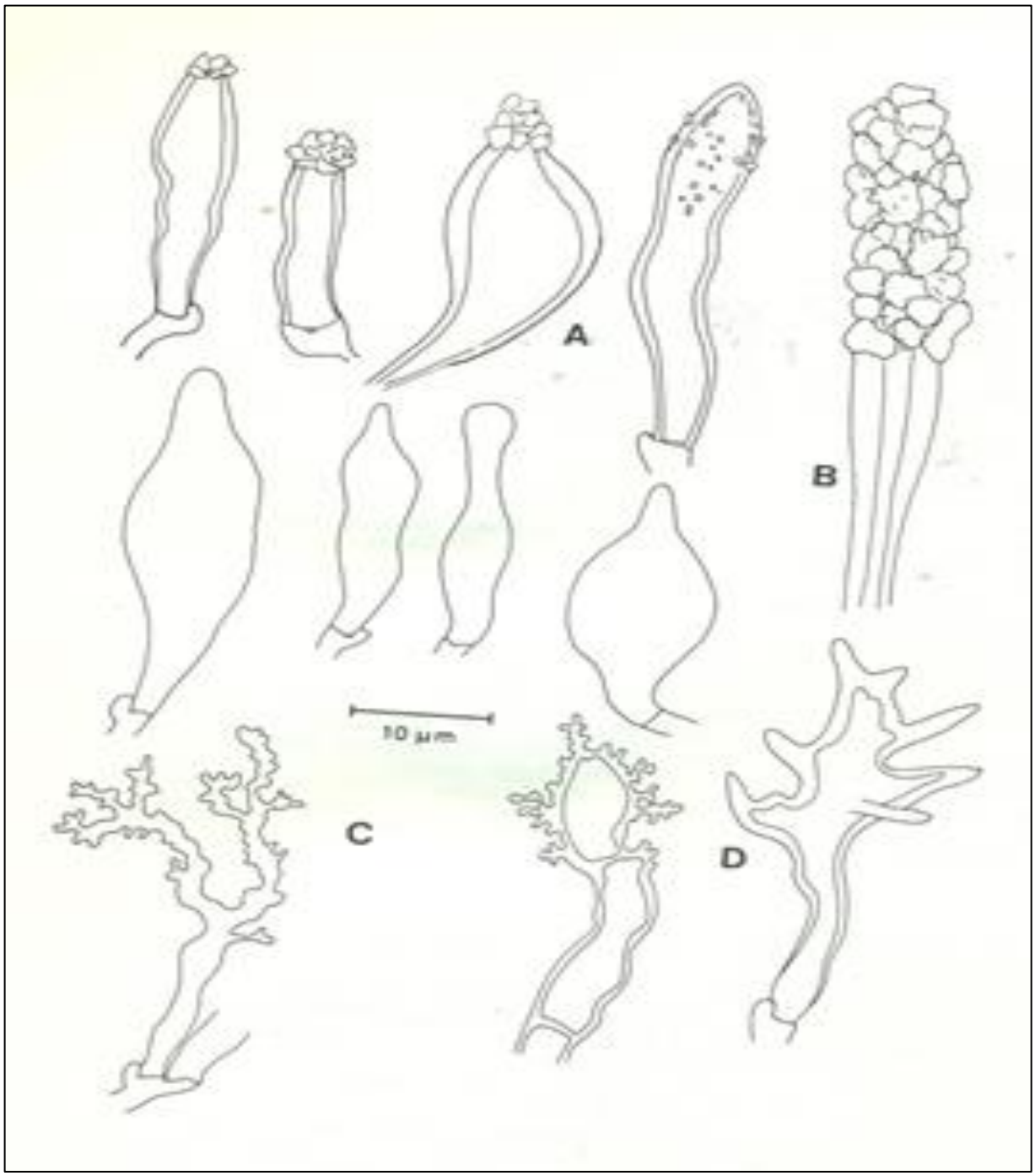

Figure 7. Types of cystidia. A. Apically encrusted cystidia. B. Coarsely encrusted. C. Thin-walled smooth cystidia. D. Dendrohyphidium (Ryvarden and Johansen 1980).

CHAPTER 2

WOOD-ROTTING BASIDIOMYCETES ASSOCIATED WITH DECLINING NATIVE TREES IN TIMBER-HARVESTING COMPARTMENTS OF THE GARDEN ROUTE NATIONAL PARK OF SOUTH AFRICA

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ABSTRACT

Trees in the Garden Route National Park (GRNP) indigenous forests in South Africa are selectively harvested for timber based on criteria that include signs and symptoms induced by wood-rotting fungi. However, virtually nothing is known regarding the identity and host associations of these macro-fungi in this natural ecosystem. Surveys were conducted in three harvesting compartments in the GRNP to investigate the taxonomic affiliation and species richness of these fungi on standing and recently harvested trees. Samples were collected from basidiomes on infected trees and tree stumps, and from diseased tissues on symptomatic trees. Phylogenetic analyses using ITS sequences characterized the isolates obtained into 26 Operational Taxonomic Units (OTUs) belonging to 17 genera after clustering the sequences at a 97% identity threshold. *Ganoderma* (Ganodermataceae) and *Inonotus* (Hymenochaetaceae) were the most species-rich genera and the Bloukrans compartment, with 22 OTUs, showed the highest species richness. A fungus (OTU1) affiliated with Ganoderma pfeifferi was the most abundant in the surveyed areas. Its predominance was also evidenced on host trees since it occurred on 15 of the 20 tree species sampled, with *Olea capensis* subsp. *macrocarpa* (Oleaceae) being the most colonized host. Given the wide variety of wood-rotting basidiomycetes revealed by this study and particularly the preponderance of species with pathogenic potential, more attention should be given to better understand their ecological role in this natural ecosystem as well as the effects of logging that may enhance their dissemination or negatively affect their diversity and the health of trees in the region.

2.1. INTRODUCTION

Wood-rotting fungi play multiple beneficial roles in natural ecosystems. Native wood-rotters in their natural environment play a crucial role in nutrient recycling as primary decomposers of dead plant materials arising from interspecific competition and succession (Schmidt 2006; Boddy et al. 2008). Some, however, pose a threat to the health and sustainability of natural forest ecosystems and are the major causes of wood-rot diseases on live trees (Castello et al. 1995; Gilbert 2002). They are capable of parasitizing and killing live young and mature trees (Hansen and Goheen 2000; Garbelotto 2004). This usually occurs as a result of the disruption of the equilibrium between hosts and their co-evolving fungi in these ecosystems.

In managed forests, for example factors such as fire, forest management activities, including logging, pruning and/or thinning, as well as other stand manipulations are factors that alter forest ecosystems and create suitable conditions that favour the pathogenic effect of these fungi (Goheen and Otrosina 1998; Garbelotto 2004; Gregory et al. 2010; Garbelotto and Gonthier 2013; Warren et al. 2013). Wood-rotting fungi can also become pathogenic when non-native species are introduced into new environments, or when genotypes become hypervirulent by acquiring virulence factors through horizontal gene transfer and/or through hybridization with indigenous species (Gonthier et al. 2007; Ghelardini et al. 2016).

Most wood-rotting fungi are Basidiomycetes. Well-known genera include *Armillaria* (Fr.) Staude, *Ganoderma* P. Karst., *Heterobasidium* Massee and *Phellinus* Quél. Species in these genera are important pathogens of a wide range of hosts including ornamental and agricultural crops as well as plantation and native forest trees (Shaw and Kile 1991; Goheen and Otrosina 1998; Coetzee et al. 2001; Bendz-Hellgren and Stenlid 1997; Rajchenberg and Robledo 2013;

Coetzee et al. 2015). Their pathogenic effect can lead to significant economic losses to timber industries due to decline in the value and volume of commercial wood, as well as to drastic shifts in the composition and structure of natural forest communities (Holah et al. 1993, 1997; Castello et al. 1995).

Wounds on trees, such as those from broken branches, falling trees, exposed upper surfaces of stumps, as well as wounds caused by wildlife damaging the bark, represent ideal entry points through which wood-rotting fungi can infect and colonize their hosts. This usually occurs through airborne dispersion of basidiospores (Nordén and Larsson 2000; Garbelotto 2004; Shortle and Dudzik 2012). However, some root-rotting species, for example *Armillaria* species, are equipped with specialized structures known as rhizomorphs and are capable of directly infecting unwounded trees (Shaw and Kile 1991). Dissemination of wood-rotting basidiomycetes can also occur through root contacts or grafts (Garbelotto et al. 1997), mycelium growing on the soil surface or from previously infected substrates (Fricker et al. 2008), or via contaminated plant material (Coetzee et al. 2001) and insects (Persson et al. 2011).

The infection cycle of Basidiomycete fungi usually begins with germination and infiltration of basidiospores into the host tissues, followed by growth and establishment of hyphal networks, which gradually colonize and digest the tree's woody tissues (Luley 2005; Schwarze 2007; Shortle and Dudzik 2012). Infected trees exhibit symptoms such as loss of crown, bark exudations (bleeding/resinosis and gummosis), dieback, wood discoloration, loss of consistency and vigour, as well as decay of almost all woody parts including roots, butts, stems and branches. Signs associated with decay include the presence of white mycelial mats beneath the bark of trees and in some instances the formation of basidiomes (Morrison et al. 1991; Goheen and Otrosina 1998; Garbelotto 2004).

African forests are under tremendous pressure due to deforestation, debarking of trees and timber harvesting. These are known activities that can promote the spread and infection of trees by wood-rotting Basidiomycetes (Morrison et al. 2001). Despite the abundant knowledge of the different ecological functions that these macro-fungi can play in forest ecosystems (Castello et al. 1995; Gilbert 2002), information pertaining to their occurrence in native African forests remains poorly documented (Ryvarden and Johansen 1980; Douanla-Meli 2007).

In South Africa, one of the largest sections of indigenous forests occurs in the Garden Route National Park (GRNP) area in the Southern Cape region in the Western and Eastern Cape Provinces and is managed by the South African National Parks (SANParks). These forests belong to the Southern Cape Afrotemperate Forest Type, cover an area of approx. 35 765 ha, and are composed of a wide variety of indigenous trees. Some of the major canopy species include *Apodytes dimidiata* E. Mey. ex Arn., *Curtisia dentata* C. A. Sm., *Ocotea bullata* E. Mey., *Olea capensis* subsp. *macrocarpa* (C. H. Wright) I. Verd. and *Podocarpus falcatus* (Thunb.) R. Br. ex Mirb., to name but a few (Geldenhuys 1991; Seydack et al. 1995; Von Maltitz et al. 2003; Durrheim 2006; SANParks 2014).

The GRNP native forests are subdivided into management classes in which specific activities are carried out, including nature reserves, recreation, research and timber utilization (Seydack et al. 1995). In the management class allocated for timber utilization, native trees are selectively harvested for timber based on criteria that include signs and symptoms induced by wood-rotting fungi (Seydack et al. 1995). However, despite the abundant evidence of the presence of these macro-fungi, such as basidiomes reminiscent of *Ganoderma* and Hymenochaetaceae species (Roux et al. 2013), growing on stumps, stems, trunks and roots of declining as well as healthy looking trees, no detailed study has been undertaken to examine their identity and host

associations in this natural ecosystem. Thus, the aim of this study was to produce base-line information regarding the identity and species richness of the basidiomycetous wood-rotting fungi associated with trees showing wood-rot symptoms in timber-harvesting compartments of the GRNP indigenous forests, to contribute to the ecological knowledge and management of this natural forest ecosystem. This was done by examining the isolation frequency of these macro-fungi at the level of forest compartments, tree species and their status, as well as the material from which the fungi were isolated. The effect of these parameters was also examined in relation to the most abundant fungus.

2.2. MATERIALS AND METHODS

2.2.1. Study area

This study was conducted over a 2-year period (2014–2015) in the GRNP indigenous forests in South Africa (Figure 1). Also referred to as the Knysna and/or Tsitsikamma forest, the native forests of the Garden Route extend from the coastal plateau to the foothills of the Outeniqua and Tsitsikamma mountains. The elevation above sea level fluctuates between 200 and 500 m, and the climatic conditions are characterized by a humid to sub-humid and warm temperate climate, with precipitation ranging from 500 to 1000 mm per year and an average temperature of about 15.6°C (Seydack et al. 1995). The floristic composition broadly consists of patches of multi-species indigenous forests, which in some areas are surrounded by extensive planted areas of non-native *Pinus* and/or Eucalyptus species, and in smaller areas with naturalizing stands of the invasive *Acacia melanoxylon*. Natural fire prone Fynbos shrublands are also present (Geldenhuys 1991).

Sampling of wood-rotting Basidiomycetes was carried out in three timber-harvesting compartments located, respectively, in the forest areas known as Bloukrans (S33°56.7470 E23°35.2200), Diepwalle (S33°57.1680 E23°10.6800) and Gouna (S33°56.1630 E23°03.4510) (Figure 1). These three compartments were those designated by SANParks for timber-harvesting during the aforementioned period of this study. Harvesting in a compartment occurs only once every 10 years.

2.2.2. Wood-rotting basidiomycete assessment and collection

South African National Parks have subdivided timber-harvesting compartments into transects of approx. 20 m wide, separated by 1 m wide parallel cut-lines (Seydack et al. 1995). It was within these pre-established transects that wood-rotting Basidiomycete assessment was carried out. A total of 120 transects were sampled at a rate of 40 per compartment. The length of a transect at Diepwalle and Gouna was about 150–175 m, whereas it ranged between 200 and 250 m at Bloukrans. In each of the three compartments, sampling was mainly focused on freshly cut stumps, standing dead trees and living trees showing wood-rot symptoms such as crown suppression, dieback, exudations from the bark, the presence of basidiomes, white mycelial mats beneath tree bark as well as root, butt or stem rots. When a symptomatic tree was found in a transect, inspection was extended to all trees within a 5–10 m range around it. In addition to the above-mentioned signs and symptoms, other information such as the tree species, the crown class as well as the DBH (Diameter at breast height) for living trees were recorded. Sampling of freshly cut stumps concerned only those with living basidiomes and/or showing heart, butt or root rot, whereas that of standing dead trees only considered those with living basidiomes. Where signs of canker and rot, characterized by bark cracking, gum exudation and sunken lesions were visible on living trees, the epidermis of the bark was removed to expose the leading edge of the

infection (intersection between dead and living bark/wood) and sections collected into paper sampling bags. Similarly, basidiomes were collected in paper bags for culturing.

2.2.3. Fungal isolation and purification

To isolate wood-rotting Basidiomycetes from symptomatic plant tissues and basidiomes, four pieces of approx. 2–3mm^3 each were excised from the leading edges of lesions and from freshly exposed inner hymenium of basidiomes. The pieces from plant tissues were surface sterilized in 7% bleach (NaClO) for ~90 s and then rinsed twice in sterile distilled water, blotted dry on a clean paper towel and inoculated onto a basidiomycete selective medium which consisted of 2% malt extract agar (MEA) [20 g malt extract and 15 g agar, Biolab, Midrand, South Africa] supplemented with benomyl, dichloran and streptomycin (BDS) as described by Worrall (1991). Sections of basidiomes were transferred directly to the Basidiomycete selective medium. Petri dishes containing the isolations were incubated for approx. 7–10 days at room temperature (22–24°C), and sub-culturing was routinely performed until pure colonies were obtained. The appearance of colonies, the presence of sporulation as well as lack of clamp connections were used as criteria to screen out non-basidiomycetous colonies. Pure fungal colonies of the putative basidiomycete fungi were maintained on 2% MEA, and representatives of each fungal isolate were conserved in the fungal collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Basidiomes were dried in an oven at 70°C for about 12 h to preserve them for subsequent morphological studies.

2.2.4. DNA extraction, amplification and sequencing

2.2.4.1. DNA extraction

The mycelium of cultures maintained on 2% MEA was harvested in 2 mL Eppendorf tubes, freeze-dried and ground to a powder using a cell disruptor machine (Retsch Gmbh, Germany). Extraction of the genomic DNA from powdered mycelium was performed following the CTAB protocol as described by Möller et al. (1992). From the extracted genomic DNA, working concentrations for DNA amplification were prepared by adjusting the initial concentrations to 100 ng μL^{-1} using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.2.4.2. DNA amplification

Amplifications from the genomic DNA of each fungal isolate targeted the internal transcribed spacer (ITS) gene region, including the 5.8S subunit of the ribosomal DNA. This was done using primers ITS1 (50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50-TCCTCCGCTTATTGATATGC-30) (White et al. 1990). Polymerase chain reactions (PCRs) were carried out in 25 µL reaction volumes containing 5 µL of 5 x MyTaq^{TM} Reaction Buffer supplied with the enzyme, 0.5 µL (10 mM) of ITS1, 0.5 µL (10 mM) of ITS4, 0.5 µL (2.5 units) of MyTaq^{TM} DNA polymerase (Bioline), 1 µL of genomic DNA and 17.5 µL of SABAX sterile water (Adcock Ingram Ltd, Bryanston, S.A.). Amplification reactions were performed in a thermal cycler (Veriti; Applied Biosystems, Foster City, CA, USA) under the following cycling conditions: a first denaturation step at 96°C for 4 min, followed by 40 cycles at 96, 58 and 72°C, respectively, for 30 s, 30 s and 1 min. The programme ended with a final elongation step at 72°C for 7 min. Prior to sequencing, amplicon sizes were estimated. This was done by staining 3 µL of

PCR products with 1.5 µL of GelRed nucleic acid dye (Biotium Incorporation, USA). The mixture was used to perform electrophoresis on 2% agarose gels along with a DNA molecular ruler (100 bp) (Fermentas O 'Gene Ruler) and visualized under ultraviolet light. PCR products were then purified using G-50 Sephadex (Sigma, Steinheim, Germany) columns as recommended by the manufacturer.

2.2.4.3. DNA sequencing

Sequencing PCRs were performed for forward and reverse primers in 12 µL reaction mixtures composed of 1 µL of either ITS1 or ITS4 (10 mmol L^{-1}), 0.5 µL Big Dye (Perkin-Emmer, Warrington, UK), 3 µL of the purified DNA amplicons, 2.5 µL sequencing buffer and 5 µL SABAX sterile water. The thermal cycling programme consisted of 25 cycles at 96°C for 10 s, 58°C for 10 s and 60°C for 4 min. Sequencing PCR products were purified using the same approach as for the PCR products. DNA sequencing was done on a DNA Analyzer ABI PRISM 3100 (Applied BioSystems) at the sequencing facility of the University of Pretoria.

2.2.5. Processing of sequences and Operational Taxonomic Units delineation

The quality of sequence data was assessed using CLC Main Workbench v.7.6.1 and consensus sequences of each complementary sequence were constructed. The generated consensus sequences were analysed in Mothur v.1.38.0 (Schloss et al. 2009) to delineate the different OTUs (Operational Taxonomic Units). In Mothur, all unique sequences were first identified using the command 'unique.seqs'. Subsequently, pairwise distances between unique sequences were calculated using the 'pairwise.seqs' command with the following parameters calc = eachgap (all gaps are penalized) and countends = F (terminal gaps are ignored). Using the cluster command and the calculated pairwise distances, sequences were then clustered into species-level OTUs at

97% identity cut-off which is regarded as the acceptable similarity threshold for which the number of species of an ITS data set can be reliably estimated (Blaalid et al. 2013). Finally, the 'get.oturep' command was used to determine the abundance of each OTU (=total number of sequences belonging to each OTU) as well as the representative sequence of each OTU.

2.2.6. Definition of OTU taxonomic affiliation

The taxonomic affiliation of the delineated OTUs was inferred using a phylogenetic approach. To do this, the nucleotide Basic Local Alignment Search Tool (BLASTn) in UNITE (https://unite.ut.ee/analysis.php), using the UNITE (fungi) + INSD (=GenBank, EMBL, DDBJ) data-bases was used to query the representative sequence of each OTU. Reference sequences for the phylogenetic analysis were selected among the first 15 hits, which showed the most significant alignment with the queried sequences (Table 1). The data set containing the query sequences, along with their best-aligned reference sequences, was then used to perform multiple sequence alignments using the online programme MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/index.html) and subsequently subjected to Maximum Likelihood (ML) calculations in RaxML (Stamatakis 2006) using raxmlGUI 1.3 (Silvestro and Michalak 2012). Ten runs were performed and 1000 bootstrap replications were used to estimate the levels of confidence at branch nodes with the GTRGAMMAI substitution model obtained from JModeltest v.2.1.7. The phylogenetic tree was rooted with two isolates of *Puccinia psidii* (AB470483 and EF599768) and the resulting tree was visualized with MEGA 5.05 (Tamura et al. 2011).

2.2.7. Statistical analyses

Operational Taxonomic Unit richness was calculated for each sampled tree. To determine whether OTU richness was influenced by the number of samples between compartments,

rarefaction curves were calculated. The 'vegan' (Oksanen et al. 2010) and 'iNEXT' (Chao et al. 2014; Hsieh et al. 2016) packages of the R software (R Core Team 2014) were used to calculate OTU richness and the rarefaction curves respectively. The isolation frequency of the different OTUs was compared between forest compartments, tree species, tree status and material of isolation. The comparisons among isolation frequency of the different OTUs in each group were done by means of a contingency table using Fisher exact test (R Core Team 2014).

The effect of forest compartments, tree species and material of isolation on the presence of the most abundant OTU was analysed with a generalized linear model (GLM). The dependent variable 'most abundant OTU' fitted a binomial distribution. The forest compartments, tree species and material of isolation were included as explanatory variables. The model also included the covariation with tree diameter, crown class (suppressed, intermediate, co-dominant or dominant), bleeding (present or absent), rots (stem, butt or root rot) and basidiocarps (present or absent). Only living trees were included in the analysis. The variables crown thinning and dieback were not included in the analysis because 96% of the trees were affected by crown thinning and dieback with no significant differences between groups. The R software (R Core Team 2014) was used for GLM.

2.3. RESULTS

2.3.1. Wood-rotting basidiomycete collection

In total, 403 isolates of basidiomycete fungi were recovered from an equivalent number of trees (i.e. one isolate per tree) in the three studied compartments. Of these, 306 were obtained directly from basidiomes and 97 from direct isolations from infected woody tissues (Table 2). The symptomatic trees sampled (belonging to 20 different species) showed various signs and

symptoms ranging from sap exudations from the bark (Figure 2a), mycelial mats under the bark (Figure 2b) and basidiomes (Figure 2c). Wood-rots from different parts of the tree including heart, butt, stem and roots were also noted as well as dieback of aerial parts and living trees and/or stumps having wood with a bleached appearance and of a stringy and spongy consistency.

2.3.2. Sequencing and OTU delineation

The ITS 1 and ITS 2 regions, including the 5.8S operon, of all 403 isolates of basidiomycete fungi were successfully amplified using PCR and sequenced. Sequencing yielded 324 unique sequences as identified using the program Mothur. Clustering at a similarity threshold of 97% resulted in the distinction of 26 OTUs (Figure 3).

The phylogenetic tree generated in this study grouped the representative sequence of each OTU, including the reference sequences with the most significant similarity based on BlastN searches in UNITE, into 24 distinct and well-supported phylogenetic groups belonging to 17 genera in 13 families of the Agaricomycetes (Basidiomycota). Only OTUs 9 and 17 did not cluster with any reference sequences despite the fact that the Blast search outputs in the consulted databases suggested their close relatedness with *Gloeostereum incarnatum* for OTU9 and to *Phellinus betulinus* and *Phellinus piceicola* for OUT17 (Figure 4). Twenty-two OTUs showed taxonomic affiliation with identified species; one (OTU12) was affiliated to species identified at the genus level, and one (OTU19) had genetic affinities with unclassified basidiomycete taxa (Figure 4).

The families Ganodermataceae and Hymenochaetaceae emerged as the most dominant taxonomic groups, and *Ganoderma* and *Inonotus*, with, respectively, three and four lineages each, including OTUs 1, 4 and 22 for the former and OTUs 8, 15, 18 and 26 for the latter, represented the genera that contained the largest fraction of detected fungal species (Figure 4).

Fuscoporia had two lineages consisting of OTUs 7 and 21, whereas the remaining genera were represented by a single lineage each (Figure 4). Percentage detection (or occurrence) calculated over the total number of sequences/isolates (i.e. 403), indicated that *Ganoderma* was the most abundant genus with 79%; *Wrightoporia* accounted for 4%, *Fomitiporia* 3%, *Coprinellus* and *Inonotus* with 2% each and the remaining genera represented no more than 1% each.

2.3.3. OTU richness and frequency of isolation

Of the total 26 OTUs obtained, only five, affiliated with *Ganoderma pfeifferi* (OTU1), *Wrightoporia tropicalis* (OTU2), *Fomitiporia mediterranea* (OTU5), *Inonotus rickii* (OTU8) and *Cylindrobasidium* sp. (OTU12) were present in all three compartments (Table 2). Four other taxa comprising species assigned to *Coprinellus micaceus* (OTU6), *Fuscoporia cinchonensis* (OTU7), *Pseudolagarobasidium acaciicola* (OTU11) and OTU17 occurred in two of the compartments. The remaining taxa, representing 17 OTUs, were recovered only once (Table 2). Based on rarefaction curve analyses, OTU richness was significantly higher in the Bloukrans forest compartment than in Gouna (Figure 5, Table 2).

The isolation frequency of the detected OTUs was significantly different between the forest compartments, tree species, the health status of trees, as well as the materials from which the fungi were isolated. The Bloukrans forest compartment, with 209 isolates recorded, represented the site with the highest frequency of isolation of the detected OTUs in comparison with Diepwalle and Gouna ($P < 0.001$, Table 2). *Olea capensis* subsp. *macrocarpa*, with 240 isolates (83, 66 and 91, respectively, at Bloukrans, Diepwalle and Gouna), represented the tree species that harboured a higher number of the recovered OTU isolates in comparison with the other tree species ($P < 0.001$, Table 2). Similarly, a higher number of the OTU isolates was recovered from

living trees (171 isolates) compared to stumps (128 isolates) and standing dead trees (104 isolates) ($P < 0.001$, Table 2). A lower number of isolates (97 isolates) was recovered from infected plant materials compared to basidiomes (306 isolates) ($P < 0.001$, Table 2).

2.3.4. Most abundant OTU

In all groups, the *G. pfeifferi*-like taxon (OTU1) was the most frequently isolated fungus. The GLM focusing only on living trees showed that the presence of this fungus was significantly influenced by the tree species ($P < 0.01$) and the materials of isolation (i.e. basidiomes and infected woody tissues) ($P < 0.01$) (Table 3). The *G. pfeifferi*-like taxon (OTU1) occurred on 15 of the 20 tree species sampled (75%), with *O. capensis* subsp. *macrocarpa* being the most colonized host tree (209 isolates recovered from this host). A much larger proportion of its isolates were obtained from basidiomes (268 isolates), compared to infected woody plant material (35 isolates).

2.4. DISCUSSION

This study provides base-line information about the identity and species richness of the basidiomycetous fungal taxa associated with trees showing wood-rot symptoms in timber-harvesting compartments of the GRNP indigenous forests in South Africa. Twenty-four of the 26 delineated OTUs clustered into well-defined phylogenetic clades with high bootstrap values, except for OTUs 9 and 17. The calculation of bootstrap values in phylogenetic analyses is a useful tool to assess the confidence of a phylogeny (Hillis and Bull 1993); the higher the bootstrap values, the more reliable the phylogenetic clades will be and so the affiliation of taxa. Based on this assumption, it can therefore, be assumed that the affiliation of taxa of wood-rotting basidiomycetes obtained in this study is validated as they formed together with their best-aligned

reference sequences, well-supported phylogenetic clusters with high bootstrap values. The non-clustering of OTUs 9 and 17 with any reference sequences of the known taxa included in the phylogenetic analyses suggests that these taxa might represent novel genus/species that have not yet been documented in any of the fungal databases (UNITE + INSD). However, with regard to OTU17, its placement within the Hymenochaetaceae clade confirmed its taxonomic proximity to species of this family as indicated in the Blast search results, as having affinity to *P. betulinus* and *P. piceicola*.

The fact that Ganodermataceae and Hymenochaetaceae were the taxa with the largest number of fungal species associated with trees showing wood-rot symptoms agrees with the outcome of the exploratory survey by Roux et al. (2013) in the same area, as well as with similar studies that investigated the ecological importance of wood-rotting macro-fungi (Holah et al. 1997; Hansen and Goheen 2000). Indeed, species of these families have been widely reported as causal agents of wood-rot diseases, decay and death of a wide variety of host trees in diverse ecosystems (Robles et al. 2011; Singh et al. 2013; Roccotelli et al. 2014). For instance Gilbert et al. (2002) reported that *Phellinus apiahynus* was the most abundant species on declining *Ocotea whitei* trees in the moist tropical forests of Panama. Robles et al. (2011) found that species of *Ganoderma*, and especially those of *Inonotus*, were widely associated with wood-rot symptoms and biodegradation of *Platanus acerifolia* in urban landscapes in Buenos Aires city (Argentina). Similarly, Singh et al. (2013) listed species of the same genera, and that of *Phellinus*, as causal agents of foliage discoloration, stem bark cracking, bark exudations and dieback of aerial parts of several tree species in the arid zone forests of north-western India. Other studies, including that of Roccotelli et al. (2014), also identified *Phellinus* species and particularly *F. mediterranea*, as the major causal agent of white-rot symptoms on Citrus trees in orchards in Italy. Although most

of these symptoms were observed on trees infested by these fungal species in the GRNP, caution remains the rule as to their cause, at least until pathogenicity trials are carried out, since some of these symptoms can also be triggered by factors such as abiotic stresses.

The physical size of the sampled compartments in this study needs to be considered in the interpretation of the isolation frequency and species richness of the basidiomycetous wood-rotting fungi recorded. As noted by O'Hanlon and Harrington (2012) and Yamashita et al. (2015), the frequency of site visitation, length of visits, as well as physical size of sampling area are among the key factors that may significantly affect macro-fungal species richness. In this study, the three timber-harvesting compartments investigated were visited only once each: June and July 2014 for Diepwalle and Gouna, respectively, and July 2015 for Bloukrans. As a result of this limited number and duration of visits, these two factors (frequency of site visitation and length of visits) are inappropriate for interpreting the variability in the total number of wood-rotting fungi recorded in each of the sampled compartments. However, the actual physical size of the sampled compartments might have had a significant effect on the total species richness found in each of the compartments. Although the width of sampling transects was the same across the sampled compartments, there was variation in their length/depth. This created non-uniformity in terms of total size of the physical areas sampled, which might have affected the sampling intensity and subsequently the species richness of the basidiomycete fungi recorded in the different compartments (transects in Bloukrans were longer than those of Diepwalle and Gouna). It is, therefore, likely that this disparity in transect length might have constituted a bias favouring more sampling effort in the Bloukrans site that resulted in the greater species richness (22 OTUs) recorded in this compartment. Nevertheless, despite longer transect length in Bloukrans, species

rarefaction curves in all three compartments indicated that additional sampling in these compartments are not likely to reveal many more fungal taxa.

The higher number of species obtained in Bloukrans can potentially be explained by logging intensity. Historically, Diepwalle and Gouna compartments were established during the 1930s, earlier than Bloukrans that was established relatively recently between 1988 and 1992 (SANParks 2012, 2014). Apparently, more intensive logging activities have been carried out in Diepwalle and Gouna in the past, due to increased demand for timber. Such practice represents a huge disturbance to forest ecosystems with a direct effect not only on host species diversity, but also on diversity of the fungal communities that are associated with them (Sippola et al. 2001; Hattori 2005; Müller et al. 2007; Hattori et al. 2012; Adarsh et al. 2015). Reports indicate that long-term logging has a marked effect on species richness of wood-rotting macro-fungi due to decreased natural tree falls that leads to less dead trees (especially those with large diameter) that serve as substrates or niches for these fungi (Bader et al. 1995; Hattori and Lee 2003; Müller et al. 2007; Yamashita et al. 2008). This was, for example the case in the Malaysian Pasoh Forest Reserve where intensive logging undertaken in this forest since the 1950s has had a negative impact on the species number of polypore fungi compared with that of an adjacent primary forest that experienced less logging (Hattori and Lee 2003). It thus appears from the foregoing that forests undergoing less logging are likely to display greater species diversity. However, natural decline in the number of tree species also occurs from east to west in the Southern Cape Afrotemperate forests (Von Maltitz et al. 2003; Mucina and Geldenhuys 2006). This could also be a possible explanation to the decrease in the macro- fungal species richness as perceived along this gradient (22 OTUs at Bloukrans in the east vs. nine, respectively, for Diepwalle and Gouna in the west).

The 26 basidiomycetous wood-rotting fungi found in this study were isolated on a wide range of tree species (20 different tree species in total). This is consistent with previous reports, which have shown that wood-inhabiting macro-fungal species richness was positively correlated with host tree species diversity, as high species richness of trees would offer a wide variety of wood substrates for colonization (Ferrer and Gilbert 2003; Yamashita et al. 2008). However, the frequency of isolation of the OTUs was different between the tree species. *Olea capensis* subsp. *macrocarpa* was the most colonized host tree in all three sampled compartments, and it was mostly infested by the *G. pfeifferi* (OTU1)-like taxon, which was also the most dominant fungal species isolated at all three sites. Host trees with high population density are likely to support more specific fungal species of Basidiomycetes especially the most common taxa (Gilbert et al. 2002, 2008; Hattori and Lee 2003). Findings of Gilbert et al. (2008) noted, for example that there were strong host preferences between the most dominant species of polypores and the denser tree species in Mangrove forests in Micronesia. This seems to be the same scenario occurring between *O. capensis* subsp. *macrocarpa* and the *G. pfeifferi* (OTU1)-like taxon in the GRNP indigenous forests. This tree species is one of the common canopy tree species in these forests, which owing to its fast growth rate, represents one of the most commonly exploited trees for timber (Seydack et al. 1995; SANParks 2014). Thus, because of its high population density and the high number of leftover stumps resulting from its logging, this tree species would be predisposed to more infection by several wood-rotting fungi and preferentially by the most dominant species, the *G. pfeifferi* (OTU1)-like taxon. High host density represents ideal conditions for propagation and infection via root contacts for root-rotting species such as *Ganoderma* and leftover stumps constitute for these species, not only more substrates for colonization, but also important inoculum reservoirs for the infection of next generations of

plants (Garbelotto 2004; Glen et al. 2009). Interestingly, another frequently encountered fungus, the *Phellinus merrillii* (OTU3)-like taxon was recovered exclusively on *Elaeodendron croceum* and only in Bloukrans. However, due to the small number of trees (13) on which this fungus was recovered and its site restriction, it is difficult to conclude on its possible host preference or host specificity.

The isolation frequency of the OTUs based on status of host trees indicated that the basidiomycetous fungi detected occurred mostly as parasites rather than saprophytes, as 22 were obtained from living trees against, respectively, 12 and 7 from stumps and standing dead trees. This is probably due to the sampling strategy, which for standing dead trees was limited to collection of basidiocarps as material for fungal isolations, whereas both basidiocarps and infected woody plant samples were recovered as materials for fungal isolations from symptomatic living trees and stumps in some instances. Thus, the use of both sources as isolation material for fungal detection from living trees might have enhanced the detection of more fungal taxa from these trees.

Different studies (Allmér et al. 2006; Rajala et al.2012; Jang et al. 2015) have highlighted the fact that type of material from which fungi are being detected can influence the species richness and diversity of basidiomycete fungi. Often, fungi detected by basidiocarps are seldom recovered by mycelium isolated from infected woody tissues. This is because the different approaches are subject to specific constraints. Jang et al. (2015) noted, for example that basidiomycetes detection based on fruiting body collection could be hampered by the ephemeral character of basidiocarps of some species, which due to high water content persist for a short period compared to those of perennial species and thus may not be present at the time of sampling. Allmér et al. (2006) and Rajala et al. (2012) also argued that sampling size can affect species

richness of basidiomycetes, especially when collection is restricted to basidiocarps since bigger sampling size will lead to the detection of several more taxa. For macro-fungi revealed by mycelium isolated from infected woody tissues, Taylor (2002) indicated that the number of species of a particular sample is based on the size of the sample, the abundance of species in the sample and the distribution of these species in the substrate where the sample was originally collected. Another constraint related to this approach may also be the selective medium used for fungal isolation, because, even though benomyl is effective in inhibiting the growth of a large range of fungi other than basidiomycetes, some basidiomycetous species may be sensitive to it and their growth can be inhibited (Jang et al. 2015).

This study has revealed high species diversity of wood-rotting basidiomycetes occurring on declining native trees in timber-harvesting compartments of the GRNP indigenous forests in South Africa. It has also shown that species richness of the basidiomycetous fungal taxa recovered was not evenly distributed across the sampled compartments, with significantly higher species richness recorded in the Bloukrans compartment that had undergone less long-term logging. Although pathogenicity trials were not con- ducted in the course of this study, nearly all the trees that showed wood-rot symptoms were predominantly associated with basidiomycetous wood-rotting species with pathogenic potential, particularly species of *Ganoderma* and Hymenochaetaceae. Thus, given the wide variety of wood-rotting basidiomycetes revealed by this study in the harvesting compartments, and particularly the preponderance of species with pathogenic potential, more attention should be given to better understand their ecological role in this natural ecosystem as well as the effect of logging that may enhance their dissemination or negatively affect their diversity and the health of trees in the region.

2.5. REFERENCES

Adarsh CK, Kumar V, Vidyasagaran K, 2015. Decomposition of wood by polypore fungi in Tropics – biological, ecological and environmental factors – a case study. *Research Journal of Agriculture and Forestry Sciences* 3, 15–37.

Allmér J, Vasiliauskas R, Ihrmark K, Stenlid J, Dahlberg A, 2006. Wood-inhabiting fungal communities in woody debris of Norway spruce (Picea abies (L.) Karst.), as reflected by sporocarps, mycelial isolations and T-RFLP identification. *FEMS Microbiology Ecology* 55, 57–67.

Bader P, Jansson S, Jonsson BG, 1995. Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests. *Biological Conservation* 72, 355–62.

Bendz-Hellgren M, Stenlid J, 1997. Decreased volume growth of *Picea abies* in response to *Heterobasidion annosum* infection. *Canadian Journal of Forest Research* 27, 1519–24.

Blaalid R, Kumar S, Nilsson RH, Abarenkov K, Kirk PM, Kauserud H, 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources* 13, 218–24.

Boddy L, Frankland JC, van West P, 2008. Ecology of Saprotrophic Basidiomycetes. Academic Press/Elsevier, London.

Castello JD, Leopold DJ, Smallidge PJ, 1995. Pathogens, patterns, and processes in forest ecosystems. *Bioscience* 45, 16–24.

Chao A, Gotelli NJ, Hsieh TC et al., 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs* 84, 45–67.

Coetzee MPA, Wingfield BD, Harrington TC, Steimel J, Coutinho TA, Wingfield MJ, 2001. The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* 10, 387–96.

Coetzee MPA, Marincowitz S, Muthelo VG, Wingfield MJ, 2015. *Ganoderma* species, including new taxa associated with root rot of the iconic *Jacaranda mimosifolia* in Pretoria, South Africa. *IMA Fungus* 6, 249–56.

Douanla-Meli C, 2007. Fungi of Cameroon. Ecological diversity with emphasis on the taxonomy of non-gilled Hymenomycetes from the Mbalmayo Forest Reserve. *Bibliotheca Mycologica* 202, 1–410.

Durrheim GP, 2006. Regional indigenous forest management plan for the Southern Cape and Tsitsikamma: a summary. South African National Parks, Knysna, 1–17.

Ferrer A, Gilbert GS, 2003. Effect of tree host species on fungal community composition in a tropical rain forest in Panama. *Diversity and Distributions* 9, 455–68.

Fricker MD, Bebber D, Boddy L, 2008. Mycelia networks: structure and dynamics. In: Ecology of Saprotrophic Basidiomycetes (eds L. Boddy, J. C. Frankland & P. van West) pp. 3–18. Academic Press/Elsevier, London.

Garbelotto M, 2004. Root and butt rot diseases. In: Encyclopedia of Forest Sciences, Vol. 2 (eds J. Burley, J. Evans & J. A. Youngquist) pp. 750–8. Elsevier, Oxford.

Garbelotto M, Gonthier P, 2013. Biology, epidemiology and control of *Heterobasidion* species worldwide. *Annual Review of Phytopathology* 51, 39–59.

Garbelotto M, Slaughter G, Popenuck T, Cobb FW, Bruns TD, 1997. Secondary spread of *Heterobasidion annosum* in white fir root-disease centers. *Canadian Journal of Forest Research* 27, 766–73.

Geldenhuys CJ, 1991. Distribution, size and ownership of forests in the southern Cape. *South African Forestry Journal* 158, 51–66.

Ghelardini L, Pepori AL, Luchi N, Capretti P, Santini A, 2016. Drivers of emerging fungal diseases of forest trees. *Forest Ecology and Management* 381, 235–46.

Gilbert GS, 2002. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* 40, 13–43.

Gilbert GS, Ferrer A, Carranza J, 2002. Polypore fungal diversity and host density in a moist tropical forest. *Biodiversity and Conservation* 11, 947–57.

Gilbert GS, Gorospe J, Ryvarden L, 2008. Host and habitat preferences of polypore fungi in Micronesian tropical flooded forests. *Mycological Research* 112, 674–80.

Glen M, Bougher NL, Francis AA et al., 2009. *Ganoderma* and *Amauroderma* species associated with root- rot disease of *Acacia mangium* plantation trees in Indonesia and Malaysia. *Australasian Plant Pathology* 38, 345–56.

Goheen DJ, Otrosina WJ, 1998. Characteristics and consequences of root diseases in forests of western North America. In: User's Guide to the Western Root Disease Model, Version 3.0 (ed. S. Frankel) pp. 3–8. USDA, Albany, CA.

Gonthier P, Nicolotti G, Linzer R, Guglielmo F, Garbelotto M, 2007. Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. *Molecular Ecology* 16, 1389–400.

Gregory M, Maffei HM, Chadwick K, Max TA, 2010. Armillaria root disease-caused tree mortality following silvicultural treatments (shelterwood or group selection) in an Oregon mixed-conifer forest: insights from a 10-year case study. *Western Journal of Applied Forestry* 25, 136–43.

Hansen EM, Goheen EM, 2000. *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in western North America. *Annual Review of Phytopathology* 38, 515–39.

Hattori T, 2005. Diversity of wood-inhabiting polypores in temperate forests with different vegetation types in Japan. *Fungal Diversity* 18, 73–88.

Hattori T, Lee SS, 2003. Community structure of wood- decaying Basidiomycetes in Pasoh. In: Pasoh: Ecology of a Lowland Rain Forest in Southeast Asia (eds T. Okuda, N. Manokaran, Y. Matsumoto, K. Niiyama, S. C. Thomas & P. S. Ashton) pp. 161–70. Springer, Tokyo.

Hattori T, Yamashita S, Lee SS, 2012. Diversity and conservation of wood-inhabiting polypores and other aphyllophoraceous fungi in Malaysia. *Biodiversity and Conservation* 21, 2375–96.

Hillis DM, Bull JJ, 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42, 182–92.

Holah JC, Wilson MV, Hansen EM, 1993. Effects of a native forest pathogen, *Phellinus weirii*, on Douglas-fir forest composition in western Oregon. *Canadian Journal of Forest Research* 23, 2473–80.

Holah JC, Wilson MV, Hansen EM, 1997. Impacts of a native root-rotting pathogen on successional development of old-growth Douglas fir forests. *Oecologia* 111, 429–33.

Hsieh TC, Ma KH, Chao A, 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution* 7, 1451–156.

Jang Y, Jang S, Min M et al., 2015. Comparison of the diversity of basidiomycetes from dead wood of the Manchurian fir(*Abies holophylla*) as evaluated by fruiting body collection, mycelial isolation, and 454 sequencing. *Microbial Ecology* 70, 634–45.

Luley CJ, 2005. Basidiomycetes wood decay life cycle. [Cited 24 May 2015.] Available from URL: http://www. mwcog.org/uploads/committee-documents/a15bX1d Z20090608122014.pdf

Möller EM, Bahnweg G, Sandermann H, Geiger HH, 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 20, 6115–6.

Morrison DJ, Merler H, Norris DJ, 1991. Detection, recognition and management of *Armillaria* and *Phellinus* root diseases in the southern interior of British Columbia. FRDA Report 179. 25 p.

Morrison DJ, Pellow KW, Nemec AFL, Norris DJ, Semenoff P, 2001. Effects of selective cutting on the epidemiology of Armillaria root disease in the southern interior of British Columbia. *Canadian Journal of Forest Research* 31, 50–70.

Mucina L, Geldenhuys CJ, 2006. Afrotemperate, subtropical and azonal forests. In: The Vegetation of South Africa, Lesotho and Swaziland (eds L. Mucina & M. C. Rutherford). Strelitzia 19, pp. 584–614. South African National Biodiversity Institute, Pretoria.

Müller J, Hothorn T, Pretzsch H, 2007. Long-term effects of logging intensity on structures, birds, saproxylic beetles and wood-inhabiting fungi in stands of European beech *Fagus sylvatica* L. *Forest Ecology and Management* 242, 297–05.

Nordén B, Larsson KH, 2000. Basidiospore dispersal in the old-growth forest fungus *Phlebia centrifuga* (Basidiomycetes). *Nordic Journal of Botany* 20, 215–9.

O'Hanlon R, Harrington TJ, 2012. The macrofungal diversity and community of Atlantic oak (Quercus petraea and Q. robur) forests in Ireland. *Anales del Jardín Botánico de Madrid* 69, 107–17.

Oksanen J, Guillaume Blanchet F, Kindt R et al., 2010. Vegan: Community Ecology Package. R package version 1.17-4.

Persson Y, Ihrmark K, Stenlid J, 2011. Do bark beetles facilitate the establishment of rot fungi in Norway spruce? *Fungal Ecology* 4, 262–9.

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Rajala T, Peltoniemi M, Pennanen T, Mäkipää R, 2012. Fungal community dynamics in relation to substrate quality of decaying Norway spruce (*Picea abies* [L.] Karst.) logs in boreal forests. *FEMS Microbiology Ecology* 81, 494–05.

Rajchenberg M, Robledo G, 2013. Pathogenic polypores in Argentina. *Forest Pathology* 43, 171–84.

Robles CA, Carmaráan CC, Lopez SE, 2011. Screening of xylophagous fungi associated with *Platanus acerifolia* in urban landscapes: biodiversity and potential biodeterioration. *Landscape and Urban Planning* 100, 129–35.

Roccotelli A, Schena L, Sanzani SM et al., 2014. Characterization of basidiomycetes associated with wood rot of citrus in southern Italy. *Phytopathology* 104, 851–8.

Roux J, Wingfield MJ, Coetzee MPA, 2013. Exploratory studies of *Ganoderma* species in the Garden Route of South Africa. Proceedings of the 10th International Congress of Plant Pathology; 25–30 Aug 2013, Beijing, China.

Ryvarden L, Johansen I, 1980. A Preliminary Polypore Flora of East Africa, pp. 636. *Fungiflora*, Oslo.

SANParks, 2012. Garden Route Park management plan. [Cited 12 October 2016.] Available from URL: http:// www.sanparks.co.za/assets/docs/conservation/park_ma n/grnp-approved-plan.pdf. Accessed 12 October 2016.

SANParks, 2014. Monitoring for sustainable indigenous forest management in the Garden Route National Park. [Cited 12 October 2016.] Available from URL: https://www.sanpa rks.org/assets/docs/parks_grnp/about/monitoring-summary. pdf. Accessed 12 October 2016.

Schloss PD, Westcott SL, Ryabin T et al., 2009. Introducing Mothur: open-source, platform-independent, community- supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75, 7537–41.

Schmidt O, 2006. Wood and tree fungi biology, damage, protection, and use. In: Wood and tree fungi biology, damage, protection, and use (eds D. D. Czeschlik & A. Schlitzberger) pp. 237–49. Springer, Heidelberg.

Schwarze FWMR, 2007. Wood decay under the microscope. *Fungal Biology Reviews* 21, 133–70.

Seydack AHW, Vermeulen WJ, Heyns HE et al., 1995. An unconventional approach to timber yield regulation for multi-aged, multispecies forests. II. Application to a South African forest. *Forest Ecology and Management* 77, 155–68.

Shaw CG, Kile GA, 1991. Armillaria Root Disease. Agriculture Handbook No. 691. Forest Service, United States Department of Agriculture, Washington, DC.

Shortle WC, Dudzik KR, 2012. Wood decay in living and dead trees: a pictorial overview. Gen. Tech Rep. NRS-97. U.S. Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA, 26 p.

Silvestro D, Michalak I, 2012. RaxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12, 335–7.

Singh SK, Doshi A, Pancholy A, Pathak R, 2013. Biodiversity in wood-decay macro-fungi associated with declining arid zone trees of India as revealed by nuclear rDNA analysis. *European Journal of Plant Pathology* 136, 373–82.

Sippola AL, Lehesvirta T, Renvall P, 2001. Effects of selective logging on coarse woody debris and diversity of wood-decaying polypores in eastern Finland. *Ecological bulletins* 49, 243–54.

Stamatakis A, 2006. RAxML-VI-HPC: maximum likelihood- based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–90.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–9.

Taylor AFS, 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. *Plant Soil* 244, 19–28.

Von Maltitz G, Mucina L, Geldenhuys CJ et al. 2003. Classification system for South African indigenous forests: an objective classification for the Department of Water Affairs and Forestry. *Environmentek Rep. ENV-PC* 17, 1–284.

Warren G, Baines P, Plamondon J, Pitt DG, 2013. Effects of precommercial thinning on the forest value chain in northwestern New Brunswick: Part 3 – incidence of root and butt decay. *The Forestry Chronicle* 89, 464–73.

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications (eds M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White) pp. 315–22. Academic Press, New York.

Worrall JJ, 1991. Media for selective isolation of hymenomycetes. *Mycologia* 83, 296–02.

Yamashita S, Hattori T, Momose K, Nakagawa M, Aiba M, Nakashizuka T, 2008. Effects of forest use on aphyllophoraceous fungal community structure in Sarawak, Malaysia. *Biotropica* 40, 354–62.

Yamashita S, Hattori T, Lee SS, Okabe K, 2015. Estimating the diversity of wood-decaying polypores in tropical lowland rain forests in Malaysia: the effect of sampling strategy. *Biodiversity and Conservation* 24, 393–06.

Table 1. List of the reference sequences from Blast searches in UNITE which showed the most significant alignment with the representative sequence of each OTU recovered in the GRNP

Closest Blast match taxa	MycoBank classification		Accession number	Origin	Host
	Genus	Family			
Agaricales sp.	Unspecified	Unspecified	KU530334	Mexico	Unspecified
Basidiomycete sp.	Unspecified	Unspecified	AY781276	Sweden	*Picea abies*
Basidiomycota sp.	Unspecified	Unspecified	KJ713986	South Korea	Unspecified
Coprinellus micaceus	*Coprinellus*	Psathyrellaceae	FJ850971	Argentina	Unspecified
Coprinellus micaceus	*Coprinellus*	Psathyrellaceae	FJ850970	Argentina	Unspecified
Coprinellus sp.	*Coprinellus*	Psathyrellaceae	FJ571499	unspecified	Unspecified
Cylindrobasidium sp.	*Cylindrobasidium*	Corticiaceae	JQ654101	New Zealand	*Pinus radiata*
Cylindrobasidium sp.	*Cylindrobasidium*	Corticiaceae	KT201653	New Zealand	unspecified
Cystidiodontia laminifera	*Cystidiodontia*	Cystostereaceae	EU118622	Costa Rica	Unspecified
Cystidiodontia sp.	*Cystidiodontia*	Cystostereaceae	JN198404	Unspecified	Unspecified
Fistulina hepatica	*Fistulina*	Fistulinaceae	AY571038	USA	Unspecified
Fistulina hepatica	*Fistulina*	Fistulinaceae	LN714544	Slovakia	Unspecified
Fomitiporia mediterranea	*Fomitiporia*	Hymenochaetaceae	AY849303	Italy	*Platanus x acerifolia*
Fomitiporia mediterranea	*Fomitiporia*	Hymenochaetaceae	AY849304	Italy	*Platanus x acerifolia*
Fulvifomes sp.	*Fulvifomes*	Hymenochaetaceae	JX104709	Unspecified	*Xylocarpus granatum*
Fulvifomes sp.	*Fulvifomes*	Hymenochaetaceae	JX104710	Unspecified	*Xylocarpus granatum*
Fuscoporia cinchonensis	*Fuscoporia*	Hymenochaetaceae	AY558613	unspecified	Unspecified
Fuscoporia gilva	*Fuscoporia*	Hymenochaetaceae	KU139196	USA	*Acer saccharum*
Fuscoporia gilva	*Fuscoporia*	Hymenochaetaceae	KU139195	USA	*Acer saccharum*
Fuscoporia sp.	*Fuscoporia*	Hymenochaetaceae	KJ677113	Russia	Unspecified
Fuscoporia sp.	*Fuscoporia*	Hymenochaetaceae	KJ677115	Russia	Unspecified

Table 1. (continued)

Closest Blast match taxa	MycoBank classification		Accession number	Origin	Host
	Genus	Family			
Ganoderma applanatum	*Ganoderma*	Ganodermataceae	AJ608709	Australia	*Acacia mangium*
Ganoderma australe	*Ganoderma*	Ganodermataceae	AY884180	UK	Unspecified
Ganoderma australe	*Ganoderma*	Ganodermataceae	KF605665	unspecified	Unspecified
Ganoderma cupreum	*Ganoderma*	Ganodermataceae	JN105701	Cameroon	*Cassia* sp.
Ganoderma cupreum	*Ganoderma*	Ganodermataceae	KX055560	Unspecified	Unspecified
Ganoderma fornicatum	*Ganoderma*	Ganodermataceae	JX840347	China	Unspecified
Ganoderma fornicatum	*Ganoderma*	Ganodermataceae	JX840348	China	Unspecified
Ganoderma mutabile	*Ganoderma*	Ganodermataceae	JN383977	China	Unspecified
Ganoderma pfeifferi	*Ganoderma*	Ganodermataceae	AY884181	UK	Unspecified
Ganoderma pfeifferi	*Ganoderma*	Ganodermataceae	AM906059	Czech Republic	*Fagus sylvatica*
Hymenochaetales sp.	Unspecified	Hymenochaetaceae	JQ038903	South Africa	grapevine
Hymenochaetales sp.	Unspecified	Hymenochaetaceae	JQ038904	South Africa	grapevine
Inonotus linteus	*Inonotus*	Hymenochaetaceae	JX985738	China	*Xylosoma* sp.
Inonotus linteus	*Inonotus*	Hymenochaetaceae	JX985739	Ethiopia	Unspecified
Inonotus rickii	*Inonotus*	Hymenochaetaceae	FJ667753	China	*Hevea brasiliensis*
Inonotus rickii	*Inonotus*	Hymenochaetaceae	HM362905	Spain	Unspecified
Inonotus setuloso-croceus	*Inonotus*	Hymenochaetaceae	KP279292	South Africa	Unspecified
Inonotus setuloso-croceus	*Inonotus*	Hymenochaetaceae	KP279293	South Africa	Unspecified
Inonotus sp.	*Inonotus*	Hymenochaetaceae	KR002878	India	Unspecified
Inonotus sp.	*Inonotus*	Hymenochaetaceae	KT800054	Unspecified	Unspecified
Inonotus sp.	*Inonotus*	Hymenochaetaceae	JF895464	Ethiopia	Unspecified
Inonotus sp.	*Inonotus*	Hymenochaetaceae	JF895465	Ethiopia	Unspecified

Table 1. (continued)

Closest Blast match taxa	MycoBank classification		Accession number	Origin	Host
	Genus	Family			
Inonotus tropicalis	*Inonotus*	Hymenochaetaceae	KP307009	Unspecified	Unspecified
Inonotus tropicalis	*Inonotus*	Hymenochaetaceae	AY599487	Unspecified	Unspecified
Oudemansiella canarii	*Oudemansiella*	Physalacriaceae	GQ892789	Argentina	Unspecified
Oudemansiella canarii	*Oudemansiella*	Physalacriaceae	AY216473	Unspecified	Unspecified
Oudemansiella cubensis	*Oudemansiella*	Physalacriaceae	KU170955	Guyana	Unspecified
Peniophorella pubera	*Peniophorella*	Corticiaceae	KP768315	USA	*Picea mariana* wood
Peniophorella pubera	*Peniophorella*	Corticiaceae	KP814549	USA	Decayed wood
Phellinus betulinus	*Phellinus*	Hymenochaetaceae	KU139154	USA	*Betula alleghaniensis*
Phellinus betulinus	*Phellinus*	Hymenochaetaceae	KU139152	USA	*Betula papyrifera*
Phellinus merrillii	*Phellinus*	Hymenochaetaceae	EU035313	Unspecified	Unspecified
Phellinus merrillii	*Phellinus*	Hymenochaetaceae	EU035311	Unspecified	Unspecified
Phellinus piceicola	*Phellinus*	Hymenochaetaceae	JQ828910	China	Unspecified
Phellinus piceicola	*Phellinus*	Hymenochaetaceae	JQ828909	China	Unspecified
Pseudolagarobasidium acaciicola	*Pseudolagarobasidium*	Phanerochaetaceae	DQ517883	South Africa	*Acacia cyclops*
Pseudolagarobasidium acaciicola	*Pseudolagarobasidium*	Phanerochaetaceae	DQ517882	South Africa	*Acacia cyclops*
Pseudolagarobasidium sp.	*Pseudolagarobasidium*	Phanerochaetaceae	KM053237	India	Mango industrial waste
Punctularia subhepatica	*Punctularia*	Punctulariaceae	KP814559	USA	Decayed wood
Sistotrema brinkmannii	*Sistotrema*	Corticiaceae	KM232461	Unspecified	Unspecified
Sistotrema brinkmannii	*Sistotrema*	Corticiaceae	KF218967	Unspecified	Unspecified
Stereum hirsutum	*Stereum*	Stereaceae	KR909200	Unspecified	grapevine
Stereum hirsutum	*Stereum*	Stereaceae	LN714607	Czech Republic	Unspecified
Trametes hirsuta	*Trametes*	Polyporaceae	KF513163	China	Unspecified
Trametes versicolor	*Trametes*	Polyporaceae	KF054739	Unspecified	Bamboo

Table 1. (continued)

Closest Blast match taxa	MycoBank classification		Accession number	Origin	Host
	Genus	Family			
Trametes villosa	*Trametes*	Polyporaceae	KF850163	Unspecified	Unspecified
Trametes villosa	*Trametes*	Polyporaceae	KF850162	Unspecified	Unspecified
uncultured Agaricales	Unspecified	Unspecified	KU175685	Mexico	Unspecified
Wrightoporia tropicalis	*Wrightoporia*	Wrightoporiaceae	FJ904857	Kenya	*Grevillea robusta*
Wrightoporia tropicalis	*Wrightoporia*	Wrightoporiaceae	KJ807072	Unspecified	Unspecified
†*Puccinia psidii*	*Puccinia*	Pucciniaceae	AB470483	Japan	Unspecified
†*Puccinia psidii*	*Puccinia*	Pucciniaceae	EF599768	Hawaii	Unspecified

†, outgroup species.

Table 2. Contingency table showing OTU richness based on tree status, material of isolation, forest compartments and host trees

		Operational Taxonomic Units (OTUs) and their frequencies of isolation by categories						
		OTU1	OTU2	OTU3	OTU4	OTU5	OTU6	OTU7
Tree status	Alive	106	10	11	3	6	7	1
	Dead standing	93	-	1	2	3	-	3
	Stump	104	5	1	6	2	1	1
Material of isolation	Basidiocarp	268	-	12	11	4	-	4
	Infected wood	35	15	1	-	7	8	1
Compartments	Diepwalle	65	3	-	-	2	4	-
	Gouna	98	3	-	-	2	-	2
	Bloukrans	140	9	13	11	7	4	3
Tree species	*Acacia melanoxylon*	2	-	-	3	-	-	-
	Apodytes dimidiata subsp.*dimidiata*	15	6	-	-	-	1	-
	Canthium mundianum	-	-	-	-	-	1	-
	Cunonia capensis	6	1	-	-	-	1	1
	Elaeodendron croceum	8	-	13	-	-	-	-
	Gonioma kamassi	3	-	-	-	-	-	-
	Halleria lucida	2	-	-	-	-	-	-
	Ilex mitis	-	-	-	-	-	1	-
	Maytenus peduncularis	2	-	-	-	-	-	-
	Nuxia floribunda	1	-	-	-	-	-	-
	Ocotea bullata	2	-	-	1	-	-	-
	Olea capensis subsp. *capensis*	9	-	-	-	1	-	3
	Olea capensis subsp. *macrocarpa*	209	7	-	1	10	1	-
	Olinia ventosa	-	-	-	-	-	-	-
	Platylophus trifoliatus	-	-	-	-	-	-	-
	Podocarpus falcatus	6	-	-	-	-	3	-
	Psydrax obovata subsp.obovata	6	-	-	-	-	-	1
	Pterocelastrus tricuspidatus	31	1	-	5	-	-	-
	Rapanea melanophloeos	1	-	-	-	-	-	-
	Robsonodendron eucleiforme	-	-	-	1	-	-	-

Table 2. (Continued)

		Operational Taxonomic Units (OTUs) and their frequencies of isolation by categories						
		OTU8	OTU9	OTU10	OTU11	OTU12	OTU13	OTU14
Tree status	Alive	3	4	3	-	2	2	2
	Dead standing	-	-	-	-	-	-	-
	Stump	1	-	-	3	1	-	-
Material of isolation	Basidiocarp	1	-	-	-	-	-	-
	Infected wood	3	4	3	3	3	2	2
Compartments	Diepwalle	1	-	3	1	1	-	-
	Gouna	2	-	-	2	1	-	-
	Bloukrans	1	4	-	-	1	2	2
Tree species	*Acacia melanoxylon*	-	-	-	-	-	-	-
	Apodytes dimidiata subsp.*dimidiata*	1	1	-	-	-	-	-
	Canthium mundianum	-	-	-	-	-	-	-
	Cunonia capensis	-	2	-	-	-	-	-
	Elaeodendron croceum	-	-	-	-	-	-	-
	Gonioma kamassi	-	-	-	-	-	-	-
	Halleria lucida	-	-	-	-	-	-	-
	Ilex mitis	-	-	-	-	-	-	-
	Maytenus peduncularis	-	-	-	-	-	-	-
	Nuxia floribunda	-	-	-	-	-	-	-
	Ocotea bullata	-	-	-	-	1	-	-
	Olea capensis subsp. *capensis*	-	-	-	-	-	-	1
	Olea capensis subsp. *macrocarpa*	3	-	3	-	1	-	-
	Olinia ventosa	-	-	-	-	1	-	-
	Platylophus trifoliatus	-	-	-	-	-	-	-
	Podocarpus falcatus	-	-	-	3	-	-	-
	Psydrax obovata subsp.obovata	-	-	-	-	-	-	
	Pterocelastrus tricuspidatus	-	1	-	-	-	2	1
	Rapanea melanophloeos	-	-	-	-	-	-	-
	Robsonodendron eucleiforme	-	-	-	-	-	-	-

Table 2. (Continued)

		Operational Taxonomic Units (OTUs) and their frequencies of isolation by categories						
		OTU15	OTU16	OTU17	OTU18	OTU19	OTU20	OTU21
Tree status	Alive	-	2	1	2	1	1	-
	Dead standing	-	-	-	-	-	-	1
	Stump	2	-	1	-	-	-	-
Material of isolation	Basidiocarp	-	-	2	2	-	-	1
	Infected wood	2	2	-	-	1	1	-
Compartments	Diepwalle	-	-	-	-	-	-	-
	Gouna	2	-	1	-	-	-	-
	Bloukrans	-	2	1	2	1	1	1
Tree species	*Acacia melanoxylon*	-	-	-	-	-	-	-
	Apodytes dimidiata subsp.*dimidiata*	-	-	-	-	-	-	1
	Canthium mundianum	-	-	-	-	-	-	-
	Cunonia capensis	-	-	-	-	-	1	-
	Elaeodendron croceum	-	-	-	-	-	-	-
	Gonioma kamassi	-	-	-	-	-	-	-
	Halleria lucida	-	-	-	-	-	-	-
	Ilex mitis	-	-	-	-	-	-	-
	Maytenus peduncularis	-	-	-	-	-	-	-
	Nuxia floribunda	-	-	-	-	1	-	-
	Ocotea bullata	-	-	-	-	-	-	-
	Olea capensis subsp. *capensis*	-	-	1	-	-	-	-
	Olea capensis subsp. *macrocarpa*	-	-	-	2	-	-	-
	Olinia ventosa	-	-	-	-	-	-	-
	Platylophus trifoliatus	-	2	-	-	-	-	-
	Podocarpus falcatus	2	-	-	-	-	-	-
	Psydrax obovata subsp.obovata	-	-	1	-	-	-	-
	Pterocelastrus tricuspidatus	-	-	-	-	-	-	-
	Rapanea melanophloeos	-	-	-	-	-	-	-
	Robsonodendron eucleiforme	-	-	-	-	-	-	-

Table 2. (Continued)

		Operational Taxonomic Units (OTUs) and their frequencies of isolation by categories					Total isolates	Richness
		OTU22	OTU23	OTU24	OTU25	OTU26		
Tree status	Alive	1	-	1	1	1	**171**	**22**
	Dead standing	-	1	-	-	-	**104**	**7**
	Stump	-	-	-	-	-	**128**	**12**
Material of isolation	Basidiocarp	-	1	-	-	-	**306**	**10**
	Infected wood	1	-	1	1	1	**97**	**21**
Compartments	Diepwalle	-	1	-	-	-	**81**	**9**
	Gouna	-	-	-	-	-	**113**	**9**
	Bloukrans	1	-	1	1	1	**209**	**22**
Tree species	*Acacia melanoxylon*	-	-	-	-	-	**5**	**2**
	Apodytes dimidiata subsp.*dimidiata*	-	-	-	-	-	**25**	**6**
	Canthium mundianum	-	-	-	-	-	**1**	**1**
	Cunonia capensis	-	-	-	-	-	**12**	**6**
	Elaeodendron croceum	-	-	-	-	-	**21**	**2**
	Gonioma kamassi	-	-	1	-	-	**4**	**2**
	Halleria lucida	-	-	-	-	-	**2**	**1**
	Ilex mitis	-	-	-	-	-	**1**	**1**
	Maytenus peduncularis	-	-	-	-	-	**2**	**1**
	Nuxia floribunda	-	-	-	-	-	**2**	**1**
	Ocotea bullata	-	-	-	-	-	**4**	**3**
	Olea capensis subsp. *capensis*	-	-	-	-	-	**15**	**5**
	Olea capensis subsp. *macrocarpa*	-	1	-	1	1	**240**	**12**
	Olinia ventosa	-	-	-	-	-	**1**	**1**
	Platylophus trifoliatus	-	-	-	-	-	**2**	**2**
	Podocarpus falcatus	-	-	-	-	-	**14**	**4**
	Psydrax obovata subsp.obovata	-	-	-	-	-	**8**	**3**
	Pterocelastrus tricuspidatus	1	-	-	-	-	**42**	**7**
	Rapanea melanophloeos	-	-	-	-	-	**1**	**1**
	Robsonodendron eucleiforme	-	-	-	-	-	**1**	**1**

Table 3. Results of Generalized Linear Model (GLM) analysis of presence/absence of OTU1

	DF	*F*-ratio	*P* value
Forest compartments	2	0.001	0.999
Tree species	15	2.743	***P* < 0.01 ****
Material of isolation	1	9.153	***P* < 0.01 ****
Tree DBH (cm)	1	0.233	0.630
Crown class	3	2.276	0.084
Bleeding	1	0.927	0.338
Rots	3	2.619	0.055
Basidiocarps	1	2.119	0.148

DF, Degrees of Freedom; *F*-ratios are shown; Significance (*P* value) is indicated in Bold (*$P < 0.05$).

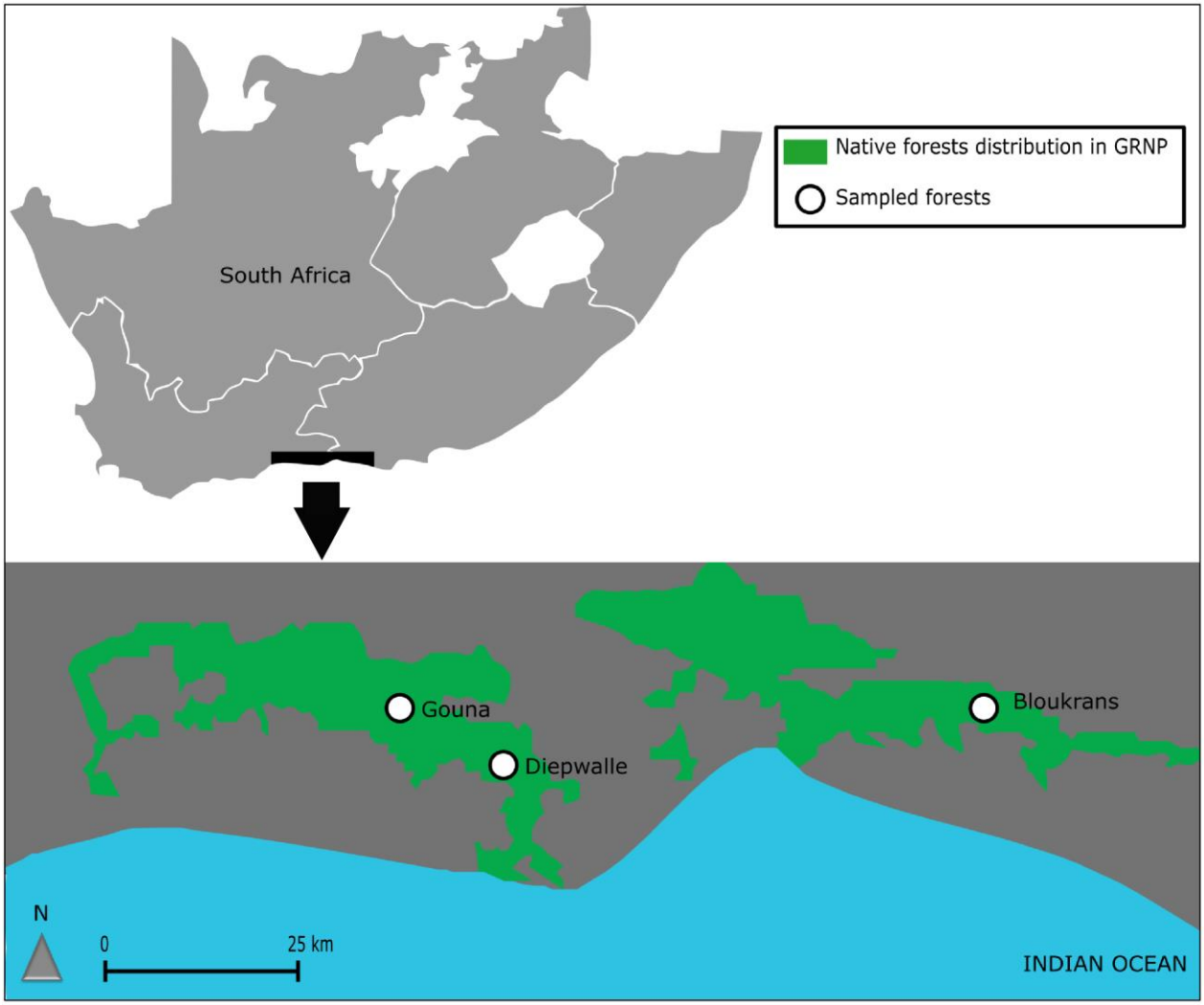

Figure 1. Map showing the location of the three sampled timber-harvesting compartments in the GRNP indigenous forests in the Western and Eastern Cape Provinces of South Africa.

Figure 2. Symptoms and signs observed on sampled trees in the GRNP. A. Sap exudation and basal stem rot. B. Bracket-like basidiomes of a *Ganoderma* species. C. White mycelial mat under the bark of tree with basal stem rot.

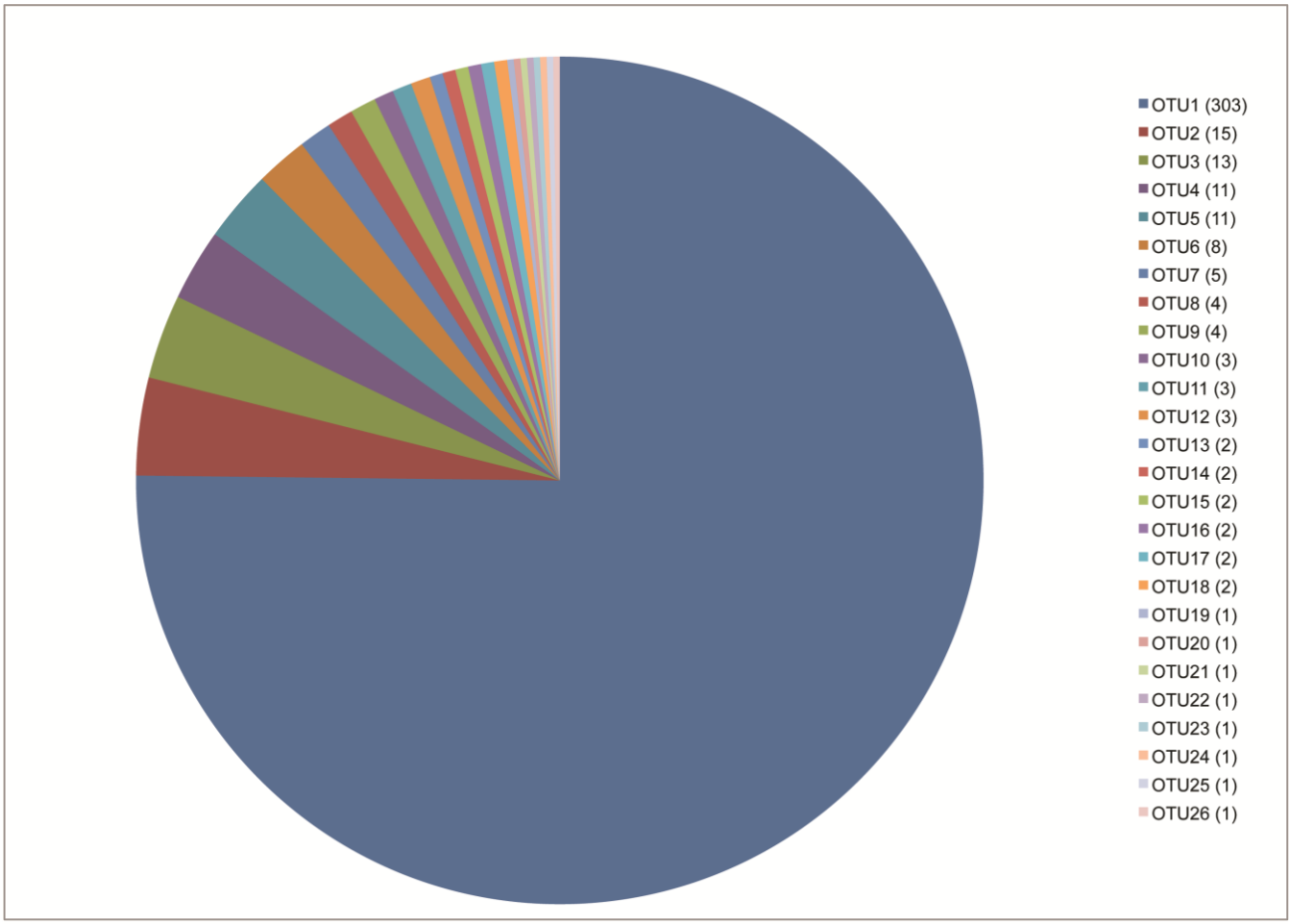

Figure 3. Abundance of Operational Taxonomic Units (OTUs) detected (number in parenthesis) as determined in Mothur.

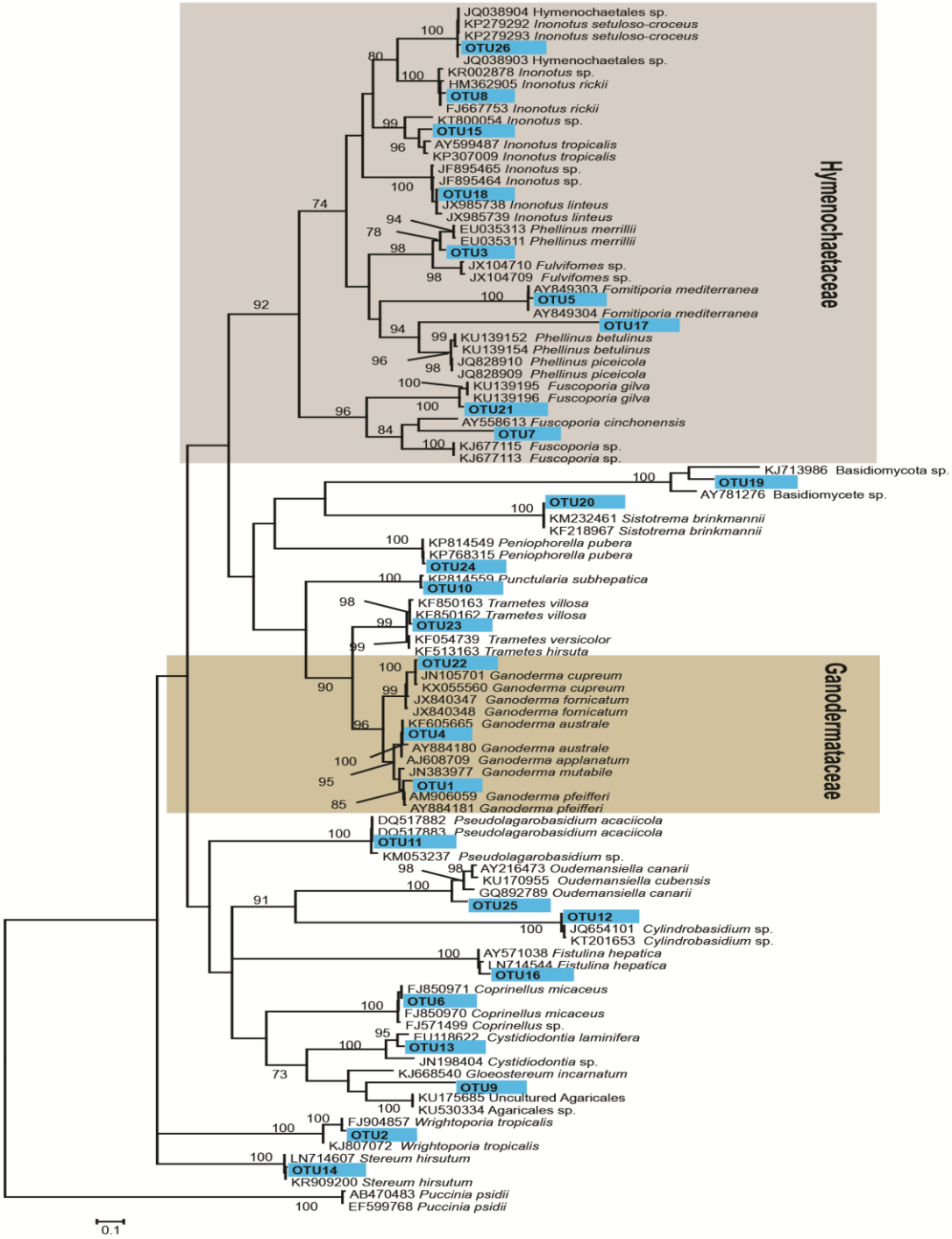

Figure 4. Maximum Likelihood (ML) phylogenetic tree inferred from the ITS gene region showing the taxonomic affiliation between the representative sequence of each OTU from the GRNP with the best-aligned reference sequences. The dataset was composed of 100 taxa and 1218 characters. The model implemented was GTR+I+G and the tree was rooted with sequences of *Puccinia psidii* (AB470483 and EF599768). Highlighted are the two dominant taxonomic groups containing species with pathogenic potential.

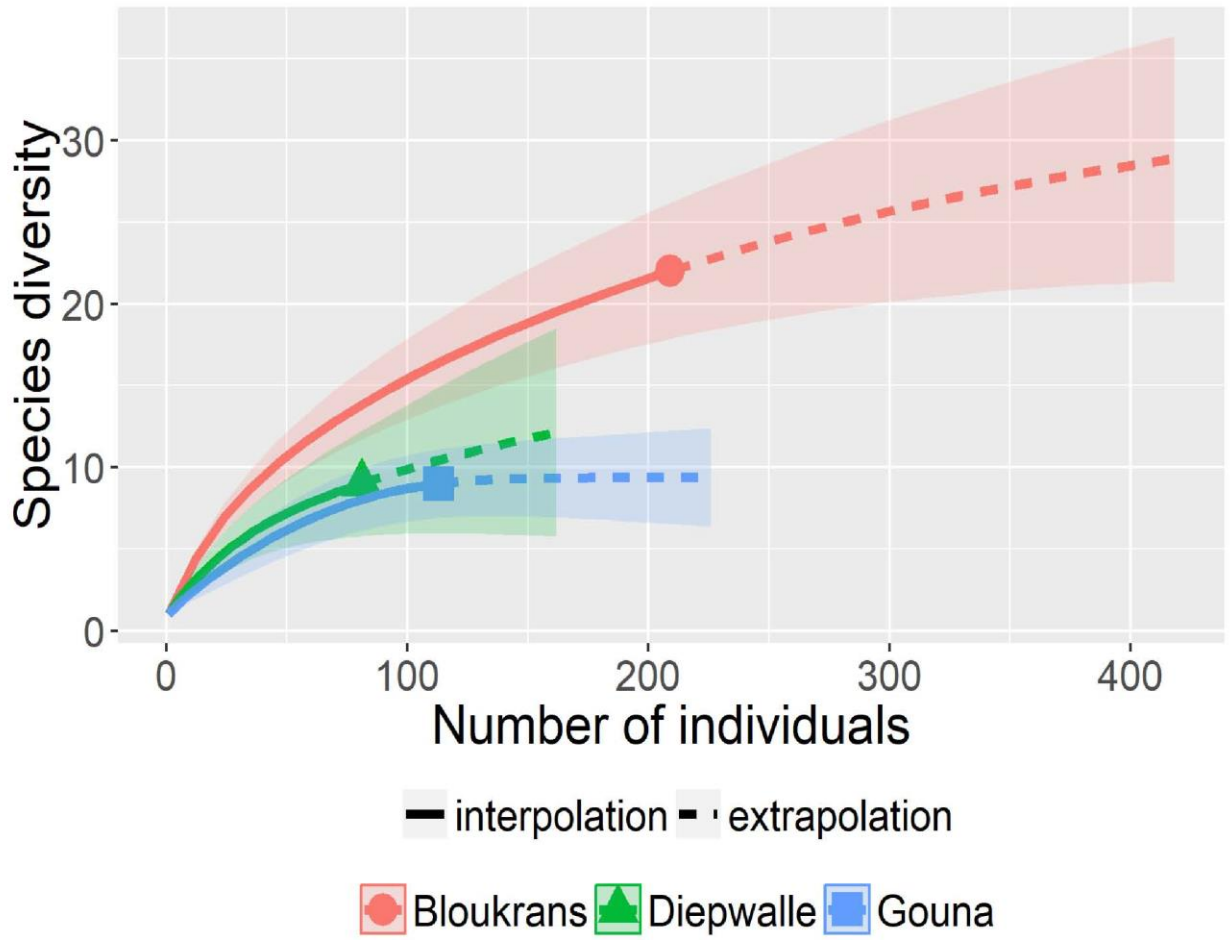

Figure 5. Rarefaction curves of the three sampled timber-harvesting compartments (Bloukrans, Diepwalle and Gouna). Species diversity shows the OTU richness depending on the sampling intensity (number of individuals).

CHAPTER 3

THREE *GANODERMA* SPECIES, INCLUDING *GANODERMA DUNENSE* SP. NOV., ASSOCIATED WITH DYING *ACACIA CYCLOPS* TREES IN SOUTH AFRICA

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ABSTRACT

Large numbers of *Acacia cyclops* trees are dying along the coastal plains of the Eastern and Western Cape Provinces of South Africa. The cause of the deaths has been attributed to a root and butt rot disease caused by the basidiomycete fungus *Pseudolagarobasidium acaciicola*. However, many signs (e.g. basidiomes) and symptoms reminiscent of Ganoderma root-rot are commonly associated with the dying trees. In this study, isolates collected from basidiomes resembling species of *Ganoderma,* as well as from root and butt samples from diseased *A. cyclops* trees were subjected to DNA sequencing and morphological studies to facilitate their identification. Multi-locus phylogenetic analyses and morphological characterisation revealed that three species of *Ganoderma* are associated with dying *A. cyclops* trees. These included *G. destructans*, a recently described species causing root-rot on trees elsewhere in South Africa. The remaining two were novel species, one of which is described here as *G. dunense*. The novel species is distinguished by its mucronate basidiomes, laccate shiny pileus surface, duplex context and ovoid basidiospores. Only an immature specimen was available for the second species and a name was consequently not provided for it. Interestingly, only a single isolate representing *P. acaciicola* was recovered in this study, suggesting that further investigations are needed to ascertain the role of each of the four basidiomycetous root-rot fungi in the death of *A. cyclops* trees.

3.1. INTRODUCTION

Acacia cyclops A. Cunn.: G. Don (Fabaceae) is a woody shrub that occurs mainly in arid and coastal areas due to its ability to withstand severe environmental pressures such as drought, soil salinity and sand blasts (Gill 1985). In South Africa, the first trees of this species were introduced from Australia in the early 1830's to contain and stabilise the movement of sand dunes in the coastal areas of the country, including the Eastern and Western Cape Provinces (Avis 1989). Subsequently, the shrub developed rapidly to become an invasive weed and a serious environmental threat because it forms tall, thick, and almost impenetrable stands that stifle the establishment of native plants (Henderson 1998, 2007). However, despite being detrimental to the diversity of local plants, *A. cyclops* trees also provide an important source of firewood, charcoal and construction materials (Shackleton et al. 2006).

During the course of the last four decades, *A. cyclops* trees have been dying along the coastal planes in the Eastern and Western Cape Provinces of South Africa, particularly between the towns of George and Stilbaai (Taylor 1969; Wood and Ginns 2006; Kotzé et al. 2015). The dying trees suffer from a rapidly developing root and butt-rot disease that results in die-back, wilt, and a white rot of the affected roots and root collars. The disease has been attributed to the basidiomycete root-rot fungus *Pseudolagarobasidium acaciicola* Ginns (Wood and Ginns 2006; Kotzé et al. 2015). However, basidiomes of another fungus resembling a species of *Ganoderma* are also regularly seen attached to the bases of the dying trees. No attempt has been made to determine the identity of that fungus (Taylor 1969; Wood and Ginns 2006).

The genus *Ganoderma* P. Karst. (Basidiomycota, Polyporales, Ganodermataceae) has a worldwide distribution and includes both saprophytic and parasitic species. They cause white rot

on a wide range of host trees (Flood et al. 2000), but are also of significant medicinal and cultural importance (Bishop et al. 2015). *Ganoderma* species are known to kill a wide variety of trees, including those with high economic value such as rubber (*Hevea brasiliensis* Müll.Arg.), tea [*Camellia sinensis* (L.) Kuntze], oil palm (*Elaeis guineensis* Jacq.) and ornamental and forest trees (Ramasamy 1972; Paterson 2007; Kinge and Mih 2011; Coetzee et al. 2015). They are also the main causal agents of root and butt rot diseases of numerous *Acacia* species in tropical regions of the world (Glen et al. 2009; Coetzee et al. 2011).

Identification and circumscription of species of *Ganoderma* has mainly relied on the description of morphological characteristics of the basidiomes, resulting in considerable taxonomic confusion (Richter et al. 2015). Most recently, the Phylogenetic Species Recognition (PSR) concept has improved species delimitation in the genus (Hong and Jung 2004, Zhou et al. 2015). Analyses of DNA sequences for loci such as the internal transcribed spacer (ITS: ITS1-5.8S-ITS2), the translation elongation factor 1-α (TEF1-α) and β-tubulin, among others, have facilitated the inference of relationships between species of *Ganoderma* (Park et al. 2012; Zhou et al. 2015; Xing et al. 2016). At least 438 specific and infraspecific names are listed for *Ganoderma* in Index Fungorum (http://www.indexfungorum.org/names/Names.asp, 08 July 2017). It has however, been suggested that less than one third of these names are valid (Kirk et al. 2008; Richter et al. 2015).

Studies by Taylor (1969) and Wood and Ginns (2006) revealed the recurring presence of basidiomes reminiscent of *Ganoderma* attached to the bases of dying *A. cyclops* trees in the Western Cape Province of South Africa. However, having demonstrated in pathogenicity trials that *P. acaciicola* was the causal agent of the tree death, the authors did not attempt to determine the identity of the *Ganoderma* species associated with the declining trees (Wood and Ginns

2006). The report of Wood and Ginns (2006) suggests that a single *Ganoderma* species is associated with dying *A. cyclops* trees although this might not be the case. The objective of this study was to resolve the identity of the unknown *Ganoderma* species occurring on declining *A. cyclops* trees using DNA sequence comparisons for multiple gene regions as well as morphological observations.

3.2. MATERIALS AND METHODS

3.2.1. Fungal collection and isolation

Basidiomes and recently infected roots and basal sections were collected from dying *A. cyclops*. Collection sites included areas close to the towns of Heroldsbaai and Stillbaai in the Western Cape Province and the Nelson Mandela Bay Metropolitan area (Port Elizabeth, PE) in the Eastern Cape Province (Figure 1). Isolations were performed on a basidiomycete selective medium composed of 2% Malt Extract Agar (MEA) [20 g/L malt extract and 15 g/L agar, Biolab, Midrand, South Africa] supplemented with benomyl, dichloran and streptomycin (BDS) as outlined in Worrall (1991). Small pieces of wood (approximately 2–3 mm^3) from both infected basal sections and roots were first surface-disinfested in 7% bleach (NaClO) for ~ 90 s and rinsed twice with sterile distilled water before transferring them to the selective medium. Isolations from basidiomes were carried out by placing small sections (2–3 mm) obtained from the hymenophore onto the selective medium.

After 7–10 days of incubation at room temperature (22–24°C), pure cultures were obtained by aseptically transferring small pieces of the growing margin of the fungal isolates onto fresh 2% MEA. Growth of the pure cultures was monitored for a further 7–10 days at room temperature (22–24°C). Two mature cultures of each isolate were preserved in the culture collection (CMW)

of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Representative isolates were deposited in the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Dried basidiomes were also deposited in the herbarium of the South African National Collection of Fungi (PREM), Roodeplaat, South Africa.

3.2.2. Genomic DNA extraction, amplification and sequencing

Genomic DNA was extracted from cultures (Table 1) following the Cetyltrimethylammonium Bromide (CTAB) extraction protocol described by Möller et al. (1992). DNA concentrations were determined with a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Working DNA concentrations for polymerase chain reactions (PCRs) were obtained by adjusting the initial concentrations of the extracted genomic DNA to 100 ng/µL. The complete internal transcribed spacer (ITS) region, including ITS-1, ITS-2 and the 5.8S small subunit gene was amplified for all isolates using primers ITS1 and ITS4 (White et al. 1990). Amplifications of the partial translation elongation factor 1-α (TEF1-α) and partial β-tubulin gene regions were obtained using primer pairs EF595F/EF1160R (Kauserud and Schumacher 2001) and β-tubulin_F/β-tubulin_R (Park et al. 2012), respectively.

All PCR reactions were carried out in 25 µL total mixtures consisting of 17.5 µL sterile SABAX water (Adcock Ingram Ltd, Bryanston, RSA), 1 µL genomic DNA (100 ng/µL), 0.5 µL (2.5 units) of MyTaq^{TM} DNA polymerase (Bioline), 5 µL 5 x MyTaq^{TM} Reaction Buffer supplied with the enzyme and 0.5 µL (10 mM) of each primer. The PCR conditions used with primers ITS1/ITS4 and EF595F/EF1160R were as follows: an initial denaturation step at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s, 30 s of annealing at 55°C and 60 s of

extension at 72°C. The reactions were completed with a final elongation step at 72°C for 7 min. Amplifications with the β-tubulin_F/β-tubulin_R primers were carried out with the same parameters as those used by Park et al. (2012). PCR products were electrophoresed on 2% agarose gels after staining with GelRed™ nucleic acid dye (Biotium Incorporation, USA) and visualized under UV illumination. Sephadex G-50 columns (Sigma, Steinheim, Germany) were then used to purify the PCR products following the recommendations of the manufacturer.

Purified PCR products were sequenced in 12 μL reaction volumes using the same primer pairs as in the amplification reactions. DNA sequencing reactions were done using Big Dye (Perkin-Emmer, Warrington, UK), following the protocol outlined by the manufacturer. Purification of the sequencing PCR products followed the same approach as for the PCR products. DNA sequencing was carried out on a DNA Analyzer ABI PRISM™ 3100 (Applied BioSystems, Foster City, CA, USA) at the sequencing facility of the University of Pretoria. CLC Main Workbench v7.6.1 was used to assess the quality of the electropherograms and to construct consensus sequences. DNA sequences of the β-tubulin and TEF1-α gene regions of *G. destructans* and *G. enigmaticum*, previously described from South Africa (Coetzee et al. 2015), were also generated for comparative purposes. These sequences as well as those of the novel species were deposited in GenBank (Table 1).

3.2.3. DNA sequence datasets and multi-gene phylogenetic analysis

Preliminary identification based on BLASTn searches of ITS sequences against those of reference sequences in GenBank was made to confirm that the sequences of the fungal isolates from *A. cyclops* were species of *Ganoderma.* Phylogenetic analyses were subsequently performed to determine the phylogenetic placement of the isolates from *A. cyclops*. For this

purpose, four sequence datasets were generated. Of these, three represented the ITS, β-tubulin and TEF1-α gene regions alone, and one consisted of the combined sequences for all three loci. In addition to sequences generated in this study, the datasets also included reference sequences used in previous studies, including those of Glen et al. (2009), Douanla-Meli and Langer (2009), Kinge et al. (2012), Park et al. (2012), Zhou et al. (2015), Coetzee et al. (2015) , Xing et al. (2016) (Table 1). *Tomophagus colossus* (Fr.) C.F. Baker and *Trametes suaveolens* (L.) Fr. were selected as outgroup taxa. Sequence alignments were performed using the online version of MAFFT (http://mafft.cbrc.jp/alignment/server/index.html) v7 (Katoh and Standley 2013), and the phylogenetic relationships between taxa were evaluated using Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) analyses. The GTR+G+I substitution model determined by JModeltest v2.1.6 (Darriba et al. 2012) on CIPRES (Miller et al. 2010) under the Akaike Information Criterion (AIC) was selected as the best-fit nucleotide substitution model and incorporated in the phylogenetic analyses. Alignments and resulting phylogenetic trees were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S22037?x-access-code=fb98b1676b4efdbbc310326e01d0ad3c&format=htm).

Maximum likelihood analyses were performed in RaxML (Stamatakis 2006) using raxmlGUI 1.3 (Silvestro and Michalak 2012) with 10 parallel runs. Maximum likelihood bootstrap (MLB) analysis was done using 1000 replications. For BI analyses, four parallel runs, each with four Monte Carlo Markov Chains (MCMC) were performed using MrBayes v3.2 (Ronquist et al. 2012). Sampling of trees was carried out for four million generations with trees sampled at every 100^{th} generation. The first 25% of the sampled trees were discarded as burn-in and the Bayesian posterior probabilities (BPP) were calculated for the remaining trees. Maximum Parsimony analyses were performed in PAUP* version 4.0b10 (Swofford 2002). A heuristic search option

with a tree bisection-reconnection (TBR) branch swapping algorithm, involving 100 random stepwise additions of sequences, was used to generate the most parsimonious trees. All characters were of equal weight, gaps were treated as missing data and tree branches of zero length were collapsed. The robustness of clades at the branch nodes was estimated using bootstrap (MPB) with 1000 replicates and using the same settings to obtain the fundamental tree but with the addition of sequences set to closest. Other estimated parameters included tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI). The generated phylogenetic trees were visualized using MEGA 5.05 (Tamura et al. 2011).

3.2.4. Morphology

Microscopic observations were made from fine sections of basidiomes mounted on microscope slides in 5% KOH (potassium hydroxide) with and without 1% phloxine and Melzer's reagent (IKI). The observations were made using a Nikon Eclipse N*i* compound microscope (Nikon, Japan) at a magnification of up to 100x under an oil-immersion objective, and on a Zeiss Stemi SV6 stereo microscope. Images of the microscopic structures were captured with a Nikon DS-Ri2 camera fitted on the microscope and the measurements were made using the program NIS-Elements BR (Nikon Instruments Software-Elements Basic research).

3.3. RESULTS

3.3.1. Signs and Symptoms

Typical signs and symptoms of Ganoderma root rot (Glen et al. 2009; Coetzee et al. 2011; Gill et al. 2016) were observed on the declining *A. cyclops* trees. These included crown wilt and die-back, fresh basidiomes of *Ganoderma* attached to the bases of the affected trees (Figure 2a),

basal cankers and butt rot (Figure 2b), and roots with reddish mycelial sheaths covering a whitish mycelial mat (Figure 2c).

3.3.2. Fungal Isolates

In total, 38 isolates resembling species of *Ganoderma* were obtained from a total of 55 diseased and dying trees. Of these, three were isolated from roots covered with the reddish mycelial sheaths; three from roots without the reddish mycelial sheaths; five from tree bases and the remaining 27 from fresh basidiomes (Table 1). The isolates in culture were either white to yellowish or completely yellow and ranged from having flat to fluffy mycelial growth.

3.3.3. DNA sequence comparisons

The ITS, β-tubulin and TEF1-α gene regions of the 38 isolates were successfully amplified using PCR, and DNA sequences were obtained for all the amplicons. Results of BLASTn searches based on ITS revealed that 36 isolates had a high level of DNA sequence similarity (99 - 100%) with *Ganoderma destructans.* Two others (CMW45100, CMW45101) had a high level of sequence similarity (99%) with *G. applanatum* (Pers.) Pat. and *G. gibbosum* (Blume & T. Nees) Pat. Besides the 38 *Ganoderma* isolates, a single isolate that was obtained from roots without rhizomorphic sheets was identified as *P. acaciicola,* and it was excluded from the rest of the study.

3.3.4. Sequence data and multi-gene phylogenetic analyses

The ITS dataset comprised of 89 ingroup taxa and one outgroup taxon. The dataset resulted in an alignment length of 651 characters, of which 357 were constant, 61 parsimony-uninformative and 233 parsimony-informative. The heuristic search resulted in eight parsimonious trees with

TL = 540, CI = 0.622, RI = 0.899 and RC = 0.559. Since the tree topologies resulting from the BI, ML and MP analyses were nearly identical, only the ML tree is presented, along with the statistical values (BPP and Bootstrap values) of the two other analyses (Figure 3). In the phylogenetic tree of the ITS sequence data, the isolates generated from *A. cyclops* formed two groups, clustering at distant positions. The first group, composed of 36 isolates, formed a monophyletic clade with isolates representing *G. destructans* and *G. multipileum*. This clade had statistical support only in the ML analysis (75% bootstrap support). The second group, comprised of isolates CMW45100 and CMW45101, nested in a strongly supported clade (MLB = 93%, MPB = 100% and BPP = 1) with isolates representing *G. applanatum*, *G. gibbosum* and *G. lobatum* (Schwein.) G.F. Atk. However, the two *A. cyclops* isolates formed a well-resolved sub-clade, although not sufficiently supported by BI analysis (BPP $\leq$ 0.95).

The β-tubulin sequence dataset consisted of 68 taxa, which resulted in an alignment length of 401 total characters. Of the total number of characters, 277 were constant, 44 were parsimony-uninformative and 80 parsimony-informative. The heuristic search yielded 100 parsimonious trees with TL = 206, CI = 0.544, RI = 0.887 and RC = 0.482. Only the ML consensus tree incorporating the statistical values of BI and MP is presented (Figure 4) since the three analyses yielded almost congruent topologies. In the β-tubulin phylogeny, unlike that for the ITS dataset, the isolates obtained from *A. cyclops* clustered at three different positions (Figure 4). Thirty-three isolates (generated in this study) formed a monophyletic clade having strong statistical support with sequences representing *G. destructans* (MLB = 82%, MPB = 98%, BPP = 0.96). Three other isolates (CMW42149, CMW42150 and CMW42157) grouped in a well-resolved and strongly supported monophyletic cluster (MLB and MPB = 99%, BPP = 1), while the third group comprising isolates CMW45100 and CMW45101 formed another strongly supported clade

(MLB and MPB = 100%, BPP = 1) that was closely related to the lineage including *G. mirabile* and *G. applanatum*.

The dataset from TEF1-α contained 71 ingroup taxa and one outgroup taxon. The total number of characters after alignment of the dataset was 579, of which 375 were constant, 35 were parsimony-uninformative and 169 parsimony-informative. The heuristic search resulted in 100 parsimonious trees with TL = 435, CI = 0.584, RI = 0.885 and RC = 0.517. The three analyses resulted in almost identical tree topologies. Hence, only the ML tree is shown alongside with BI and MP statistical values. As with β-tubulin, the TEF1-α phylogeny placed the isolates from *A. cyclops* at three different positions (Figure 5). The first group, consisting of the same 33 isolates as in the β-tubulin gene tree, formed a strongly supported monophyletic clade (MLB = 99%, MPB = 100%, BPP = 0.98) with sequences representing *G. destructans* (generated in this study). This group was closely related to the lineage representing *G. multipileum*. The second group, including isolates CMW42149, CMW42150 and CMW42157, formed a well-supported monophyletic clade (MLB and MPB = 100%, BPP = 1). The third group of isolates, including CMW45100 and CMW45101, formed a cluster with *G. lobatum*, but represented a distinct lineage with high bootstrap values (MLB and MPB = 100%, BPP=1).

The combined dataset of the ITS, β-tubulin and TEF1-α sequences comprised 115 taxa and 1631 total characters. In this dataset the ITS, β-tubulin and TEF1-α respectively contributed 651, 401 and 579 characters. Of the total number of characters, 1152 were constant, 94 parsimony-uninformative and 385 were parsimony-informative. The heuristic search generated 100 parsimonious trees with TL = 1005, CI = 0.541, RI = 0.878, and RC = 0.475. Since the three analyses resulted in similar topologies, only the topology of the ML, incorporating BPP and MP bootstrap values, is presented (Figure 6). The phylogeny for the combined dataset supported that

of β-tubulin and TEF1-α, placing the isolates obtained from *A. cyclops* at three different positions. A first group, consisting of the same 33 isolates as in the β-tubulin and TEF1-α phylogenies, formed a well-resolved monophyletic clade with strong statistical support (MLB = 88% and MPB = 98%) in ML and MP analyses with isolates representing *G. destructans*. This clade was closely related to *G. multipileum*, but both formed distinct lineages. The second group, comprised of isolates CMW42149, CMW42150 and CMW42157, clustered in a clearly resolved monophyletic clade with strong statistical support (MLB and MPB =100%, BPP = 1) in all three analyses. The third group, composed of isolates CMW45100 and CMW45101, formed a highly supported clade (MLB and MPB = 99%, BPP = 1), which was closely related to *G. lobatum* but with marginal support.

3.3.5. Taxonomy

Based on a phylogenetic species recognition concept, the 38 isolates of *Ganoderma* from *A. cyclops* in South Africa, represent three distinct taxa. One of these was *G. destructans* M.P.A. Coetzee, Marinc. & M.J. Wingf. (Coetzee et al. 2015) and two others represented novel species. One of these novel taxa (CMW45100 and CMW45101) is not described because only a rudimentary and immature basidiome (Figure 7) could be found for it. The other new species, represented by isolates CMW42149, CMW42150 and CMW42157 is described as follows:

Ganoderma dunense Tchotet, Rajchenb., & Jol. Roux sp. nov. Figure 8

MB823686

Etymology: Name refers to the coastal sand dunes where this species was collected on dying *A. cyclops* trees.

Diagnosis: Ganoderma dunense is morphologically similar to species in the *Ganoderma lucidum* complex. It is characterised by a light and spongy, perennial, applanate, dimidiate or reniform, mucronate basidiome, a laccate shiny pilear surface and a thick, soft-spongy duplex context. *Hymenial surface* poroid, white when fresh. *Hyphal system* trimitic and *basidiospores* ovoid, 10.9–12.3 × 7.3–8.5 µm, double walled with hyaline exosporium and yellowish brown coarse echinulae endosporium, IKI-.

Type: **South Africa**, Western Cape Province, George, Heroldsbaai (S34° 03.242' E22° 22.711'), at the base of dying *Acacia cyclops* A. Cunn. ex G. Don (Fabaceae), 13 April 2014, J.M. Tchotet Tchoumi and J. Roux, JMT137 (holotype-PREM61936, culture ex-type CMW42157 = CBS142831). GenBank accession number: ITS = MG020255, β-tubulin = MG020150, EF1-α = MG020227.

Description: ***Basidiomes*** light, with spongy consistency, perennial, applanate, dimidiate or reniform, up to 14–20 cm wide, 11–15 cm radius, and 2–2.5 cm thick at base, mucronate; mucro lateral, more or less ellipsoid, 2.3–5 cm long, 1.8–4 cm wide. Margins more or less circular, slightly lobate and round. ***Pileus surface*** laccate shiny, dark brown to dark brown chestnut, mostly covered by a dull brown deposit of basidiospores, glabrous, sulcate with wide furrows that become narrow towards the margins, and with irregular protuberances and a thin crust. ***Hymenial surface*** poroid, white when fresh turning brown to dark brown upon bruising; pores round to somewhat angular and elongated, 3–4 per mm; dissepiments thin. ***Context*** soft-spongy, 4–8 mm thick, duplex, with a light brown upper layer and a chocolate brown lower layer towards the tubes, absence of black resin-like deposits. ***Tubes*** light greyish brown, not stratified, up to 1.5 cm long. ***Hyphal system*** trimitic; generative hyphae not easily found, colourless, thin-walled, bearing clamp septa; skeletal hyphae poorly branched, brown to brown chestnut, thick-walled,

3.1–8 µm thick, lumen 1.4–2.2 µm wide; skeleto-binding hyphae present, branched at the apex with long flagelliform branches, basal stem unbranched. ***Cutis*** a hymeniodermis composed of a palisade of vertical and closely packed clavate cells; cells yellowish brown, thick–walled, some inflated at the apex and with rare growths of protuberances on the lateral parts, amyloid, 22.5–34.4 × 5.4–10.6 µm. ***Cystidia*** and ***basidia*** not seen. ***Basidiospores*** ovoid with indistinct truncate apex, 10.9–12.3 × 7.3–8.5 µm (11.6 ± 0.7 × 7.9 ± 0.6 µm), IKI-, double-walled, exosporium hyaline with inter-wall pillars, endosporium yellowish brown with coarse echinulae.

Additional specimen: South Africa, Western Cape Province, George, Heroldsbaai (S34° 03.249' E22° 22.720'), at the base of dying *A. cyclops*. 13 April 2014, J.M. Tchotet Tchoumi and J. Roux, JMT124 (paratype-PREM 61937, culture ex-type CMW42149 = CBS142945). GenBank accession number: ITS = MG020248, β-tubulin = MG020153, EF1-α = MG020226.

Remarks: *Ganoderma dunense* and *G. destructans* cannot be distinguished from each other based on ITS phylogeny (Figure 3). They segregate only in phylogenies for the β-tubulin and TEF1-α gene regions as well as in the combined genes (Figures 4, 5, 6). This phylogenetic divergence based on these three analyses is also reflected in some morphological characters. Morphologically, *G. destructans* differs from *G. dunense* in having a stipitate basidiome, a laccate shiny reddish brown pilear surface with well-developed protuberances, a not completely homogenous context, slightly smaller pores (3–5 per mm) and larger basidiospores (11–14 × 7–9 µm, Coetzee et al. 2015; Table 2).

Ganoderma dunense is macroscopically similar to *G. enigmaticum* M.P.A. Coetzee, Marinc. & M.J. Wingf., *G. aridicola* J.H. Xing & B.K. Cui and *G. austroafricanum* M.P.A. Coetzee, M.J. Wingf., Marinc. & Blanchette that also occur in South Africa and with which it is genetically

related in the combined phylogeny but without statistical support (Figure 6). They share a white poroid hymenophore surface when fresh, irregular pores and absence of black melanoid bands in the context (Crous et al. 2014; Coetzee et al. 2015; Xing et al. 2016; Table 2). However, *G. enigmaticum* and *G. aridicola* differ from *G. dunense* in their ecology. *Ganoderma enigmaticum* occurred as a parasite at the base of the trunk of *Ceratonia siliqua* L. in Pretoria, Gauteng Province (Coetzee et al. 2015), while *G. aridicola* was recovered on charred wood of *Ficus* in Durban, KwaZulu-Natal Province (Xing et al. 2016). In addition, *G. enigmaticum* differs morphologically from *G. dunense* by having a stipitate basidiome, homogenous context and narrower ellipsoid basidiospores (8–11 × 3.5–6 µm, Coetzee et al. 2015; Table 2). Similarly, *G. aridicola* differs from *G. dunense* by having a sessile basidiome, a fuscous homogenous context, distinctly stratified tube layers, smaller pores (6–8 per mm) and broadly ellipsoid basidiospores (9.7–11.2 × 7–7.8 µm, Xing et al. 2016; Table 2). *G. austroafricanum* has an annual sessile basidiome, a dimitic hyphal system and smaller (8–11 × 5.5–7 µm) subglobose basidiospores (Crous et al. 2014; Table 2).

When compared morphologically with other laccate *Ganoderma* species from South Africa and tropical Africa based on published descriptions (Steyaert 1961, 1962, 1967, 1972, 1980; Table 2), *G. dunense* shares few characteristics with most of these species. Exceptions are *G. namutambalaense* Steyaert (1962), collected in Uganda and *G. megalosporum* Steyaert (1962) collected in Kenya, which share minor similarities with *G. dunense*. They can, however, easily be distinguished from *G. dunense* in that *G. namutambalaense* has a substipitate basidiome, smaller pores and larger basidiospores, while *G. megalosporum* has larger basidiospores and much smaller sub-cylindric cuticular cells (Table 2).

3.4. DISCUSSION

Three distinct *Ganoderma* species were found associated with dying *Acacia cyclops* trees in the Eastern and Western Cape Provinces of South Africa. Two of these species represent novel taxa but a name was provided for only one due to absence of mature basidiomes needed to describe the second species. The description of the new species brings to 11 the *Ganoderma* spp. recorded from South Africa (Van der Bijl 1921; Reid 1973; 1974; 1975; Moncalvo and Ryvarden 1997; Crous et al. 2014; Coetzee et al. 2015 and Xing et al. 2016). Of these, only five have been described with the support of DNA sequence data.

The majority of *Ganoderma* isolates obtained in this study (33 = ~ 87%) represented *G. destructans*. This fungus was recently described as the causal agent of a serious root disease of non-native Jacaranda (*Jacaranda mimosifolia* D. Don) trees in the city of Pretoria (Tshwane Metropolitan area, Gauteng Province) in South Africa (Coetzee et al. 2015). The common occurrence of *G. destructans* on *A. cyclops* in the Western Cape, together with its occurrence on *J. mimosifolia* more than 1000 km further north in the country suggests that it has a wide host range, but also a wide geographical distribution. Coetzee et al. (2015) suggested that this fungus might be a native species and *A. cyclops* thus represents the second non-native host that it has successfully colonized. Its wide geographic distribution also supports the contention that it is a native South African fungus.

The two novel species of *Ganoderma* obtained from *A. cyclops* in this study were represented by only five isolates. Two were of the *Ganoderma* sp. that was not described and three were of the newly described *G. dunense*. Based on the appearance of the pilear surface, *G. dunense* could be

linked to the *G. lucidum* complex, while the undescribed *Ganoderma* sp. was related to species in the *G. applanatum* complex (Imazeki 1952; Richter et al. 2015).

As has been found in many other studies (i.e. Gottlieb et al. 2000 and Zhou et al. 2015) sequence data for the ITS gene regions were not sufficient to delineate between *G. dunense* and *G. destructans*. Based on the ITS phylogeny, *G. dunense* would be treated as the same as *G. destructans*. But phylogenies inferred from the β-tubulin and TEF1-α data sets, as well as that of the combined genes, showed that this taxon represented a novel species. Morphological characterization also revealed very distinct macro- and microscopic characters that could further distinguish between these two taxa.

In the global multi-locus phylogeny, *G. dunense* formed a well-resolved and isolated monophyletic clade. However, it was linked to species belonging to the *G. lucidum* complex (Li et al. 2015; Zhou et al. 2015; Richter et al. 2015) but without these relationships being statistically supported. These species included *G. enigmaticum*, *G. aridicola*, *G. carnosum*, *G. lucidum* (*s. lat.*), *G. leucocontextum*, *G. tsugae*, *G. oregonense*, *G. austroafricanum*, *G. resinaceum* and *G. sessile*. Although the placement of *G. dunense* among these species lacked statistical support, it indicates a relatedness to species in the complex, which was also supported by its morphology.

The extent of infection by *Ganoderma* species in this study raises an intriguing question about the role of *P. acaciicola* in the death of *A. cyclops*. More specifically, whether it is the only causal agent of the deaths of these trees as reported by Wood and Ginns (2006). This question is worth considering because most of the isolates recovered from recently infected trees in the current study were of *G. dunense* and only a single isolate was identified as *P. acaciicola*.

Moreover, although *Ganoderma* was included in the pathogenicity trials conducted by Wood and Ginns (2006), results of the present study show that at least three species of *Ganoderma* are associated with the dying *A. cyclops* in these areas. It is not known which of the three *Ganoderma* species was used in the aforementioned trials. Additionally, most of the sampled trees exhibited symptoms typical of Ganoderma root rot similar to those observed in previous studies (Glen et al. 2009; Coetzee et al. 2011). It is, therefore, clear that further investigations should be undertaken to determine the role of *P. acaciicola* and *Ganoderma* species in the death of *A. cyclops* trees in the coastal areas of South Africa.

3.5. REFERENCES

Avis AM, 1989. A review of coastal dune stabilization in the Cape Province of South Africa. *Landscape and Urban Planning* 18, 55–68.

Bishop KS, Kao CHJ, Xu Y, Glucina MP, Paterson RRM, Ferguson LR, 2015. From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. *Phytochemistry* 114, 56–65.

Coetzee MPA, Marincowitz S, Muthelo VG, Wingfield MJ, 2015. *Ganoderma* species, including new taxa associated with root rot of the iconic *Jacaranda mimosifolia* in Pretoria, South Africa. *IMA Fungus* 6: 249–256.

Coetzee MPA, Wingfield BD, Golani GD, Tjahjono B, Gafur A, Wingfield MJ, 2011. A single dominant *Ganoderma* species is responsible for root rot of *Acacia mangium* and *Eucalyptus* in Sumatra. *Southern Forests* 73, 175–180.

Crous PW, Wingfield MJ, Schumacher RK, Summerell BA, Giraldo A et al., 2014. Fungal Planet description sheets: 281–319. *Persoonia* 33, 212–289.

Darriba D, Taboada GL, Doallo R, Posada D, 2012. "jModelTest 2: more models, new heuristics and parallel computing". *Nature Methods* 9, 772.

Douanla-Meli C, Langer E, 2009. *Ganoderma carocalcareus* sp. nov., with crumbly-friable context parasite to saprobe on *Anthocleista nobilis* and its phylogenetic relationship in *G. resinaceum* group. *Mycological Progress* 8, 145–155.

Flood J, Bridge PD, Holderness M (Eds), 2000. Ganoderma diseases of perennial crops. (CABI Publishing: Wallingford, UK) 275 pp.

Gill AM, 1985. *Acacia cyclops* G. Don (Leguminosae: Mimosaceae) in Australia: distribution and dispersal. *Journal of the Royal Society of Western Australia* 67, 59–65.

Gill W, Eyles A, Glen M, Mohammed C, 2016. Structural host responses of *Acacia mangium* and *Eucalyptus pellita* to artificial infection with the root rot pathogen, *Ganoderma philippii*. *Forest Pathology* 46, 369–375.

Glen M, Bougher NL, Francis AA, Nigg SQ, Lee SS, Irianto R, Barry KM, Beadle CL, Mohammed CL, 2009. *Ganoderma* and *Amauroderma* species associated with root-rot disease of *Acacia mangium* plantation trees in Indonesia and Malaysia. *Australasian Plant Pathology* 38, 345–356.

Gottlieb AM, Ferrer E, Wright JE, 2000. rDNA analyses as an aid to the taxonomy of species of *Ganoderma*. *Mycological Research* 104, 1033–1045.

Henderson L, 1998. Invasive alien woody plants of the Southern and Southwestern Cape region, South Africa. *Bothalia* 28, 91–112.

Henderson L, 2007. Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). *Bothalia* 37, 215–248.

Hong SG, Jung HS, 2004. Phylogenetic analysis of *Ganoderma* based on nearly complete mitochondrial small-subunit ribosomal DNA sequences. *Mycologia* 96, 742–755

Imazeki R, 1952. A contribution to the fungus flora of Dutch New Guinea. Bulletin of the *Government Forest Experimental Station Meguro* Tokyo 57, 87–128

Katoh K, Standley DM, 2013. MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30, 772–780.

Kauserud H, Schumacher T, 2001. Outcrossing or inbreeding: DNA markers provide evidence for type of reproductive mode in *Phellinus nigrolimitatus* (Basidiomycota). *Mycological Research* 53, 220–230.

Kinge TR, Mih AM, 2011. *Ganoderma ryvardense* sp. nov. associated with basal stem rot (BSR) disease of oil palm in Cameroon. *Mycosphere* 2, 179–188.

Kinge TR, Mih AM, Coetzee MPA, 2012. Phylogenetic relationships among species of *Ganoderma* (Ganodermataceae, Basidiomycota) from Cameroon. *Australian Journal of Botany* 60, 526–538.

Kirk PM, Cannon PF, Minter DW, Stalpers JA, 2008. Dictionary of the fungi, 10th edn. CAB International, Wallingford.

Kotzé LJD, Wood AR, Lennox CL, 2015. Risk assessment of the *Acacia cyclops* dieback pathogen, *Pseudolagarobasidium acaciicola*, as a mycoherbicide in South African strandveld and limestone fynbos. *Biological Control* 82, 52–60.

Li TH, Hu HP, Deng WQ, Wu SH, Wang DM, Tsering T, 2015. *Ganoderma leucocontextum*, a new member of the *G. lucidum* complex from Southwestern China. *Mycoscience* 56, 81–85.

Miller MA, Pfeiffer W, Schwartz T, 2010. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1–8.

Möller EM, Bahnweg G, Sandermann H, Geiger HH, 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Res* 20, 6115–6116.

Moncalvo JM, Ryvarden L, 1997. A nomenclatural study of the Ganodermataceae Donk. Synopsis Fungorum 11. *Fungiflora* : Oslo, Norway.

Moncalvo JM, Ryvarden L, 1995. *Ganoderma hildebrandii*, a forgotten species. *Mycotaxon* 56, 175–180.

Park Y, Kwon O, Son E, Yoon D, Han W, Yoo Y, Lee C, 2012. Taxonomy of *Ganoderma lucidum* from Korea Based on rDNA and Partial β-tubulin Gene Sequence Analysis. *Mycobiology* 40, 71–75.

Paterson RRM, 2007. Ganoderma disease of oil palm-A white rot perspective necessary for integrated control. *Crop Protection* 26, 1369–1376.

Ramasamy S, 1972. Cross-infectivity and decay ability of *Ganoderma* species parasitic to rubber, oil palm and tea. In: Ganoderma diseases of perennial crops. Flood J, Bridge PD and Holderness M, 2000. CABI, Wallingford, UK.

Reid DA, 1973. A reappraisal of type and authentic specimens of Basidiomycetes in the Van der Byl Herbarium, Stellenbosch. *South African Journal of Botany* 39, 141–178.

Reid DA, 1974. A reappraisal of type and authentic material of the larger Basidiomycetes in the Pretoria Herbarium. *Bothalia* 11, 221–230.

Reid DA, 1975. Type studies of the larger Basidiomycetes described from southern Africa. *Contributions from the Bolus Herbarium* 7, 1–255.

Richter C, Wittstein K, Kirk P, Stadler M, 2015. An assessment of the taxonomy and chemotaxonomy of *Ganoderma*. *Fungal Diversity* 71, 1–15.

Ronquist F, Teslenko M, van der Mark P et al., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539–542.

Shackleton CM, McConnachie M, Chauke MI, Mentz J, Sutherland F, Gambiza J, Jones R, 2006. Urban fuelwood demand and markets in a small town in South Africa: Livelihood vulnerability and alien plant control. *International Journal of Sustainable Development & World Ecology* 13, 481–491.

Silvestro D, Michalak I, 2012. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12, 335–337.

Stamatakis A, 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.

Steyaert RL, 1961. Genus *Ganoderma* (Polyporaceae) taxa nova — I. In *Bulletin du Jardin Botanique de l'État à Bruxelles* 31, 69–83.

Steyaert RL, 1962. Genus *Ganoderma* (Polyporaceae) taxa nova—2. *In Bulletin du Jardin Botanique National de Belgique* 32, 89–104.

Steyaert RL, 1967. Les *Ganoderma* palmicoles. *In Bulletin du Jardin Botanique National de Belgique* 37, 465–492.

Steyaert RL, 1972. Species of *Ganoderma* and related genera mainly of the Bogor and Leiden Herbaria. *Persoonia* 7, 55–118.

Steyaert RL, 1980. Study of some *Ganoderma* species. *In Bulletin du Jardin Botanique National de Belgique* 50, 135–186.

Swofford DL, 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, MA: Sinauer Associates.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.

Taylor H, 1969. Pest plants and nature conservation in the winter rainfall region. *South African Journal of Botany* 55, 32–35.

Van der Bijl PA, 1921. A contribution to our knowledge of the Polyporeae of South Africa. *South African Journal of Science* 18, 246–293.

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds), PCR Protocols: a guide to methods and applications. Academic Press, New York. pp. 315–322.

Wood AR, Ginns J, 2006. A new dieback disease of *Acacia cyclops* in South Africa caused by *Pseudolagarobasidium acaciicola* sp. nov. *Canadian Journal of Botany* 84, 750–758.

Worrall JJ, 1991. Media for selective isolation of hymenomycetes. *Mycologia* 83, 296–02.

Xing JH, Song J, Decock C, Cui BK, 2016. Morphological characters and phylogenetic analysis reveal a new species within the *Ganoderma lucidum* complex from South Africa. *Phytotaxa* 266, 115–124.

Zhou LW, Cao Y, Wu SH, Vlasák J, Li DW, Li MJ, Dai YC, 2015. Global diversity of the *Ganoderma lucidum* complex (Ganodermataceae, Polyporales) inferred from morphology and multilocus phylogeny. *Phytochemistry* 114, 7–15.

Table 1. Isolates used in the phylogenetic analyses

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	β-tubulin	TEF1-α
Ganoderma adspersum	Yao34456	United Kingdom	AJ006685	-	-
G. applanatum	Dai 12483	China	KF494999	-	KF494977
G. applanatum	ATCC 44053	Japan	JQ520161	JQ675614	-
G. aridicola	Dai12588 (holotype)	South Africa	KU572491	-	KU572502
G. austroafricanum	CMW 41454	South Africa	KM507324	-	-
G. boninense	WD 2028	Japan	KJ143905	-	KJ143924
G. boninense	WD 2085	Japan	KJ143906	-	KJ143925
G. carnosum	MJ 21/08	Czech Republic	KU572492	-	-
G. carnosum	JV 8709/8	Czech Republic	KU572493	-	-
G. carnosum	CBS 516.96	Netherlands	-	JQ675616	-
G. cupreum	GanoTK4	Cameroon	JN105701	-	-
G. cupreum	GanoTK7	Cameroon	JN105702	-	-
G. curtisii	CBS 100131	United States of America (USA)	JQ781848	-	KJ143926
G. curtisii	CBS 100132	USA	JQ781849	-	KJ143927
G. curtisii	CBS 100132	Netherlands	-	JQ675617	-
G. destructans	CBS 139793 (type)	South Africa	NR132919	MG020151*	MG020213*
G. destructans	CMW 43671	South Africa	KR183857	MG020156*	MG020220*
G. destructans	CMW42129	South Africa	MG020232	MG020158	MG020191
G. destructans	CMW42130	South Africa	MG020233	MG020159	MG020192
G. destructans	CMW42131	South Africa	MG020234	MG020160	MG020193
G. destructans	CMW42134	South Africa	MG020235	MG020161	MG020216
G. destructans	CMW42135	South Africa	MG020236	MG020162	MG020214
G. destructans	CMW42136	South Africa	MG020237	MG020163	MG020194
G. destructans	CMW42137	South Africa	MG020238	MG020164	MG020195
G. destructans	CMW42138	South Africa	MG020239	MG020165	MG020196
G. destructans	CMW42139	South Africa	MG020240	MG020166	MG020197
G. destructans	CMW42140	South Africa	MG020241	MG020167	MG020215
G. destructans	CMW42141	South Africa	MG020242	MG020168	MG020198
G. destructans	CMW42142	South Africa	MG020243	MG020169	MG020199

Table 1. (continued)

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	β-tubulin	TEF1-α
G. destructans	CMW42143	South Africa	MG020244	MG020170	MG020221
G. destructans	CMW42146	South Africa	MG020245	MG020171	MG020200
G. destructans	CMW42147	South Africa	MG020246	MG020172	MG020201
G. destructans	CMW42148	South Africa	MG020247	MG020173	MG020202
G. destructans	CMW42151	South Africa	MG020250	MG020174	MG020203
G. destructans	CMW42152	South Africa	MG020251	MG020175	MG020204
G. destructans	CMW42153	South Africa	MG020252	MG020176	MG020205
G. destructans	CMW42154	South Africa	MG020253	MG020177	MG020206
G. destructans	CMW42155	South Africa	MG020254	MG020178	MG020217
G. destructans	CMW42158	South Africa	MG020256	MG020179	MG020224
G. destructans	CMW42159	South Africa	MG020257	MG020180	MG020223
G. destructans	CMW42160	South Africa	MG020258	MG020181	MG020207
G. destructans	CMW42161	South Africa	MG020259	MG020182	MG020208
G. destructans	CMW42162	South Africa	MG020260	MG020183	MG020209
G. destructans	CMW42163	South Africa	MG020261	MG020184	MG020218
G. destructans	CMW42164	South Africa	MG020262	MG020185	MG020210
G. destructans	CMW42165	South Africa	MG020263	MG020186	MG020211
G. destructans	CMW45109	South Africa	MG020266	MG020187	MG020225
G. destructans	CMW45110	South Africa	MG020267	MG020188	MG020222
G. destructans	CMW45113	South Africa	MG020268	MG020189	MG020212
G. destructans	CMW45114	South Africa	MG020269	MG020190	MG020219
***G. dunense* sp. nov**	**CMW42149**	**South Africa**	**MG020248**	**MG020153**	**MG020226**
***G. dunense* sp. nov**	**CMW42150**	**South Africa**	**MG020249**	**MG020154**	**MG020228**
***G. dunense* sp. nov**	**CMW42157 (Type)**	**South Africa**	**MG020255**	**MG020150**	**MG020227**
G. gibbosum	XSD-35	Unknown	EU273514	-	-
G. gibbosum	AS5.624 type 3	China	AY593856	-	-
G. enigmaticum	Dai 15970	Africa	KU572486	-	KU572496
G. enigmaticum	Dai 15971	Africa	KU572487	-	KU572497
G. enigmaticum	CBS 139792 (type)	South Africa	NR132918	MG020157*	MG020231*

Table 1. (continued)

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	β-tubulin	TEF1-α
G. leucocontextum	Dai 15601	China	KU572485	-	KU572495
G. leucocontextum	GDGM 44489	China	KM396271	-	-
G. lingzhi	Wu 1006-38 (holotype)	China	JQ781858	-	JX029976
G. lingzhi	Dai 12574	China	KJ143908	-	JX029977
G. lingzhi	Dai 12479	China	JQ781864	-	JX029975
G. lobatum	JV 0409/13J	USA	KF605675	-	-
G. lobatum	JV 1212/10J	USA	KF605676	-	KU572501
G. lobatum	ATCC 42985	Canada	-	JQ675618	-
G. lobatum	ASI 7061	USA	-	JQ675619	-
G. lucidum	Cui 9207	China	KJ143910	-	KJ143928
G. lucidum	K 175217	UK	KJ143911	-	KJ143929
G. lucidum	ASI 7117	Korea	-	JQ675633	-
G. lucidum	IUM 4303	Bangladesh	-	JQ675635	-
G. lucidum	IUM 0047	Korea	-	JQ675627	-
G. meredithae	ATCC 64492	USA	-	JQ675643	-
G. meredithae	ASI 7140	Unknown	-	JQ675644	-
G. mirabile	CBS 218.36	Philippines	-	JQ675645	-
G. multipileum	CWN 04670	China	KJ143913	-	KJ143931
G. multipileum	Dai 9447	China	KJ143914	-	KJ143932
G. multiplicatum	Dai 12320	China	-	-	KU572500
G. multiplicatum	Dai 13710	China	-	-	KU572499
G. mutabile	Yuan 2289 (type)	China	JN383977	-	-
G. neojaponicum	ASI 7032	Unknown	-	JQ675646	-
G. oerstedii	GO138	Unknown	-	DQ288098	-
G. oregonense	CBS 265.88	USA	JQ781875	NS	KJ143933
G. oregonense	ASI 7049	USA	-	JQ675647	-
G. oregonense	ASI 7067	USA	-	JQ675650	-
G. oregonense	CBS 266.88	USA	JQ781876	-	-
G. pfeifferi	CBS 747.84	Netherlands	-	JQ675651	-

Table 1. (continued)

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	**β-tubulin**	**TEF1-α**
G. philippii	E7098	Indonesia	AJ536662	-	-
G. philippii	E7092	Indonesia	AJ608710	-	-
G. resinaceum	BR 4150	France	KJ143915	-	-
G. resinaceum	CBS 194.76	Netherlands	KJ143916	-	KJ143934
G. resinaceum	ATCC 52416	Argentina	-	JQ675652	-
G. resinaceum	IUM 3651	Czech Republic	-	JQ675657	-
G. ryvardenii	HKAS 58055	Cameroon	HM138670	-	-
G. ryvardenii	HKAS 58053 (type)	Cameroon	HM138671	-	-
G. sessile	JV 1209/9	USA	KF605629	-	KJ143936
G. sessile	LDW 20121017	USA	KJ143917	-	KJ143935
G. sinense	Wei 5327	China	KF494998	-	KF494976
***Ganoderma* sp.**	**CMW45100**	**South Africa**	**MG020264**	**MG020152**	**MG020229**
***Ganoderma* sp.**	**CMW45101**	**South Africa**	**MG020265**	**MG020155**	**MG020230**
Ganoderma sp.	ASI 7150	Unknown	-	JQ675665	-
Ganoderma sp.	ASI 7151	Unknown	-	JQ675666	-
G. subamboinense	GSUB136	Unknown	-	DQ288096	-
G. subamboinense	GSUB137	Unknown	-	DQ288097	-
G. tenue	GTEN24	Unknown	-	DQ288074	-
G. tornatum	CBS 109679	Netherlands	-	JQ675670	-
G. tornatum	BAFC1172	Argentina	AH008096	-	-
G. tornatum	BAFC1139	Argentina	AH008098	-	-
G. tropicum	He 1232	China	KF495000	-	KF494975
G. tropicum	Yuan 3490	China	JQ781880	-	KJ143938
G. tsugae	Dai 12751b	USA	KJ143919	-	KJ143939
G. tsugae	Dai 12760	USA	KJ143920	-	KJ143940
G. tsugae	ATCC 64795	Canada	-	JQ675668	-
G. tsugae	ASI 7064	USA	-	JQ675669	-
G. valesiacum	CBS 428.84	USA	-	JQ675671	-
G. webeianum	CBS 219.36	Philippines	-	JQ675672	-

Table 1. (continued)

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	**β-tubulin**	**TEF1-α**
G. zonatum	FL-02	USA	KJ143921	-	KJ143941
G. zonatum	FL-03	USA	KJ143922	-	KJ143942
Tomophagus colossus	TC-02	Vietnam	KJ143923	-	KJ143943
Trametes suaveolens	-	Unknown	-	FJ410378	-

Reference sequences with star (*) are those generated in this study. In bold are isolates of the newly proposed species.

Table 2. Morphological comparison of *G. dunense* sp. nov. with other laccate *Ganoderma* taxa found in South Africa and tropical Africa

Taxon	Shape	Pores/mm	Spores (µm)	Cutis	Other	Reference
Ganoderma africanum	NI	NI	NI	NI	type not found	Moncalvo and Ryvarden 1997
G. aridicola	sessile dimidiate, pilear surface fuscous black when fresh, reddish brown to black upon drying	6–8	9.7–11.2 × 7–7.8, broadly ellipsoid	amyloid elements 30–55 × 5–8 µm	skeletal hyphae up to 2.5–5 µm wide	Xing et al. 2016
G. austroafricanum	sessile, dimidiate	3–4	8–11 × 5.5–7 broadly ellipsoid	NI	NI	Crous et al. 2014
G. capensis	sessile	5–6	9.75–10.5 × 5.75–6.2	elements 50 x 5–10 µm up to 9-12 µm when capitate	dimitic hyphal system	Reid 1975
	-	6	10–12 × 6–7 ovoid to ellipsoid	amyloid elements 35–45(–55) × 7–9 µm	trimitic with skeleteo-binding hyphae, skeletal hyphae 5.5–10 µm diam.	This publication
G. destructans	stipitate globular pileus	3–5	11–14 × 7–9	amyloid elements 13–35 × 4.5–7.5	homogeneous context	Coetzee et al. 2015
	laccate shiny reddish brown, stipe lateral to eccentric and circular to ellipsoid, pileus dimidiate to circular, with small to wide protuberances		-	-	not completely homogeneous context	This publication

Table 2. (continued)

Taxon	Shape	Pores/mm	Spores (µm)	Cutis	Other	Reference
G. dunense sp. nov.	sessile but with a lateral mucro, pileus dimidiate or reniform	3–4	10.9-12.3 × 7.3-8.5	amyloid elements clavate, 22.5–34.4 × 5.4–10.6 µm	duplex context present	This publication
G. enigmaticum	stipitate globular pileus	3–5	8–11 × 3.5–6	amyloid elements 20–46 × 5.5–9 µm	-	Coetzee et al. 2015
G. hildebrandii	small basidiome centrally stipitate	7–8	7–8(8.5) × 4.5–5.5(–6)	IKI− elements similar but wider up to 15 µm	dextrinoid skeletal hyphae	Moncalvo and Ryvarden 1995, 1997
G. reticulatosporum	centrally stipitate pileus, umbrella shaped	3–4	25–26 × 15.5–16	NI	perhaps *Humphreya* sp.	Reid 1973 Moncalvo and Ryvarden 1997
G. namutambalaense	substipitate flabellate	5–6	11.5–14 × 8–9.5	elements sphaeropedunculate, 50 x 4–7(–10) µm, sustaining hyphae 2-3 µm	from Uganda	Steyaert 1962
G. megalosporum	sessile dimidiate	3–4	11.5–13 × 8–9	elements subcylindric 30 x 4-6 µm	from Kenya	Steyaert 1962

*NI refers to no information in the literature

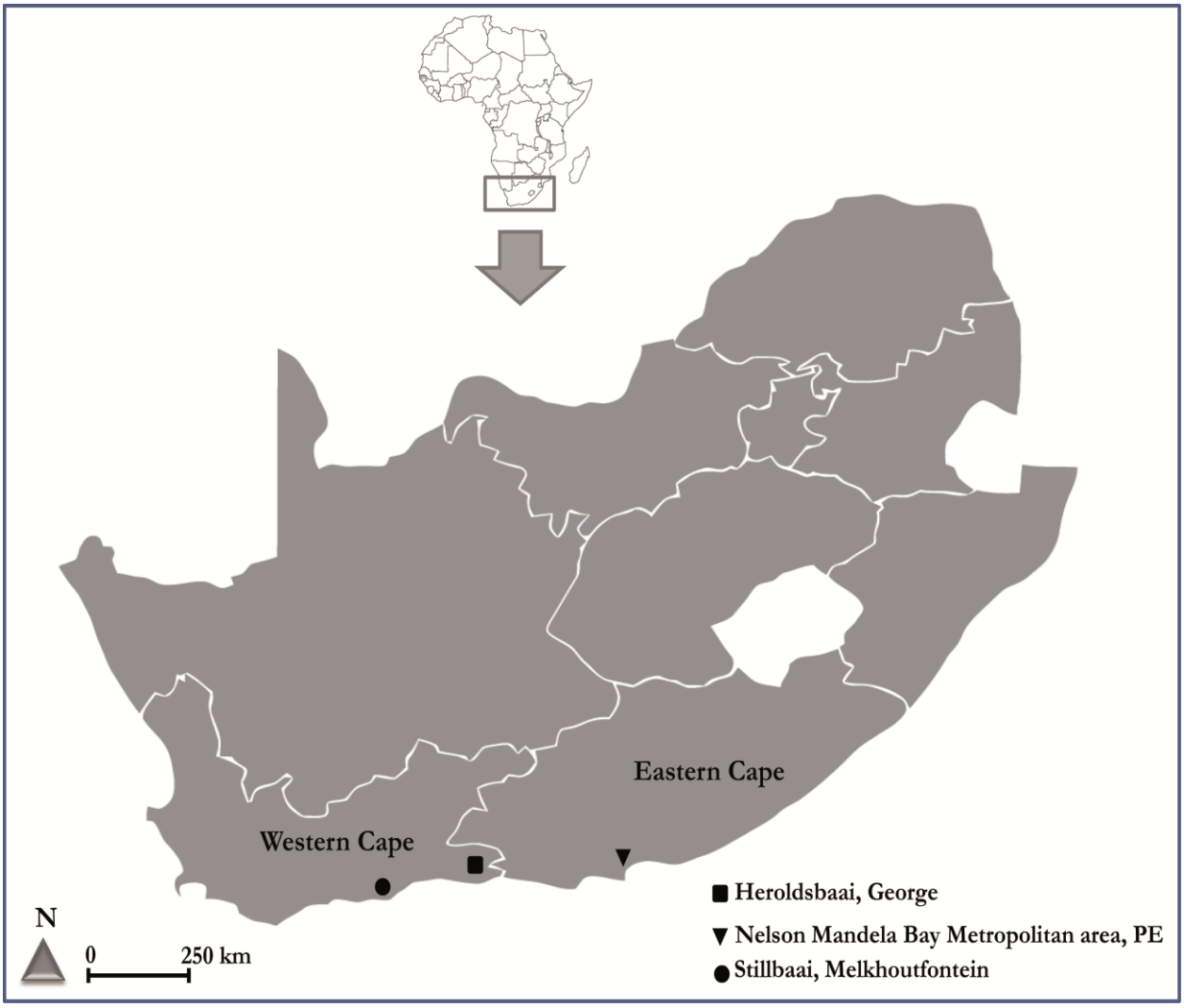

Figure 1. South Africa map showing the sites in the Eastern and Western Cape Provinces where specimens of *Ganoderma* on *Acacia cyclops* were sampled. PE stands for Port Elizabeth.

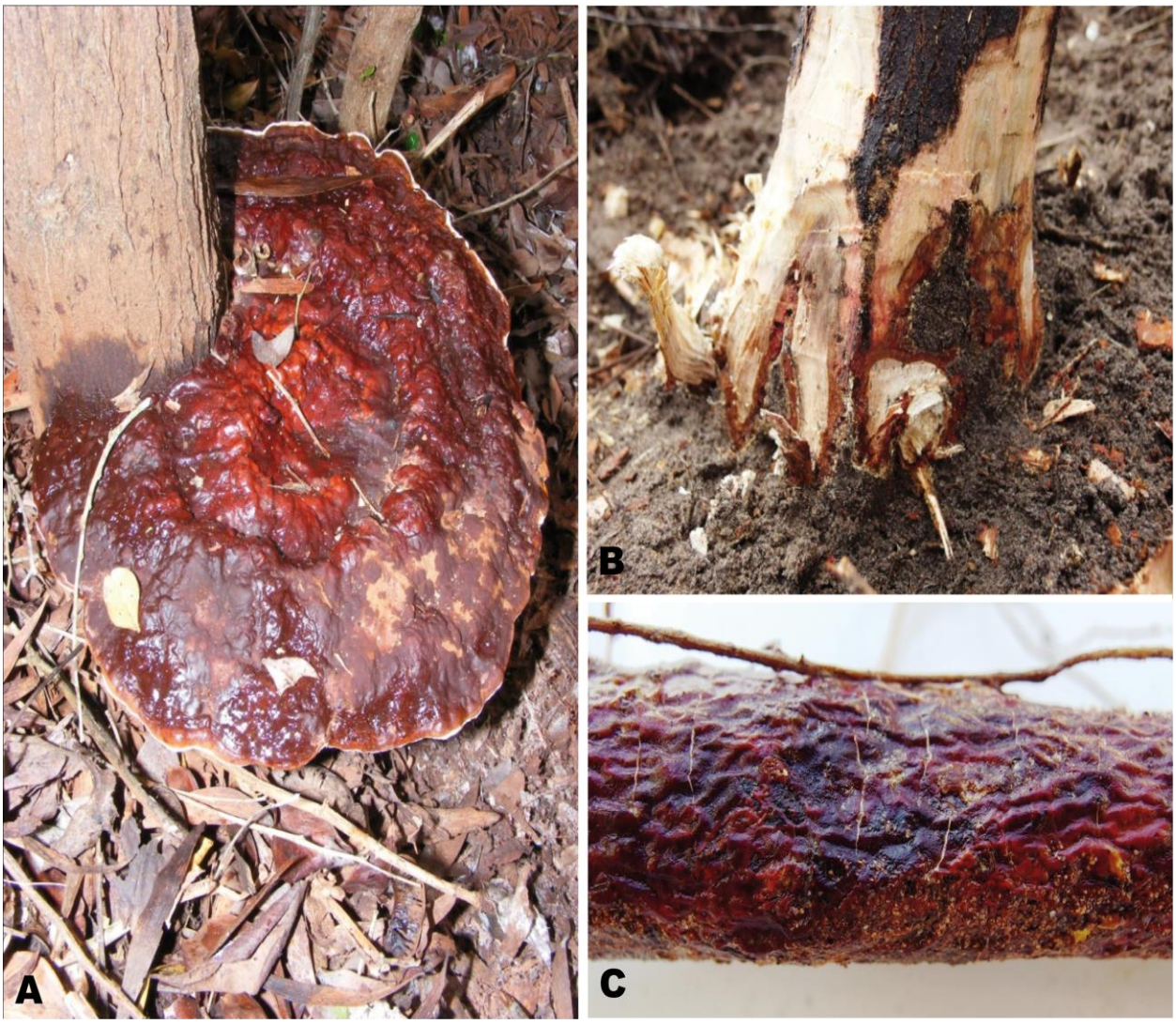

Figure 2. Signs and symptoms of *Ganoderma* species infecting *Acacia cyclops* in South Africa. A. Basidiome of *G. destructans* with reddish brown (laccate shiny) pilear surface attached to the base of wilting tree. B. Collar rot. C. Reddish brown to dark brown rhizomorphic sheet of a *Ganoderma* sp. covering root of dying tree.

Figure 3. Maximum likelihood tree based on ITS sequences, depicting the phylogenetic position of isolates obtained from *Acacia cyclops* (in bold) and related species within *Ganoderma*. Bootstrap values ≥ 70% from 1000 replicates of ML and MP analyses are displayed at the nodes. Posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated at nodes with green circles.

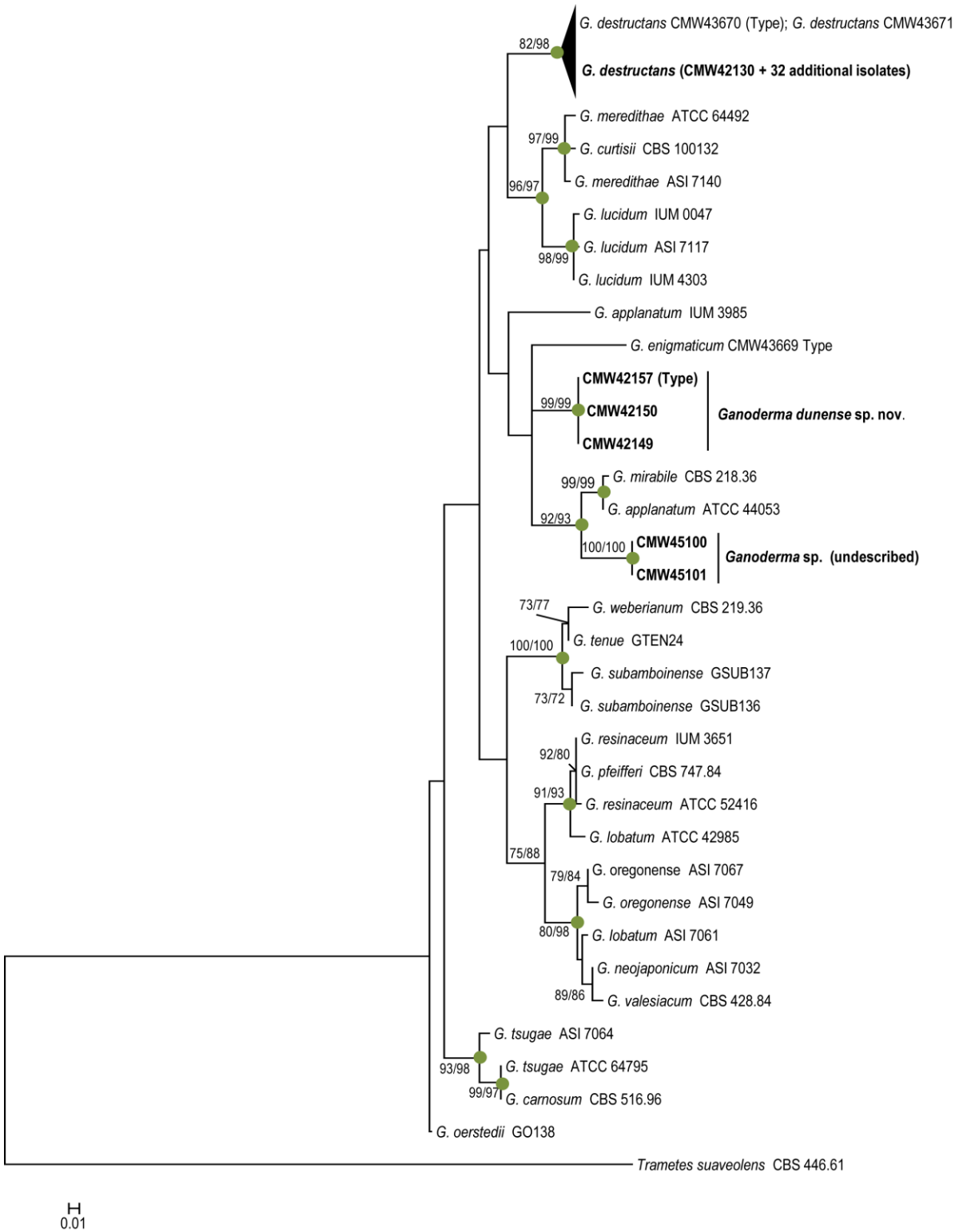

Figure 4. Maximum likelihood tree based on β-Tubulin sequences, depicting the phylogenetic position of isolates obtained from *Acacia cyclops* (in bold) and related species within *Ganoderma*. Bootstrap values ≥ 70% from 1000 replicates of ML and MP analyses are displayed at the nodes. Posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated at nodes with green circles.

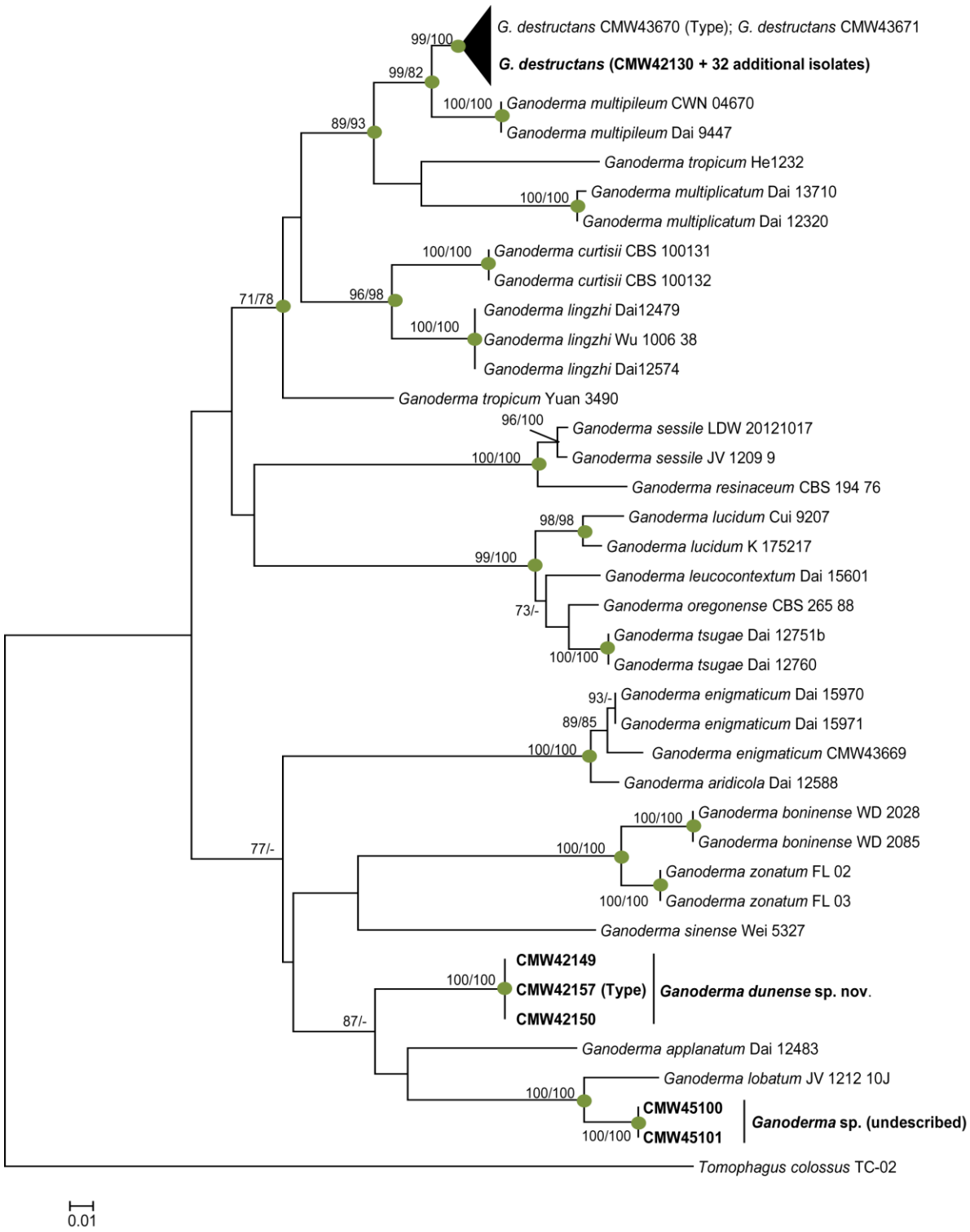

Figure 5. Maximum likelihood tree based on TEF1-α gene sequences, depicting the phylogenetic position of isolates obtained from *Acacia cyclops* (in bold) and related species within *Ganoderma*. Bootstrap values ≥ 70% from 1000 replicates of ML and MP analyses are displayed at the nodes. Posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated at nodes with green circles.

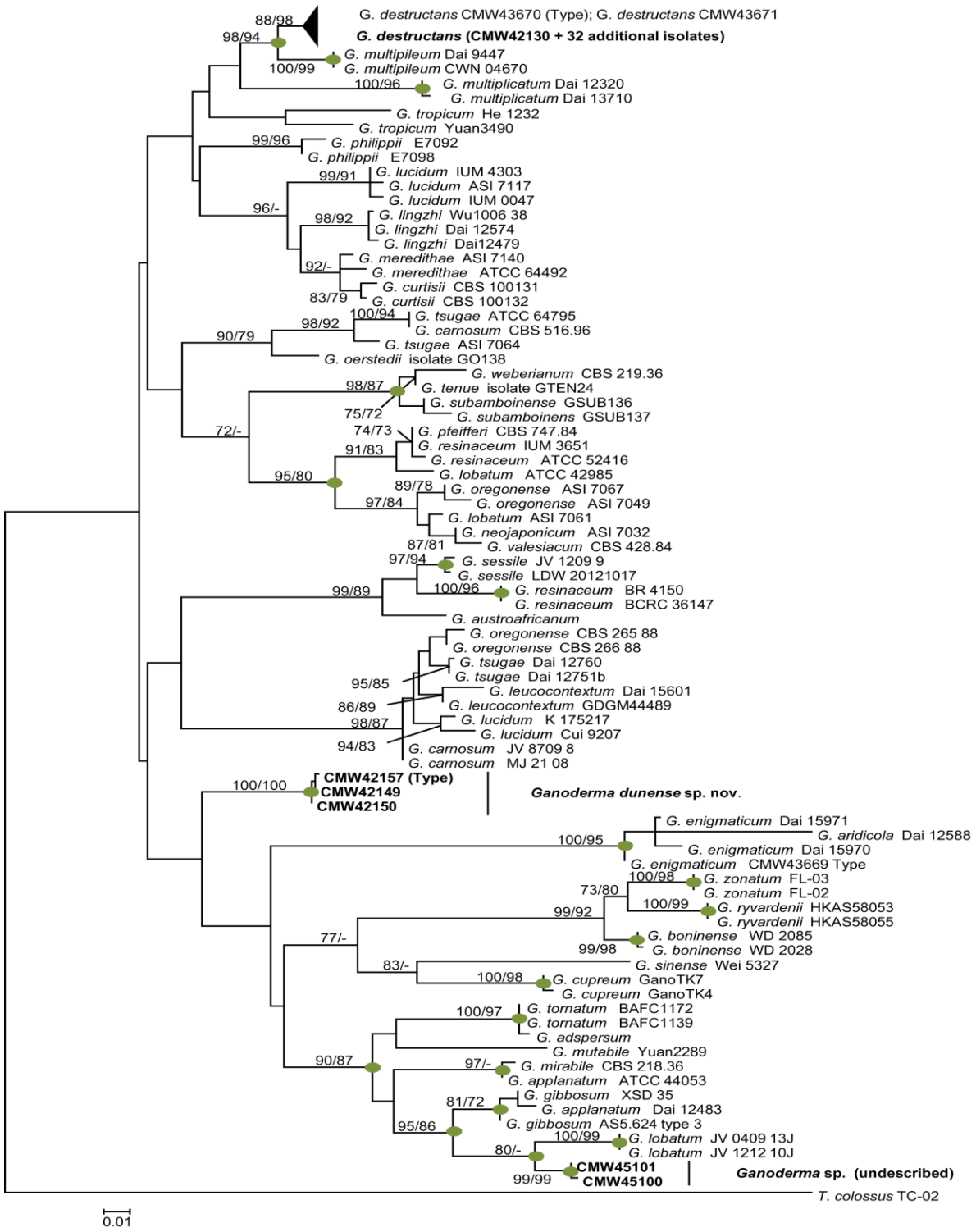

Figure 6. Maximum likelihood tree based on combined dataset of ITS, β-Tubulin and TEF1-α gene sequences, depicting the phylogenetic position of the isolates obtained from *Acacia cyclops* (in bold) and related species within *Ganoderma*. Bootstrap values ≥ 70% from 1000 replicates of ML and MP analyses are displayed at the nodes. Posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated at nodes with green circles

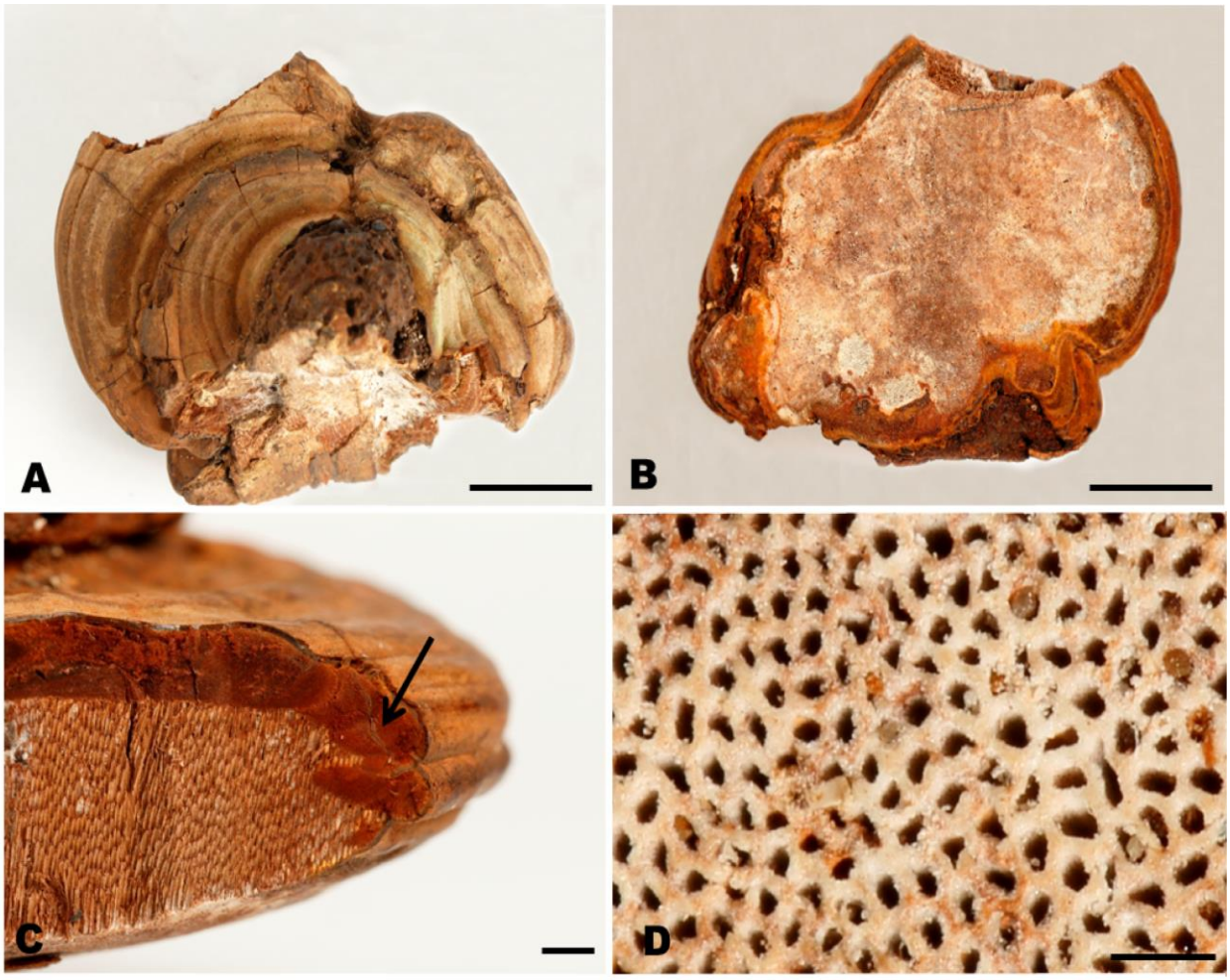

Figure 7. *Ganoderma* sp.: A–B. Pilear and hymenial surfaces of an immature basidiome. C. Homogenous chocolate brown context with discrete resin-like incrustations/deposits (arrowed). D. Pore surface. Bars: A–B = 2 cm; C = 2 mm; D = 1 mm.

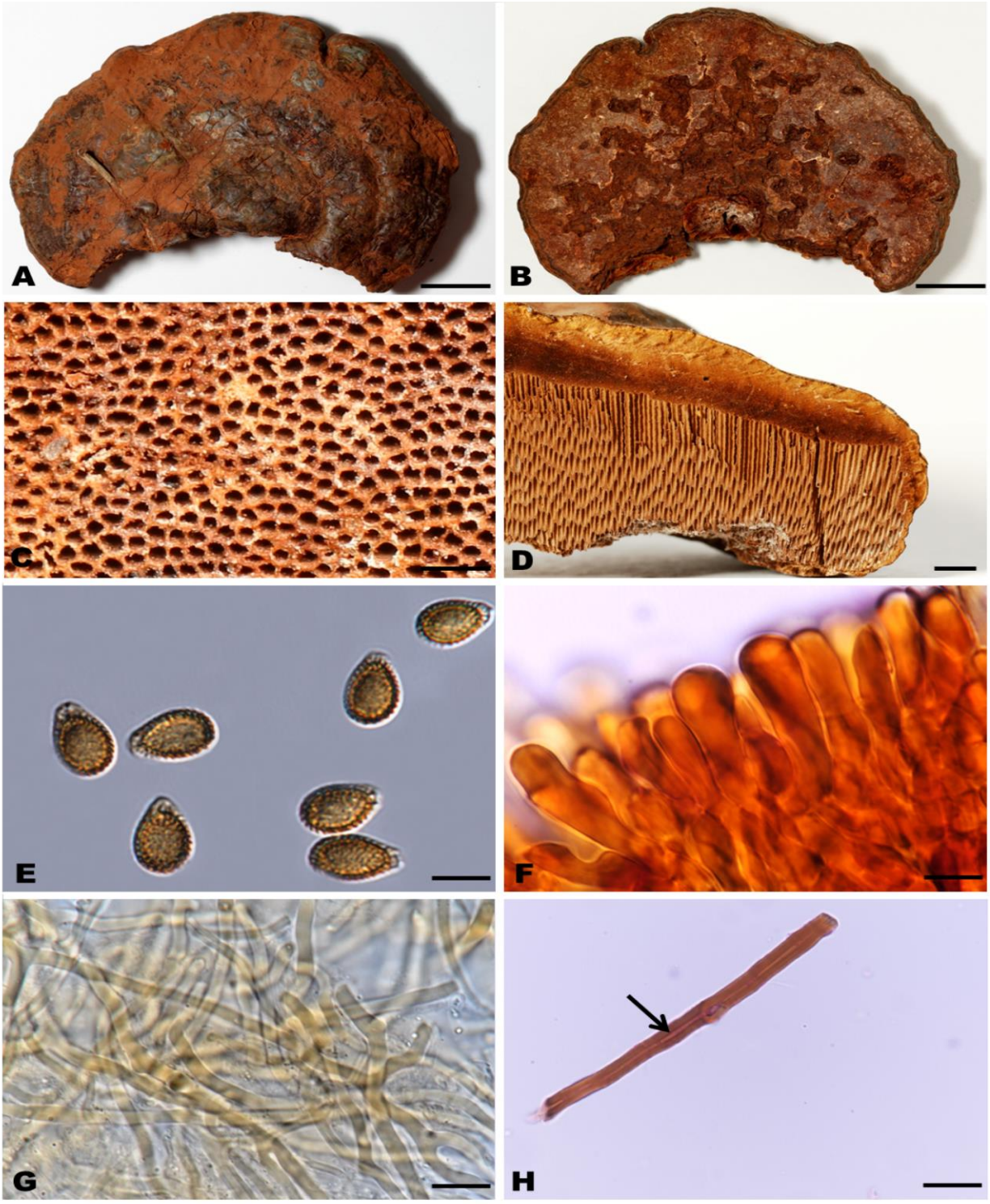

Figure 8. *Ganoderma dunense* sp. nov. (Type, PREM 61936): A–B. Mature reniform-like basidiome showing a pileus surface partially covered with a dull brown deposit of basidiospores and a poroid dark brown hymenial surface. C. Pores. D. Duplex context with a light brown upper layer and a chocolate brown lower layer towards the tubes. E. Basidiospores. F. Palisade of clavi-form cutis cells. G. Skeletal hyphae. H. Skeletal hypha showing a distinct lumen (arrowed). Bars: A–B = 5 cm; C = 1 mm; D = 1 cm; E–H = 10 µm.

CHAPTER 4

TAXONOMY AND SPECIES DIVERSITY OF *GANODERMA* SPECIES IN THE GARDEN ROUTE NATIONAL PARK OF SOUTH AFRICA INFERRED FROM MORPHOLOGY AND MULTI-LOCUS PHYLOGENIES

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ABSTRACT

Ganoderma is a cosmopolitan genus that encompasses species with cultural, economic, and pathogenic importance. Despite the importance of this genus, knowledge pertaining to the species diversity of *Ganoderma* in South Africa is limited. This study aimed at elucidating the identity and phylogenetic placements of *Ganoderma* samples obtained during a survey of woodrotting fungi in the Garden Route National Park (GRNP) of South Africa, supplemented with isolates obtained from other localities across the country. Identification was achieved by means of multilocus phylogenetic inference combined with morphological evaluation. In total, eight distinct species of *Ganoderma* were recovered from different hosts and localities across the country. Of these, *Ganoderma* cf. *cupreum* and *Ganoderma* cf. *resinaceum* represent possible new records for South Africa. Two novel species are described, namely, *G. eickeri*. and *G. knysnamense*. *Ganoderma eickeri*, sp. nov., is characterized by a triquetrous and broadly ttached basidiome, a sulcate or zonate yellowish brown to brown pilear surface, and ovoid to ellipsoid basidiospores. *Ganoderma knysnamense* is distinguished by an applanate to ungulate, sometimes convex, dimidiate to broadly attached basidiome, a chocolate-brown pilear surface covered with a hard woody-like crust and ellipsoid, broadly ellipsoid to ovoid basidiospores. The discovery of two new *Ganoderma* species in this study raises the known *Ganoderma* species in South Africa to 13.

4.1. INTRODUCTION

Ganoderma P. Karst. (Basidiomycota, Polyporales) is a globally distributed genus that encompasses important species for forestry, medicine, and cultural traditions. In forestry, many *Ganoderma* species grow parasitically on a wide variety of woody plants, including those with economic and aesthetic values, on which they cause root rot diseases and/or basal stem rot (Paterson 2007; Glen et al. 2009; Kinge and Mih 2011; Coetzee et al. 2015). Other species of this genus live as saprophytes (Ariffin et al. 2000; Flood et al. 2000). Based on attributed medicinal properties of some of its species, including the treatment of cancer and certain microbial/viral infections (Hapuarachchi et al. 2017), *Ganoderma* species have long been considered amongst some of the most important therapeutic fungi in the world (Pegler 2002; Lai et al. 2004). In Asian cultures, including China for instance, species of *Ganoderma* have been used in traditional medicine for over two millennia and are revered for supposedly mystical powers that bring good fortune, success, and long life (McMeekin 2004). As a result, many Ganoderma-based products are manufactured and marketed globally (Lai et al. 2004).

Ganoderma is an old genus with a taxonomic history dating back to 1881, when the Finnish mycologist Petter Adolf Karsten erected the genus to accommodate a single species, *Polyporus lucidus* [= *G. lucidum* (Curtis: Fr.) P. Karst.], which then became the generic type (Karsten 1881). It is one of the most taxonomically scrutinized genera among the Ganodermataceae and even among the Polypores as a whole (Richter et al. 2015), with nearly 449 epithets listed in Index Fungorum (http://www.indexfungorum.org/names/Names.asp, 9

Apr 2019). However, contemporary fungal-taxonomists still consider its nomenclatural state as controversial and argue for a revision (Ryvarden 1991; Moncalvo and Ryvarden 1997; Kirk et al. 2008; Richter et al. 2015).

For several years, taxonomic studies in *Ganoderma* relied on the analysis of macro- and microscopic morphological characters of the basidiomes to identify and delineate species. However, the use of the "Morphological Species Recognition (MSR) concept" has been proven insufficient to accurately define *Ganoderma* species (Ryvarden 1995; Moncalvo and Ryvarden 1997), as they are highly variable and overlapping, often influenced by geographical origin and environmental conditions (Moncalvo 2000, Kim et al. 2002; Torres-Torres and Gúman-Dávalos 2012). The use of the "Biological Species Recognition (BSR) concept" and the "Phylogenetic Species Recognition (PSR) concept" has significantly improved species identification and delimitation in the genus (Adaskaveg and Gilbertson 1986, Moncalvo et al. 1995; Kinge et al. 2012; Zhou et al. 2015). With the application of the genealogical concordance phylogenetic species recognition (GCPSR) approach, in particular, many *Ganoderma* species poorly bounded morphologically have been recognized and described (Douanla-Meli and Langer 2009; Cao et al. 2012; Zhou et al. 2015; Tchotet Tchoumi et al. 2018). The strength of this approach lies in the fact that it uses the concordance of more than one gene genealogy to indicate a lack of genetic exchange and thus evolutionary independence of lineages that cannot easily be separated based on morphology or that show incomplete intersterility (Taylor et al. 2000; Cai et al. 2011). As a result, this approach has been adopted for the recognition and delineation of *Ganoderma* species in this study.

Despite the advancement in taxonomic studies of *Ganoderma* species diversity, many species are still being discovered, especially in Africa (Douanla-Meli and Langer 2009; Kinge et al. 2012;

Coetzee et al. 2015; Tchotet Tchoumi et al. 2018). According to Baxter and Eicker (1995), 20 *Ganoderma* species are present in Southern Africa (generally accepted to include Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, and Zimbabwe), but this estimation solely relied on morphological criteria for species identification. In South Africa, 11 *Ganoderma* species have been reported (Van der Bijl 1921; Reid 1973; 1974; 1975; Moncalvo and Ryvarden 1997; Crous et al. 2014; Coetzee et al. 2015; Xing et al. 2016; Tchotet Tchoumi et al. 2018). Of these, only six were described based on DNA sequences (Crous et al. 2014; Coetzee et al. 2015; Xing et al. 2016; Tchotet Tchoumi et al. 2018). There is, therefore, a compelling need to update the taxonomy of *Ganoderma* species from Africa with molecular data. It is expected that, as with other fungi, this will lead to description or at least phylogenetic definition of several *Ganoderma* species previously unknown to Science.

During a survey of wood-rot fungi in timber-harvesting compartments of the Garden Route National Park (GRNP) of South Africa, species in the genus *Ganoderma* constituted the most abundant wood-rot fungi associated with trees showing signs of wood-rot (Tchotet Tchoumi et al. 2017). Their affiliation to the genus was confirmed by nuc rDNA ITS1-5.8S-ITS2 (ITS) barcoding, but their species level identities were not established (Tchotet Tchoumi et al. 2017). The objective of this study was to further the identification of these isolates using a combination of morphological and multi-gene DNA sequence data. Unidentified *Ganoderma* samples/specimens, collected from other localities in South Africa, including those preserved at the CMW culture collection of the Tree Protection Cooperative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, were also included in the study.

4.2. MATERIALS AND METHODS

4.2.1. Isolate collection and primary identification

Isolates resembling species of *Ganoderma* from the GRNP were collected from three timber-harvesting compartments during a survey of wood-rotting Basidiomycetes in 2014 and 2015. The 315 obtained isolates were classified into three species level Operational Taxonomic Units (OTUs) based on analysis of sequence variation in the internal transcribed spacer (ITS) region of the rDNA gene cluster, as described by Tchotet Tchoumi et al. (2017). Of the 315 isolates, 303 belonged to OTU1, 11 to OTU4 and one to OTU22 (Tchotet Tchoumi et al. 2017). Twenty-five representative isolates of the three OTUs, from different hosts and different compartments were selected for further analysis (Table 1). Eighteen isolates were from the most abundant OTU (303 isolates recovered), six from the second OTU (11 isolates recovered) and one from the third OTU that was represented by a single isolate (Table 1).

Isolates resembling species of *Ganoderma*, obtained from other regions in South Africa were also included in the study. These isolates were represented by 12 accessions maintained in the CMW culture collection of the University of Pretoria. They were revitalized on 2% malt extract agar (MEA) (Biolab, Midrand, South Africa). Twenty-three more isolates, collected as basidiomes for inclusion in this study, were processed into pure culture plates, following the procedure described by Tchotet Tchoumi et al. (2017). Isolates obtained from basidiomes were preserved in the CMW culture collection, and herbarium specimens were deposited in the herbarium of the South African National Collection of Fungi (PREM), Roodeplaat, Pretoria, South Africa.

All isolates obtained from the CMW culture collection and the newly collected basidiomes were subjected to the same procedure of primary identification as previously described for the GRNP isolates (Tchotet Tchoumi et al. 2017). Briefly, DNA was extracted following the CTAB protocol described by Möller et al. (1992). The ITS (including the ITS-1, 5.8S gene and ITS-2) region was amplified and sequenced, using the ITS1/ITS4 universal primers combination (White et al. 1990). Species level OTUs were delineated based on ITS sequence alignment at a minimum identity threshold of 97% similarity using the software MOTHUR 1.38.0 (Schloss et al. 2009). The taxonomic affiliation of the newly generated OTUs to the genus *Ganoderma* was evaluated by means of Blastn searches in the GenBank nucleotide sequence database, and 24 representative isolates of the delineated OTUs from different hosts and geographic regions were retained for further analyses (Table 2).

4.2.2. PCR amplifications and sequencing of additional gene regions

All South African (RSA) isolates (i.e. 25 from the GRNP and 24 from the new accessions) selected for further studies were amplified and sequenced for two additional markers, including the partial translation elongation factor 1-α (*TEF1-α*) gene and the partial *β-tubulin* gene region. The *TEF1-α* region was amplified and sequenced using primer pair EF595F/EF1160R (Kauserud and Schumacher 2001), while β-tubulin_F/β-tubulin_R (Park et al. 2012) were used for the *β-tubulin* gene region. Amplifications and sequencing protocols were the same as described by Tchotet Tchoumi et al. (2018).

4.2.3. Phylogenetic analyses

Phylogenetic analyses were conducted in two phases and involved seven datasets. In the first phase of analyses, only sequences of the RSA isolates together with out-group sequences were

investigated for the three genes (ITS, *β-tubulin* and *TEF1-α*) using Maximum Likelihood (ML) analysis. Based on the different phylogenetic groups that emerged, at least two (as available) isolates from each group were selected for the second phase of the analyses (Table 3), in which they were examined in combination with reference sequences from GenBank for closely related species. Phylogenetic relationships between these taxa were assessed on the individual gene datasets (ITS, *β-tubulin* and *TEF1-α*) and in a combined multi-locus dataset, using ML, Maximum Parsimony (MP) and Bayesian Inference (BI) analyses.

Forward and reverse sequences were assembled using CLC MAIN WORKBENCH 7.6.1 and multiple sequence alignments were performed using the online programme MAFFT (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley 2013). The resulting alignments were manually edited in MEGA 5.05 (Tamura et al. 2011), and the evolutionary model for nucleotides that best-fit the datasets were selected using JMODELTEST 2.1.6 (Darriba et al. 2012) on CIPRES (Miller et al. 2012) under the Akaike Information Criterion (AIC). The alignments are deposited at TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S24310?x-access-code=373762e611bb758776c60abd2b88c147%26format=html). ML, MP and BI analyses were carried out as outlined in Tchotet Tchoumi et al. (2018). Reference sequences retrieved from GenBank included those used in Glen et al. (2009), Douanla-Meli et al. (2009), Kinge et al. (2012), Park et al. (2012), Zhou et al. (2015), Coetzee et al. (2015), Xing et al. (2016) and Tchotet Tchoumi et al. (2018) (supplementary Table 1). Out-group taxa were represented by *Tomophagus colossus* (Fr.) C.F. Baker for the ITS and *TEF1-α* datasets, and *Trametes suaveolens* (L.) Fr. for the *β-tubulin* dataset (Xing et al. 2016; Tchotet Tchoumi et al. 2018).

4.2.4. Morphological characterization

Basidiome morphology were examined using a Zeiss Stemi SV6 stereo microscope and a Nikon Eclipse N*i* compound microscope (Nikon, Japan) at a magnification of up to 100 times under immersion oil. Characteristic micro-morphological structures were studied by mounting fine tissue sections of dried basidiomes in 5% KOH (potassium hydroxide) supplemented with or without 1% phloxine and Melzer's reagent (IKI- = nonamyloid, IKI+ = amyloid). The structures were measured using the program NIS-Elements BR (Nikon Instruments Software-Elements Basic research), and images were captured using a Nikon DS-Ri2 camera mounted on the microscope. Measurements are presented in minimum-maximum values.

4.3. RESULTS

4.3.1. ITS sequencing and primary identification of the new accessions

The ITS gene region was amplified and sequenced successfully for the 35 new accessions. Clustering of the ITS sequences of these isolates in MOTHUR resulted in the delineation of seven OTUs. Blastn search results in the GenBank nucleotide sequence database confirmed that the seven delineated OTUs were taxonomically affiliated with *Ganoderma*.

4.3.2. Sequencing and phylogenetic analyses of additional gene regions

A total of 49 isolates, including 25 from the GRNP and 24 from the new accessions, constituted the subset referred to as South African (RSA) isolates. Nucleotide sequence data of the *β-tubulin* and *TEF1-α* genes were successfully obtained for all these isolates. DNA sequencing of the isolates yielded amplicons of approximately 420 and 560 base pairs respectively for the *β-tubulin* and *TEF1-α* loci. Best models of nucleotide substitution estimated and applied on sequences of

the three individual gene (ITS, *β-tubulin* and *TEF1-α*) datasets of RSA isolates alone were GTR+G+I for the ITS dataset and GTR+G for the *β-tubulin* and *TEF1-α* datasets. ML analysis of these datasets resulted, for the three genes, in the distinction of eight consistent phylogenetic lineages (RSA1-8; see Supplementary Figures. 1- 3).

Phylogenetic information of the four datasets, ITS, *β-tubulin*, *TEF1-α* and combined gene regions, containing the representative isolates from each South African phylogenetic lineage and the accessions from GenBank, are summarized in supplementary Table 2. The topology of phylograms and the phylogenetic relationships among taxa of these datasets were overall congruent for ML, MP and BI phylogenetic analyses (Figures1 to 4). Phylograms of the phylogenetic analyses using different methods, for individual loci and the concatenated dataset, consistently recovered the same eight South African phylogenetic lineages (RSA1-8) (Figures 1 to 4). Thus, only ML topologies are presented incorporating the statistical values of the two other analyses.

Phylogenetic analysis of ITS sequences resulted in the identification and classification of South African isolates into six previously described species and two novel species (Figure 1). The phylogenetic lineages corresponding to species already described were RSA3 affiliated with G. australe (Fr.) Pat., RSA4 with *G. enigmaticum* M.P.A. Coetzee, Marinc. & M.J. Wingf., RSA5 with *G. cupreum* (Sacc.) Bres., RSA6 with *G. austroafricanum* M.P.A. Coetzee, M.J. Wingf., Marinc. & Blanchette, RSA7 with *G. resinaceum* Boud., and RSA8 with *G. destructans* M.P.A. Coetzee, Marinc. & M.J. Wingf., as well as the recently described *G. dunense* Tchotet, Rajchenb. & Jol. Roux (Tchotet Tchoumi et al. 2018). These lineages formed wellsupported clades with high bootstrap support values. The two unidentified lineages, RSA1 and RSA2, formed distinct and isolated groups in the phylogeny. Isolates of RSA1 resolved into a well-supported

monophyletic clade, whereas those of RSA2 formed a monophyletic clade with isolates of the undescribed *Ganoderm*a taxon from A. cyclops in the Eastern Cape Province of South Africa (see Tchotet Tchoumi et al. 2018).

Phylogenies derived from *β-tubulin* and *TEF1-α* loci recovered the same South African lineages as in the phylogeny of the ITS gene, despite slight conflicts in the relationships between taxa. Sequences of the *β-tubulin* and *TEF1-α* genes are not available for all reference isolates used. Thus, unlike in the phylogeny of the ITS locus, only three lineages (RSA4 and RSA8) in the *β-tubulin* phylogeny (Figure 2) and five (RSA1, RSA4, RSA5, RSA7 and RSA8) in the *TEF1-α* phylogeny (Figure 3), clustered with previously described species in their respective phylogenetic trees.

In *β-tubulin* analyses, RSA4 and RSA8 formed strongly supported phylogenetic clades with type sequences of *G. enigmaticum* and *G. destructans*, respectively. The remaining six lineages formed well-resolved phylogenetic clades. Of these, lineages RSA1 and RSA2 resolved into well-distinct and isolated monophyletic clades, with high statistical support similar to what was obtained in the ITS phylogeny. RSA3 and RSA7 also resolved into wellsupported monophyletic clades, whereas RSA5 and RSA6 did not cluster with any known *Ganoderma* species.

In the TEF1-α phylogeny, the RSA1 lineage formed a strongly supported monophyletic clade with isolates of the recently described *G. dunense*. The other lineages, comprising RSA4, RSA5, RSA7, and RSA8 grouped respectively with *G. enigmaticum*, *G. sinense* J.D. Zhao, L.W. Hsu & X.Q. Zhang, *G. resinaceum*, and *G. destructans*. The three remaining lineages, RSA2, RSA3, and RSA6, occurred at isolated positions. Isolates of lineage RSA2, as observed in the ITS and β-tubulin phylogenies, formed a strongly supported monophyletic clade with sequences of the

undescribed *Ganoderma* species from *A. cyclops*. Those of RSA3 clustered into a monophyletic clade with strong statistical support, and RSA6 did not cluster with any described species.

Phylogenetic analysis of the concatenated dataset resulted in a robust phylogeny that recovered the eight South African lineages and resolved the conflicts observed with the individual gene trees (Figure 4). Six of the RSA lineages grouped with known *Ganoderma* species, and two formed novel lineages. RSA3 clustered with *G. australe*, RSA4 with *G. enigmaticum*, and RSA5 clustered with *G. cupreum*. RSA6, RSA7, and RSA8 clustered with *G. austroafricanum*, *G. resinaceum*, and *G. destructans*, respectively. The last two lineages RSA1 and RSA2 consistently clustered into two well-resolved and distinct monophyletic clades supported by high bootstrap values. RSA1 was sister to *G. dunense*, but with no statistical support. RSA2 grouped with the undescribed *Ganoderma* species from *A. cyclops*.

4.3.3. Taxonomy

Results from phylogenetic analyses of sequences of the three combined loci revealed that the *Ganoderma* isolates from South Africa examined in this study represented eight distinct taxa, including two novel species for which morphological characterization is provided as follows:

Ganoderma knysnamense Tchotet, M.P.A. Coetzee, Rajchenb. & Jol. Roux sp. nov. Figure 5

MycoBank: MB 826941

Typification: SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, Bloukrans (S33° 56.825' E23° 35.200'), attached to a stump of *Olea capensis* subsp. *macrocarpa* (C. H. Wright) I. Verd., 06 July 2015, J.M. Tchotet Tchoumi, JMT745 (**holotype**-PREM 62155, culture ex-type

CMW47755 = PPRI 29125). GenBank accession number: ITS = MH571681, *β-tubulin* = MH567305, *TEF1-α* = MH567261.

Diagnosis: Morphologically, *Ganoderma knysnamense* resembles species in the subgen. *Elfvingia* P. Karst. It is characterized by a perennial, applanate to ungulate, sometimes convex, and dimidiate to broadly attached basidiome. Pilear surface chocolate-brown covered with a hard woody crust. Context thin with white streaks of mycelium. Hymenial surface poroid, white when fresh. Hyphal system trimitic and basidiospores ellipsoid, broadly ellipsoid to ovoid, (9.2–) 10.4–12.1 × 7.8–8.7 µm, double walled with hyaline exosporium, light brown, with tooth-like or dentate pillars endosporium, IKI-.

Etymology: knysnamense refers to the Knysna indigenous forests, also known as the Garden Route National Park (GRNP) indigenous forests, in the Southern Cape region of South Africa, where this fungus is commonly found growing on native trees.

Description: ***Basidiomes*** perennial, applanate to ungulate, sometimes convex, dimidiate to broadly attached but with narrow attachment for ungulate specimens, up to 13–30 cm wide, 7.5–18.5 cm radius, and 3.3–10.5 cm thick at base, woody-like, light when dry, hard in consistency. Margins circular and slightly lobate. ***Pilear surface*** non-laccate, covered with a crust, chocolate-brown, color more or less homogenous but darker zones present towards the base, dull, smooth, narrowly zonate, somewhat cracked or rimose towards the base; crust up to 3 mm thick at the base and thinning to 0.5–1 mm towards the margin, chocolate-brown, with a black line against the context, dull to resinous-like and sometimes cracking in older parts. ***Context*** thin, dark brown, up to 2 mm thick, inlaid with white streaks of mycelium, melanoid deposits absent. ***Tubes*** light greyish to whitish brown, not stratified, up to 3.4 cm long. ***Hymenial surface*** poroid,

white-cream, pores round to ellipsoid, 4–5 per mm; dissepiments thick. ***Pileipellis*** a crustotrichodermis composed of sclerified hyphae strongly incrusted in a resinous-like matter. ***Hyphal system*** trimitic. Generative hyphae clamped, thin-walled 2–4 µm diam. Skeletal hyphae thick-walled unbranched or branched with knob-like protuberances, honey colored to light chestnut, 3–5(–6) µm diam. Skeleto-binding hyphae with a basal unbranched stem that ramifies into several narrower flagelliform branches up to 1–2 µm diam. Ligative hyphae with protuberant, knob-like to shortly branched or with digitiform ramifications present. Dissepiments with a similar hyphal system, hyphae 4 µm as the largest diameter; ligative hyphae more abundant. Wider hyphae are darker in color than the narrower ones. ***Basidia*** not seen. ***Basidiospores*** ellipsoid, broadly ellipsoid to ovoid, truncate, double-walled, outer wall hyaline and smooth, inner wall light brown, with tooth-like or dentate pillars that are longitudinally arranged in rows, (9.2–) 10.4–12.1 × 7.8–8.7 µm, IKI–.

Other specimen examined: SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, Bloukrans (S33° 56.825' E23° 35.200'), attached to the trunk of a living *O. capensis* subsp. *macrocarpa* tree, 06 July 2015, J.M. Tchotet Tchoumi, JMT746 (**paratype**-PREM 62156, culture ex-type CMW47756 = PPRI 29126). GenBank accession number: ITS = MH571684, *β-tubulin* = MH567306, *TEF1-α* = MH567274.

Remark: *Ganoderma knysnamense*, sp. nov., is apparently restricted to the southern Cape region of South Africa. Our entire collection of this species have been derived from the GRNP forest compartments distributed in the Eastern (Tsitsikamma) and Western (Diepwalle and Gouna) Cape provinces. Table 4 presents a morphological comparison between the new species and its closest phylogenetic neighbors, including other South African species.

Ganoderma eickeri Tchotet, M.P.A. Coetzee, Rajchenb. & Jol. Roux sp. nov. Figure 6

MycoBank: MB 826942

Typification: SOUTH AFRICA, Mpumalanga, Mbombela, formerly Nelspruit (S25°26'38.486" E30°58'14.887"), at the base of *Prunus africana* (Hook.f.) Kalkman, 06 September 2016, T. Paap, JMT1315 (**holotype**-PREM 62157, culture ex-type CMW49692 = PPRI 29127). GenBank accession number: ITS = MH571690, *β-tubulin* = MH567318, *TEF1-α* = MH567287.

Diagnosis: Morphologically *Ganoderma eickeri* resembles species in the subgen. *Elfvingia.* It is characterized by a perennial, dimidiate, broadly attached and triquetrous basidiome. Pileus surface yellowish brown to brown, slightly radially cracked and sulcate up to zonate. Context homogenous and thick. Hymenial surface poroid, white when fresh. Hyphal system trimitic and basidiospores ovoid to ellipsoid with distinct truncated apex, 8.5–11 × 5–7 µm, double-walled, outer-wall hyaline with inter-wall pillars, inner-wall yellowish brown with coarse echinulae, IKI–.

Etymology: eickeri is given in honour of Professor Albert Eicker who has contributed significantly to the knowledge of Basidiomycetes in South Africa.

Description: ***Ganoderma eickeri*** is morphologically similar to species in *Ganoderma* subgen. *Elfvingia.* ***Basidiome*** perennial, dimidiate, broadly attached, triquetrous, 8–12.5 cm wide, up to 5.5–6.3 cm in radius and 4.5–5 cm thick at base; margins regular and round; of woody consistency, light when dry. ***Pileus surface*** yellowish brown to brown, dull, glabrous and slightly radially cracked; sulcate up to zonate, with each furrow built by several years of growth; crust thin. ***Pileipellis*** a crustotrichodermis. ***Hymenial surface*** poroid, white when fresh turning greyish white upon bruising; pores round to ellipsoid, 4–5 per mm; dissepiment well developed.

Context homogenous, soft corky to light woody, up to 4 mm thick, chocolate brown; white mycelial streaks present. ***Tube layer*** up to 2.5 cm long, stratified, light brown to chocolate brown. ***Hyphal system*** trimitic; generative hyphae hyaline, thin-walled and clamped, 2–4 µm diam., branched; skeletal hyphae chestnut, light brown to brown, thick-walled, with a narrow to wide lumen, unbranched to rarely so, 3–7 µm diam; skeleto-binding hyphae consisting of an unbranched basal stem that ramifies progressively forming an upper much branched, arboriform hyphae, the basal part 3–4 µm diam, light brown, the arboriform hyphae 1–2 µm diam, flagelliform to dendritically branched, 'openly' branched or very much branched, forming or not protuberances (formed at the branching of the hyphae or along the hypha), thick-walled, with hyaline walls. In dissepiments the hyphae are similar but much tightly arranged. ***Cystidia*** and ***basidia*** not seen. ***Basidiospores*** ovoid to ellipsoid with distinct truncated apex, double-walled, outer-wall hyaline with inter-wall pillars, inner-wall yellowish brown with coarse echinulae, 8.5–11 × 5–7 µm, IKI–.

Other specimen examined: South Africa, Eastern Cape Province, Port Elizabeth, Nelson Mandela Bay Metropolitan area, attached to stump of an *A. cyclops* tree, 20 April 2015, J.M. Tchotet Tchoumi, M.P.A. Coetzee and J. Roux, JMT641 (**paratype**-PREM 62158, culture ex-type CMW45101 = CBS 143578). GenBank accession number: ITS = MG020265; *β-tubulin* = MG020155; *TEF1-α* = MG020230.

Remark: Morphological comparison between Ganoderma eickeri, sp. nov., and its closest phylogenetic neighbors, including other South African species, are shown in Table 4.

4.4. DISCUSSION

Prior to this study, 11 species of *Ganoderma* had been recognized in South Africa (Van der Bijl 1921; Reid 1973, 1974, 1975; Moncalvo and Ryvarden 1997; Crous et al. 2014; Coetzee et al. 2015; Xing et al. 2016; Tchotet Tchoumi et al. 2018). Results from multilocus phylogenetic analyzes combined with morphological characterization performed during this study showed a greater species diversity, with the addition of newly described species and species not previously reported from the country. In total, eight distinct species of *Ganoderma* were recovered from different hosts and localities across the country. Of these, six were identified as belonging to *Ganoderma australe* (RSA3), *G. enigmaticum* (RSA4), *Ganoderma* cf. *cupreum* (RSA5), *Ganoderma* aff. *austroafricanum* (RSA6), *Ganoderma* cf. *resinaceum* (RSA7), and *G. destructans* (RSA8) and two others are new species described as *G. knysnamense* (RSA1) and *G. eickeri* (RSA2). The isolates from the GRNP resolved into three distinct species, namely, *Ganoderma* cf. *cupreum*, *Ganoderma australe*, and *G. knysnamense*, sp. nov. The last two species were also recovered among the isolates from other localities. *Ganoderma* cf. *cupreum* and *Ganoderma* cf. *resinaceum* are new records for South Africa. The discovery of two new species of *Ganoderma* in this study raises the total known *Ganoderma* species in South Africa to 13.

RSA5 (*Ganoderma* cf. *cupreum*) and RSA7 (*Ganoderma* cf. *resinaceum*) are new records for South Africa based on their genetic affinities, in the combined multilocus phylogeny, with two species not previously reported in the country, namely, *G. cupreum* and *G. resinaceum*. Both lineages formed well-resolved and statistically well-supported phylogenetic clades with sequences representing these two taxa (*G. cupreum* and *G. resinaceum*) in GenBank. RSA5 was obtained from infected woody tissues of Pterocelastrus tricuspidatus, a native tree in the GRNP

forest (Tchotet Tchoumi et al. 2017), whereas isolates of RSA7 were recovered from three provinces of the country and were mainly associated with Acacia species from Africa and perhaps also from Australia, as some trees could not be identified up to the specific level.

RSA5 and RSA7 were given a preliminary identity because of some sources of uncertainty regarding the identity of *G. cupreum* and *G. resinaceum*. Wang et al. (2014) demonstrated that *G. cupreum* was conspecific to several other *Ganoderma* species, including *G. mastoporum* (Lév.) Pat., *G. densizonatum* J.D. Zhao & X.Q. Zhang, *G. limushanense* J.D. Zhao & X.Q. Zhang, *G. orbiforme* (Fr.) Ryvarden, *G. subtornatum* Murrill, and *G. fornicatum* (Fr.) Pat., with *G. orbiforme* being the nomenclatural priority of this group. RSA5 and *G. orbiforme*, however, occupied distinct positions in the global multilocus phylogeny. *Ganoderma resinaceum* is known to be a Northern Hemisphere species, mainly occurring in Europe (Moncalvo et al. 1995; Ryvarden and Melo 2014), and results of the combined phylogeny indicate that it represents a species complex.

In the combined phylogeny, RSA6 lineage clustered with the type sequence of *G. austroafricanum*, a species originally described as a new species pathogenic on Jacaranda (*Jacaranda mimosifolia* D. Don) trees in Pretoria, South Africa (Crous et al. 2014). However, despite their close phylogenetic relationship, RSA6 was named *Ganoderma* aff. *austroafricanum* because the clade it formed with this taxon did not receive any statistical support in the three phylogenetic analyzes (ML, MP, and BI), and RSA6 in this clade had a long branch that indicated dissimilarity of sequences between the two lineages. This sequence dissimilarity between RSA6 and *G. austroafricanum* was confirmed by BLAST searches, which revealed that the ITS sequence of lineage RSA6 had rather high similarity (97%) to sequences representing *G. subamboinense* var. *laevisporum* Bazzalo & J.E. Wright and *G. weberianum* (Bres. & Henn. ex

Sacc.) Steyaert. in GenBank. Thus, from the foregoing, it appears that RSA6 lineage probably represents a novel species. Additional analyzes will certainly help to clarify its identity.

RSA3, RSA4, and RSA8 lineages were identified as belonging respectively to *G. australe*, *G. enigmaticum*, and *G. destructans*, three species previously reported in South Africa (Van der Bijl 1921; Moncalvo and Buchanan 2008; Coetzee et al. 2015). *Ganoderma australe* is a globally distributed white rot fungus (Ryvarden and Johansen 1980; Corner 1983; Moncalvo and Buchanan 2008), whose holotype collected in the Pacific Islands was lost (Moncalvo and Ryvarden 1997). Isolates of RSA3 were attributed to this species based on phylogenetic results consistent with those of Moncalvo and Buchanan (2008). In their study, these authors showed that two isolates representing *G. australe* from South Africa formed a strongly supported monophyletic clade with isolates of this species from other countries. Using the same reference isolates, including both reported from South Africa, the phylogenetic results of ITS and the combined loci in this study showed that RSA3 isolates were the same as those reported by these authors and therefore belong to G. australe. This phylogenetic identification was also confirmed by results of BLAST search, which revealed that ITS sequences of RSA3 had 100% similarity to those of G. australe from Brazil.

Van der Bijl (1921) was the first to report the presence of *G. australe* in South Africa, based on classical taxonomy. He highlighted some discriminating traits, including a harder crust, a scantier context, and much longer pores, that distinguish this species from its closest relative, *G. applanatum* (Pers.) Pat. However, it is through the study by Moncalvo and Buchanan (2008) that the first molecular identification of *G. australe* in South Africa was made. This study, however, is the first to produce both molecular and morphological data of *G. australe* in South Africa. The

fact that basidiomes of this taxon were collected from eight different hosts in four provinces of the country indicates that it is not host restricted and should be common in South Africa.

RSA4 and RSA8 were identified as *G. enigmaticum* and *G. destructans*, respectively, due to their strong phylogenetic relationships with the type sequences of these two taxa in the combined gene phylogeny (Coetzee et al. 2015). Both lineages also share the same geographic origin (South Africa) with the type specimens of these two taxa, and their primary identification, based on BLAST search of the three gene regions (ITS, BT, and TEF), also revealed that RSA4 and RSA8 had high sequence similarity (100%) to the type sequences of *G. enigmaticum* and *G. destructans* in GenBank. *Ganoderma enigmaticum* was recently described as a new species parasitizing the base of the trunk of *Ceratonia siliqua* in Gauteng Province, South Africa (Coetzee et al. 2015). In this study, the basidiome collected for this species was attached to the base of a *Combretum* sp. in Gauteng Province. This discovery not only enriches the collection of this species for the country but also expands its known host range. The new collection of *G. enigmaticum* on a different host also indicates that more of this species is likely to be found on other hosts and elsewhere in the country if further investigations are conducted.

Ganoderma destructans was described as a new species pathogenic on Jacaranda trees in Pretoria, Gauteng Province, South Africa (Coetzee et al. 2015). The species was recently reported as causal agent of root and butt rot disease on non-native Acacia cyclops trees in the Western Cape Province of South Africa (Tchotet Tchoumi et al. 2018). Added to the two Provinces (Gauteng and Western Cape) in which *G. destructans* had already been reported, it has now also been found in the Limpopo Province and from eight additional hosts, supporting the suggestion of Tchotet Tchoumi et al. (2018) that this fungus has a wide geographic distribution

in South Africa. This result also reinforces the hypothesis that this fungus is indigenous to South Africa (Coetzee et al. 2015; Tchotet Tchoumi et al. 2018).

Phylogenetic analyses of the three combined gene regions as well as morphological characterization did not assign lineages RSA1 and RSA2 to previously described *Ganoderma* species. Hence, they were treated as novel species and described as *G. knysnamense*, sp. nov., for RSA1 and *G. eickeri*, sp. nov., for RSA2. The newly described *G. dunense* from South Africa (Tchotet Tchoumi et al. 2018) was the closest phylogenetic relative of *G. knysnamense*, with which it shared a common ancestor in the combined phylogeny. However, this clade did not receive any statistical support, and both lineages also resolved into distinct and well-supported monophyletic subclusters. Additionally, both species differ in their habitat and host associations. *Ganoderma dunense* was associated with diseased and dying trees of the non-native *A. cyclops* in Heroldsbaai, George, Western Cape Province (Tchotet Tchoumi et al. 2018), whereas *G. knysnamense* was collected only in the GRNP where it was also the most abundant fungus associated with wood rot symptoms on native trees, particularly on *Olea capensis* subsp. *macrocarpa* (Tchotet Tchoumi et al. 2017). Both species are also easily distinguishable morphologically. Contrary to *G. knysnamense*, *G. dunense* is characterized macroscopically by a laccate pilear surface and a duplex context, and microscopically by the presence of cutis cells of a hymeniodermis and ovoid basidiospores (Tchotet Tchoumi et al. 2018; Table 4).

Ganoderma eickeri, sp. nov., was closely related to *G. gibbosum* (Blume & T. Nees) Pat. and *G. lobatum* (Schwein.) G.F. Atk. in the phylogeny inferred from the concatenated sequence data set. However, the three lineages clustered into three distinct subclades, with statistical support only for the subclades of *G. eickeri* and *G. lobatum*. The phylogenetic divergence between these two species and *G. eickeri*, sp. nov., is also supported morphologically. Although they all belong to

the subgenus *Elfvingia*, they are easily distinguishable by some macroand microscopic characters of their basidiomes. Contrary to *G. eickeri*, *G. lobatum* has a sessile, reniform to dimidiate shape, reddish brown to dark reddish brown pileus surface, a thicker context (up to 12 mm wide), and ovoid basidiospores (Steyaert 1980; Gilbertson and Ryvarden 1986; Gottlieb and Wright 1999b; Table 4). *Ganoderma gibbosum*, contrary to the new species, is characterized by stipitate, dimidiate to subflabelliform basidiomes and much smaller ovoid basidiospores (Zhao 1989; Table 4). When compared with each other, *G. knysnamense* and *G. eickeri* occupied distinct and distant positions in the global multilocus phylogenetic tree, and the morphology of their basidiomes also exhibits very distinct macro- and microscopic characters that can further differentiate the two species. Thus, based on convergent results from morphology and molecular data analyses, *G. knysnamense* and *G. eickeri* are considered to be new species to science.

It is currently widely accepted that laccate and nonlaccate *Ganoderma* species belong to subgenera Ganoderma and Eflvingia, respectively (Gottlieb and Wright 1999a, 1999b; Table 4). Smith and Sivasithamparam (2000) reported that the phylogeny derived from ITS sequences of five *Ganoderma* species from Australia also favored the retention of these two subgenera. However, the result of the global multilocus phylogeny obtained in this study calls into question this subdivision. *Ganoderma* species with laccate and nonlaccate pileus did not form two distinct groups in the phylogenetic tree, as might be expected. Moreover, the new species *G. knysnamense* was sister to the laccate *G. dunense*. A scenario already observed with the recently described laccate *G. mutabile* (Cao and Yuan 2013). This *Ganoderma* species was also sister to nonlaccate species of the *G. applanatum* group in the phylogeny by Cao and Yuan (2013). These observations, therefore, suggest that the laccate or nonlaccate trait commonly used for the

demarcation of *Ganoderma* species at the subgeneric level does not always reveal true phylogenetic relationships.

Species of *Ganoderma* have been consistently reported to cause damage to a wide variety of host trees in various ecosystems (Flood et al. 2000). In this study, the eight identified *Ganoderma* species occurred in seven of the country's nine provinces and were associated with numerous native and non-native hosts (Tables 1 and 2). The wide geographic and host distribution of *Ganoderma* species observed in this study emphasizes the potential threat and impact of these fungi. Species of *Ganoderma* can indiscriminately attack urban and forest trees, causing in some cases heavy economic losses in sectors such as agriculture, horticulture, forestry, and/or the timber industry (Sankaran et al. 2005). Notable examples include *G. ryvardense* associated with basal stem rot disease of oil palm in Cameroon (Kinge and Mih 2011); *G. destructans* associated with root and butt rot disease of ornamental Jacaranda trees in South Africa (Coetzee et al. 2015); and the newly described *G. knysnamense*, sp. nov., which was the main taxon associated with wood rot symptoms on declining native trees in the GRNP indigenous forest of South Africa (Tchotet Tchoumi et al. 2017).

Factors such as human activities and wind-borne dispersal of basidiospores (Gonthier et al. 2004;Moncalvo and Buchanan 2008) are some of the disseminating factors that may have facilitated the wide geographic distribution of *Ganoderma* species as observed in this study, particularly for species that occurred in more than one locality. This is not unprecedented because evidence of long distance dispersal by human activities and airborne basidiospores has previously been reported within root-rotting fungi in general and in *Ganoderma* in particular (e.g., Coetzee et al. 2001; Gonthier et al. 2004;Moncalvo and Buchanan 2008).

DNA multilocus phylogenetic inference combined with morphological evaluation performed in this study revealed greater species diversity in *Ganoderma* from South Africa than previously known. Isolates from the GRNP represented three species of *Ganoderma*, including the newly described *G. knysnamense*. The species of *Ganoderma* from *A. cyclops* that could not be identified in Tchotet Tchoumi et al. (2018) was also described here as *G. eickeri*, sp. nov., based on the examination of more mature and fertile specimens. The continual discovery of new species, as is shown in this study and other recent studies (e.g., Coetzee et al. 2015; Tchotet Tchoumi et al. 2018), suggests that many more *Ganoderma* species are likely to be discovered in South Africa and indicates that further research iswarranted on this important genus in the country and in Africa in general.

4.5. REFERENCES

Adaskaveg JE, Gilbertson RL, 1986. Culture studies and genetics of sexuality of *Ganoderma lucidum* and *G. tsugae* in relation to the taxonomy of the *G. lucidum* complex. *Mycologia* 78, 694–705.

Baxter AP, Eicker A, 1995. Preliminary synopsis: recorded taxa of southern African Ganodermataceae. In: *Ganoderma*: systematics, phytopathology and pharmacology (Buchanan RK, Hseu RS, Moncalvo JM, eds): 3–5. Proceedings Contributed Symposium 59A, B 5th International Mycological Congress Vancouver, August 14-21 1994. Taipei: R.S. Hseu.

Bishop KS, Kao CHJ, Xu Y, Glucina MP, Paterson RRM, Ferguson LR, 2015. From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. *Phytochemistry* 114, 56–65.

Blaalid R., Kumar S, Nilsson RH, Abarenkov K, Kirk PM, Kauserud H, 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources* 13, 218–24.

Cao Y, Wu SH, Dai YC, 2012. Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi". *Fungal Diversity* 56, 49–62.

Coetzee MPA, Marincowitz S, Muthelo VG, Wingfield MJ, 2015. *Ganoderma* species, including new taxa associated with root rot of the iconic *Jacaranda mimosifolia* in Pretoria, South Africa. *IMA Fungus* 6, 249–256.

Coetzee MPA, Wingfield BD, Harrington TC, Steimel J, Coutinho TA, Wingfield MJ, 2001. The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* 10, 387–396.

Crous PW, Wingfield MJ, Schumacher RK, Summerell BA, Giraldo A, Gené J, Guarro J, Wanasinghe DN, Hyde KD, Camporesi E, Gareth Jones EB, Thambugala KM, Malysheva EF, Malysheva VF, Acharya K, Álvarez J, Alvarado P, Assefa A et al., 2014. Fungal Planet description sheets: 281–319. *Persoonia* 33, 212–289.

Darriba D, Taboada GL, Doallo R, Posada D, 2012. "jModelTest 2: more models, new heuristics and parallel computing". *Nature Methods* 9, 772.

Douanla-Meli C, Langer E, 2009. *Ganoderma carocalcareus* sp. nov., with crumbly-friable context parasite to saprobe on *Anthocleista nobilis* and its phylogenetic relationship in *G. resinaceum* group. *Mycological Progress* 8, 145–155.

Flood J, Bridge PD, Holderness M (Eds), 2000. *Ganoderma* diseases of perennial crops. (CABI Publishing: Wallingford, UK) 275 pp.

Gilbertson RL, Ryvarden L, 1986. North American Polypores 1. *Fungiflora* Oslo Norway, 287–308.

Glen M, Bougher NL, Francis AA, Nigg SQ, Lee SS, Irianto R, Barry KM, Beadle CL, Mohammed CL, 2009. *Ganoderma* and *Amauroderma* species associated with root-rot disease of *Acacia mangium* plantation trees in Indonesia and Malaysia. *Australasian Plant Pathology* 38, 345–356.

Gonthier P, Warner R, Nicolotti G, Mazzaglia A, Garbelotto M, 2004. Pathogen introduction as a collateral effect of military activity. *Mycological Research* 108, 468–470.

Jong SC, Birmingham JM, 1992. Medicinal benefits of the mushroom *Ganoderma*. *Advances in Applied Microbiology* 37, 101–34.

Karsten PA, 1881. Enumeratio boletinearum et poly-porearum fennicarum. Systemate novo dispositarum. *Review Mycology* 3, 16–19.

Katoh K, Standley DM, 2013. MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30, 772–780.

Kauserud H, Schumacher T, 2001. Outcrossing or inbreeding: DNA markers provide evidence for type of reproductive mode in *Phellinus nigrolimitatus* (Basidiomycota). *Mycological Research* 53, 220–230.

Kim HK, Shim MY, Seo GS, Kim HG, 2002. Comparison of characteristics of *Ganoderma lucidum* according to geographical Origins (III): Classification between species of genus *Ganoderma* using dikaryon- monokaryon mating. *Mycobiology* 30, 61–64.

Kinge TR, Mih AM, 2011. *Ganoderma ryvardense* sp. nov. associated with basal stem rot (BSR) disease of oil palm in Cameroon. *Mycosphere* 2, 179–188.

Kinge TR, Mih AM, Coetzee MPA, 2012. Phylogenetic relationships among species of *Ganoderma* (Ganodermataceae, Basidiomycota) from Cameroon. *Australian Journal of Botany* 60, 526–538.

Kirk PM, Cannon PF, Minter DW, Stalpers JA, 2008. Dictionary of the fungi, 10th edn. CAB International, Wallingford.

Lai T, Gao Y, Zhou S, 2004. Global marketing of medicinal ling Zhi mushroom *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd (Aphyllophoromycetideae) products and safety concerns. *International Journal of Medicinal Mushrooms* 6, 189–194.

McMeekin D, 2004. The perception of *Ganoderma lucidum* in Chinese and Western culture. *Mycologist* 18, 165–169.

Miller MA, Pfeiffer W, Schwartz T, 2010. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1–8.

Möller EM, Bahnweg G, Sandermann H, Geiger HH, 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 20, 6115–6116.

Moncalvo JM, 2000. Systematics of *Ganoderma*. In: Ganoderma diseases of perennial crops. Flood J, Bridge PD and Holderness M, 2000. CABI, Wallingford, UK.

Moncalvo JM, Buchanan PK, 2008. Molecular evidence for long distance dispersal across the Southern Hemisphere in the *Ganoderma applanatum-australe* species complex (Basidiomycota). *Mycological Research* 112, 425–436.

Moncalvo JM, Ryvarden L, 1997. A nomenclatural study of the Ganodermataceae Donk. Synopsis Fungorum 11. *Fungiflora* : Oslo, Norway.

Moncalvo JM, Wang HF, Hseu RS, 1995. Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters. *Mycological Research* 99, 1489–1499.

Park Y, Kwon O, Son E, Yoon D, Han W, Yoo Y, Lee C, 2012. Taxonomy of *Ganoderma lucidum* from Korea Based on rDNA and Partial *β-tubulin* Gene Sequence Analysis. *Mycobiology* 40, 71–75.

Paterson RRM, 2006. Ganoderma – a therapeutic fungal biofactory. *Phytochemistry* 67, 1985–2001.

Paterson RRM, 2007. Ganoderma disease of oil palm-A white rot perspective necessary for integrated control. *Crop Protection* 26, 1369–1376.

Pegler DN, 2002. Useful Fungi of the World: the Ling-zhi - The mushroom of immortality. Mycologist 16, 100–101.

Ramasamy S, 1972. Cross-infectivity and decay ability of *Ganoderma* species parasitic to rubber, oil palm and tea. In: Ganoderma diseases of perennial crops. Flood J, Bridge PD and Holderness M, 2000. CABI, Wallingford, UK.

Reid DA, 1973. A reappraisal of type and authentic specimens of Basidiomycetes in the Van der Byl Herbarium, Stellenbosch. *Journal of South African Botany* 39, 141–178.

Reid DA, 1974. A reappraisal of type and authentic material of the larger Basidiomycetes in the Pretoria Herbarium. *Bothalia* 11, 221–230.

Reid DA, 1975. Type studies of the larger Basidiomycetes described from southern Africa. *Contributions from the Bolus Herbarium* 7, 1–255.

Richter C, Wittstein K, Kirk P, Stadler M, 2015. An assessment of the taxonomy and chemotaxonomy of *Ganoderma*. *Fungal Diversity* 71, 1–15.

Ryvarden L, 1991. Genera of polypores. Nomenclature and taxonomy. Synopsis Fungorum 5, *Fungoflora*, Oslo, Norway.

Ryvarden L, 1995. Can we trust morphology in *Ganoderma*? In: Buchanan PK, Hsea RS, Moncalvo JM (eds) Proceedings of Contributed Symposium 59. Fifth International Mycological Congress, Vancouver, Canada, pp 19–24.

Ryvarden L, Melo I, 2014. Poroid fungi of Europe. *Synopsis Fungorum* 31, 1–455.

Ryvarden, Leif, 2000. "Studies in neotropical polypores 2: a preliminary key to neotropical species of *Ganoderma* with a laccate pileus". *Mycologia* 92, 180–191.

Sankaran KV, Bridge PD, Gokulapalan C, 2005. Ganoderma diseases of perennial crops in India – an overview. *Mycopathologia* 159, 143–152.

Schloss PD, Westcott SL, Ryabin T, Justine R. Hall, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF, 2009. Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75, 7537–41.

Steyaert RL, 1972. Species of *Ganoderma* and related genera mainly of the Bogor and Leiden herbaria. *Persoonia* 7, 55–118.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731-2739.

Tchotet Tchoumi JM, Coetzee MPA, Vivas M, Rajchenberg M, Roux J, 2017. Wood-rotting basidiomycetes associated with declining native trees in timber-harvesting compartments of the Garden Route National Park of South Africa. *Austral Ecology* 42, 947–963.

Tchotet Tchoumi JM, Coetzee MPA, Rajchenberg M, Wingfield MJ, Roux J, 2018. Three *Ganoderma* species, including *Ganoderma dunense* sp. nov., associated with dying *Acacia cyclops* trees in South Africa. *Australasian Plant Pathology* 47, 431–447.

Torres-Torres MG; Gúman-Dávalos L, 2012. The morphology of *Ganoderma* species with a laccate surface. *Mycotaxon* 119, 201–216.

Van der Bijl PA, 1921. A contribution to our knowledge of the Polyporeae of South Africa. *South African Journal of Science* 18, 246-293.

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds), PCR Protocols: a guide to methods and applications. Academic Press, New York. pp. 315–322.

Worrall JJ. 1991. Media for selective isolation of hymenomycetes. *Mycologia* 83, 296–02.

Xing JH, Song J, Decock C, Cui BK, 2016. Morphological characters and phylogenetic analysis reveal a new species within the *Ganoderma lucidum* complex from South Africa. *Phytotaxa* 266, 115–124.

Zhou LW, Cao Y, Wu SH, Vlasák J, Li DW, Li MJ, Dai YC, 2015. Global diversity of the *Ganoderma lucidum* complex (Ganodermataceae, Polyporales) inferred from morphology and multi-locus phylogeny. *Phytochemistry* 114, 7–15.

TABLE 1. Representative isolates of the three OTUs from the three sampled forest compartments in the GNRP (Tchotet Tchoumi et al. 2017) used for DNA sequencing of *β-tubulin* and *TEF1-α* gene regions.

OTU	Isolates	Host	Source	Origin (Province)	Collector
OTU1	CMW44729	*Olea capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [d]	J.M. Tchotet Tchoumi, 2014
	CMW44742	*O. capensis subsp. macrocarpa*	Infected wood	GRNP, Western Cape [d]	J.M. Tchotet Tchoumi, 2014
	CMW44760	Unknown	Basidiocarp	GRNP, Western Cape [d]	J.M. Tchotet Tchoumi, 2014
	CMW44761	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [d]	J.M. Tchotet Tchoumi, 2014
	CMW45294	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [g]	J.M. Tchotet Tchoumi, 2014
	CMW45323	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [g]	J.M. Tchotet Tchoumi, 2014
	CMW45335	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [g]	J.M. Tchotet Tchoumi, 2014
	CMW45341	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [g]	J.M. Tchotet Tchoumi, 2014
	CMW45814	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [d]	J.M. Tchotet Tchoumi, 2014
	CMW47755	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47756	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47770	*Pterocelastrus tricuspidatus*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47777	*P. tricuspidatus*	Infected wood	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47783	*P. tricuspidatus*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47787	*Acacia melanoxylon*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47832	*Elaeodendron croceum*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015

TABLE 1. (continued)

OTU	Isolates	Host	Source	Origin (Province)	Collector
OTU1	CMW47878	*Apodytes dimidiata subsp.dimidiata*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW48063	*E. croceum*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
OTU4	CMW47767	*O. capensis subsp. macrocarpa*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47785	*A. melanoxylon*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47819	*Ocotea bullata*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW47821	*Robsonodendron eucleiforme*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW48137	*A. melanoxylon*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
	CMW48146	*P. tricuspidatus*	Basidiocarp	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015
OTU22	CMW48134	*P. tricuspidatus*	Infected wood	GRNP, Western Cape [b]	J.M. Tchotet Tchoumi, 2015

[b]Bloukrans forest compartment; [d]Diepwalle forest compartment; [g]Gouna forest compartment

TABLE 2. List of additional isolates from other Provinces in South Africa included in this study and their delineation into OTUs at 97% identity threshold based on ITS sequence data.

OTUs	Isolates	Identity	Host	Source	Origin (Province)	Collector
OTU1	CMW25883*	*Ganoderma destructans*	*Spathodea campanulata*	CMW culture Collection	Limpopo	J Roux, 2006
	CMW25902	*G. destructans*	*Vachellia xanthophloea*	CMW culture Collection	Gauteng	J Roux, 2007
	CMW49707*	*G. destructans*	*Dracaena braunii*	Basidiocarp	Limpopo	MPA Coetzee, 2017
	CMW49708*****	*G. destructans*	Unknown	Basidiocarp	Limpopo	MPA Coetzee, 2017
	CMW49713*	*G. destructans*	*Celtis africana*	Basidiocarp	Gauteng	F. Fru, 2017
	CMW50314	*G. destructans*	*Acacia* sp.	Basidiocarp	Gauteng	T Paap, 2017
	CMW50315	*G. destructans*	*Acacia* sp.	Basidiocarp	Gauteng	T Paap, 2017
	CMW50316	*G. destructans*	*Dracaena* sp.	Basidiocarp	Gauteng	T Paap, 2017
	CMW50317*****	*G. destructans*	*V. xanthophloea*	Basidiocarp	Gauteng	T Paap, 2017
	CMW50319*	*G. destructans*	*Senegalia nigrescens*	Basidiocarp	Gauteng	T Paap, 2017
	CMW50320*	*G. destructans*	*Searsia chirindensis*	Basidiocarp	Gauteng	T Paap, 2017
	CMW50321	*G. destructans*	*S. chirindensis*	Basidiocarp	Gauteng	T Paap, 2017
	CMW50322	*G. destructans*	*S. chirindensis*	Basidiocarp	Gauteng	T Paap, 2017
	CMW50323	*G. destructans*	*S. chirindensis*	Basidiocarp	Gauteng	T Paap, 2017
	CMW50324	*G. destructans*	*S. chirindensis*	Basidiocarp	Gauteng	T Paap, 2017

TABLE 2. (continued)

OTUs	Isolates	Identity	Host	Source	Origin (Province)	Collector
OTU2	CMW25881	*G.* cf. *resinaceum*	Unknown	CMW culture Collection	Gauteng	MPA Coetzee, 2006
	CMW25895*	*G.* cf. *resinaceum*	*Vachellia tortilus*	CMW culture Collection	Gauteng	B Slippers, 2006
	CMW25900*	*G.* cf. *resinaceum*	*Acacia* sp.	CMW culture Collection	Eastern Cape	MPA Coetzee, 2007
	CMW49711*****	*G.* cf. *resinaceum*	*Acacia* sp.	Basidiocarp	Gauteng	L Eksteen, 2017
	CMW50326*****	*G.* cf. *resinaceum*	Unknown	Basidiocarp	Kwa-zulu Natal	T Paap, 2017
OTU3	CMW25877*	*G. australe*	*Salix* sp.	CMW culture Collection	Free State	MPA Coetzee, 2005
	CMW25897*	*G. australe*	*Salix babylonica*	CMW culture Collection	Gauteng	R Heath, 2006
	CMW49694*	*G. australe*	Unknown	Basidiocarp	Western Cape	J Roux, 2016
	CMW49697*	*G. australe*	Unknown	Basidiocarp	Mpumalanga	J Roux, 2016
	CMW50311*	*G. australe*	*Olinia ventosa*	Basidiocarp	Western Cape	T Paap, 2016
OTU4	CMW49688*****	*G. knysnamense* sp. nov.	Unknown	CMW culture Collection	Western Cape	J Roux, 2014
	CMW49689	*G. knysnamense* sp. nov.	Unknown	CMW culture Collection	Western Cape	J Roux, 2014
	CMW49690	*G. knysnamense* sp. nov.	Unknown	CMW culture Collection	Western Cape	J Roux, 2014
	CMW49691*****	*G. knysnamense* sp. nov.	Unknown	CMW culture Collection	Western Cape	J Roux, 2014

TABLE 2. (continued)

OTUs	Isolates	Identity	Host	Source	Origin (Province)	Collector
OTU5	CMW49692*****	*G. eickeri* sp. nov.	*Prunus africana*	Basidiocarp	Mpumalanga	T Paap, 2016
	CMW49705*	*G. eickeri* sp. nov.	Unknown Palm species	Basidiocarp	Limpopo	J Roux, 2016
	CMW50313*	*G. eickeri* sp. nov.	Unknown	Basidiocarp	Western Cape	T Paap, 2016
	CMW50325*****	*G. eickeri* sp. nov.	*C. africana*	Basidiocarp	Kwa-zulu Natal	T Paap, 2017
OTU6	CMW50318*****	*G. enigmaticum*	*Combretum* sp.	Basidiocarp	Gauteng	T Paap, 2017
OTU7	CMW25884*****	*G.* aff. *austroafricanum*	Unknown	CMW culture Collection	Limpopo	J Roux, 2006

*Representative isolates selected for further studies

TABLE 3. GenBank accession numbers of South African isolates selected for the second phase of phylogenetic analyses

Species	**Voucher no.**	**Geographical origin**	**GenBank accession numbers**		
			ITS	***β-tubulin***	***TEF1-α***
Ganoderma australe	CMW47785	South Africa	MH571686	MH567310	MH567276
G. australe	CMW48146	South Africa	MH571685	MH567309	MH567283
G. australe	CMW49694	South Africa	MH571687	MH567312	MH567277
G. australe	CMW49697	South Africa	MH571688	MH567311	MH567280
G. aff. *austroafricanum*	CMW25884	South Africa	MH571693	MH567321	MH567296
G. cf. *cupreum*	CMW48134	South Africa	MH571696	MH567313	MH567291
G. destructans	CMW49708	South Africa	MH571694	MH567314	MH567303
G. destructans	CMW50317	South Africa	MH571695	MH567315	MH567299
G. eickeri sp. nov	CMW49692 (Type)	South Africa	MH571690	MH567318	MH567287
G. eickeri sp. nov	CMW50325	South Africa	MH571689	MH567317	MH567290
G. enigmaticum	CMW50318	South Africa	MH571697	MH567316	MH567297
G. knysnamense sp. nov	CMW47755 (Type)	South Africa	MH571681	MH567316	MH567261
G. knysnamense sp. nov	CMW47756	South Africa	MH571684	MH567305	MH567274
G. knysnamense sp. nov	CMW49688	South Africa	MH571683	MH567306	MH567266
G. knysnamense sp. nov	CMW49691	South Africa	MH571682	MH567307	MH567267
G. cf. *resinaceum*	CMW49711	South Africa	MH571691	MH567319	MH567295
G. cf. *resinaceum*	CMW50326	South Africa	MH571692	MH567320	MH567294

TABLE 4. Morphological comparison of *G. knysnamense* and *G. eickeri* spp. novs. with their closest relatives in the combined phylogeny

Taxon	Shape	Pileus	*Cutis anatomy	Subgen.	Pores	Spores (µm)	Distribution	Reference
Ganoderma aridicola	sessile, dimidiate	laccate	hymeniodermis	*Ganoderma*	round, 6–8 per mm	broadly ellipsoid, 9.7–11.2 × 7–7.8	South Africa	Xing et al. 2016
G. australe	sessile, dimidiate	non-laccate	trichodermis	*Elfvingia*	round, 4–4.5 per mm	ellipsoid to narrowly ovoid, 10–11 × 6–7	South Hemisphere countries SA, Argentina, Chile, New Zealand, Australia	This study
G. boninense	irregular, dimidiate to stipitate, sub-ungulate	laccate	hymeniodermis	*Ganoderma*	circular, 90–380 (av. 155) µm diam.	ellipsoid, 8.5–13.5 (av. 10.9) × 4.5–7 (av. 5.9)	throughout the Indonesian and Pacific Islands and eastern states of Australia	Steyaert 1967
G. cupreum (= *G. orbiforme sensu* Wang et al. 2014)	sessile to stipitate	laccate	hymeniodermis	*Ganoderma*	circular or subcircular, 5–7(–10)/mm	ellipsoid to ovoid, brown, (8.52)9.5–12.56 × (5.52)6–7(7.5)	Guinea, Bonin island, Puerto Rico, Venezuela, China	Wang et al. 2014
G. dunense	sessile but with a lateral mucro, dimidiate	laccate	hymeniodermis	*Ganoderma*	round, 3–4 per mm	ovoid, 10.9–12.3 × 7.3–8.5	South Africa	Tchotet et al. 2018

TABLE 4. (continued)

Taxon	Shape	Pileus	*Cutis anatomy	Subgen.	Pores	Spores (µm)	Distribution	Reference
G. eickeri sp. nov.	dimidiate, triquetrous	non-laccate	trichodermis	*Elfvingia*	round to ellipsoid, 4–5 per mm	ovoid to ellipsoid, 8.5–11 × 5–7	South Africa	This study
G. enigmaticum	stipitate globular	laccate	hymeniodermis	*Ganoderma*	round, 3–5 per mm	ellipsoid, 8–11 × 3.5–6	South Africa	Coetzee et al. 2015
G. gibbosum	stipitate, dimidiate to subflabelliform	non-laccate	subanamixodermis	*Elfvingia*	round, 4–5 per mm	ovoid, 6.9–8.7 × 5–5.2	Java, China	Zhao 1989
G. knysnamense sp. nov.	applanate to ungulate, convex, dimidiate	non-laccate	trichodermis	*Elfvingia*	round to ellipsoid, 4–5 per mm	ellipsoid, broadly ellipsoid to ovoid, (9.2–)10.4–12.1 × 7.8–8.7	South Africa	This study
G. lobatum	sessile, reniform to dimidiate	non-laccate	anamixodermis	*Elfvingia*	round, 4–5 per mm	ovoid, 7.5(9.3)–11 × 5(6.3)–7	USA	Steyaert 1980
G. mirabile	sessile, dimidiate	non-laccate	anamixodermis	*Elfvingia*	round, 100–125–150 µm diam.	ovoid to subspherical, 7–8.5 × 5.5–6.5	Philippine islands, Malaysia	Steyaert 1972
G. mutabile	sessile, semicircular or shell-shaped	laccate	hymeniodermis	*Ganoderma*	circular or angular, 4–5 per mm	broadly ellipsoid, (9.2–)9.7–11.2(−12) × (6.8–)7–7.8(−8.4)	China	Cao & Yuan 2013

TABLE 4. (continued)

Taxon	Shape	Pileus	*Cutis anatomy	Subgen.	Pores	Spores (µm)	Distribution	Reference
G. pfeifferi	sessile, semicircular	laccate	hymeniodermis	*Ganoderma*	circular, 5-6 per mm	ellipsoid, 9–11.5 × 6–9	south and central Europe, Indonesia	Ryvarden & Gilbertson 1993
G. sinense	stipitate, dimidiate, suborbicular or subcochleariform	laccate	hymeniodermis	*Ganoderma*	round, 5–6 per mm	ovoid, 9–12 × 6.5–8	China	Zhao 1989
G. ryvardenii	dimidiate, concave and circular	laccate	hymeniodermis	*Ganoderma*	angular; 2–4 per mm	ellipsoid, (9–)10–13(–14) × (5–)6–8	Cameroon	Kinge & Mih 2011
G. zonatum	dimidiate, sessile, applanate to convex	laccate	hymeniodermis	*Ganoderma*	regular to polygonal, (3) 4–5/mm	ellipsoid, (10–)11–14 × 5–7 (–8)	USA, Ghana, Nigeria, Gabon, Central Rep., the two Congos	Steyaert 1967; Gottlieb and Wright 1999

*Gottlieb & Wright 1999a,b

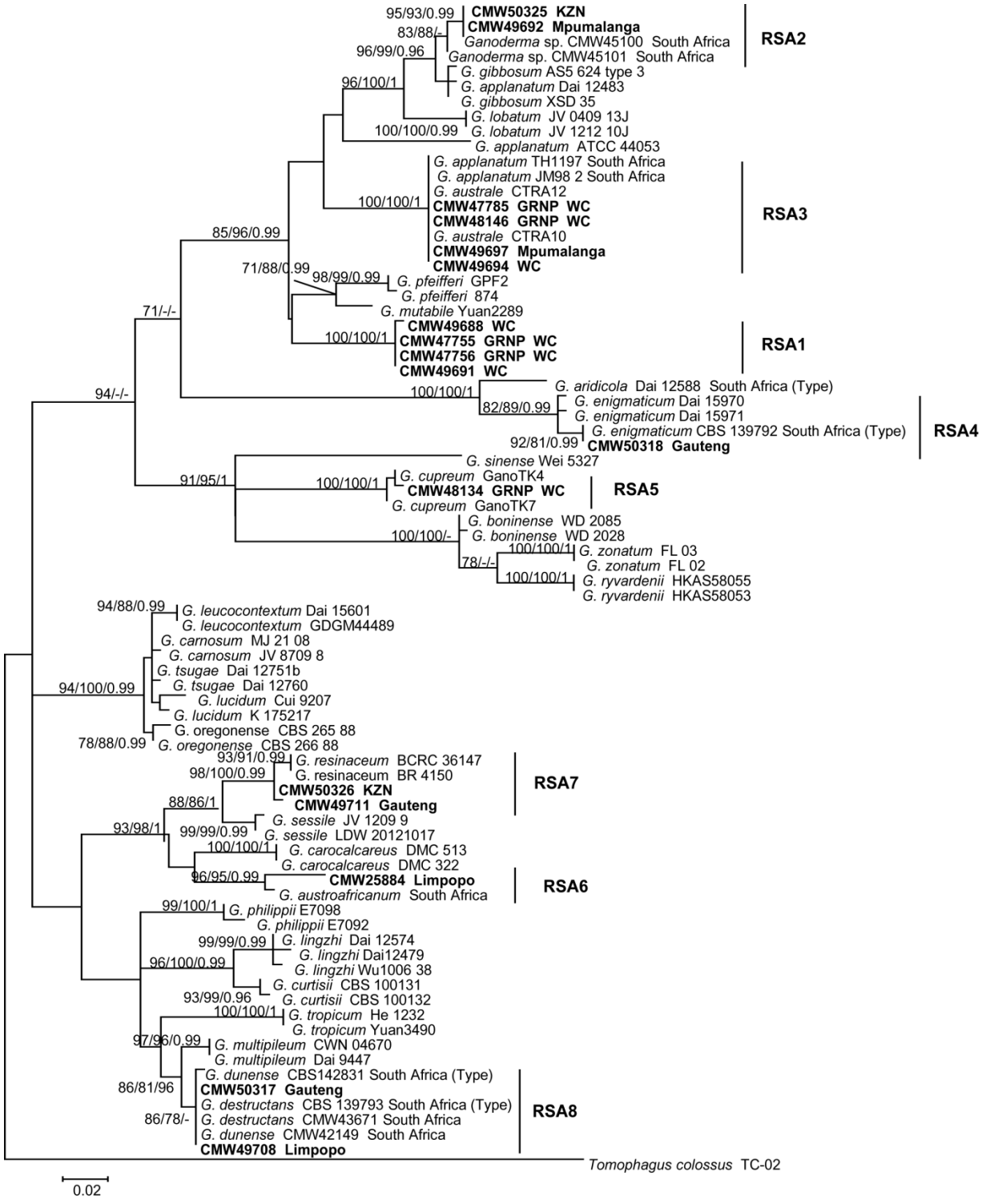


Figure 1. Phylogenetic tree derived from ML analysis of ITS gene sequences for *Ganoderma* from South Africa and sequence of relatives from GenBank. Bootstrap support values ≥ 70 % from 1000 replicates of ML and MP analyses and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are the South African isolates analyzed in this study. Isolates with GRNP are those from the Garden Route National Park.

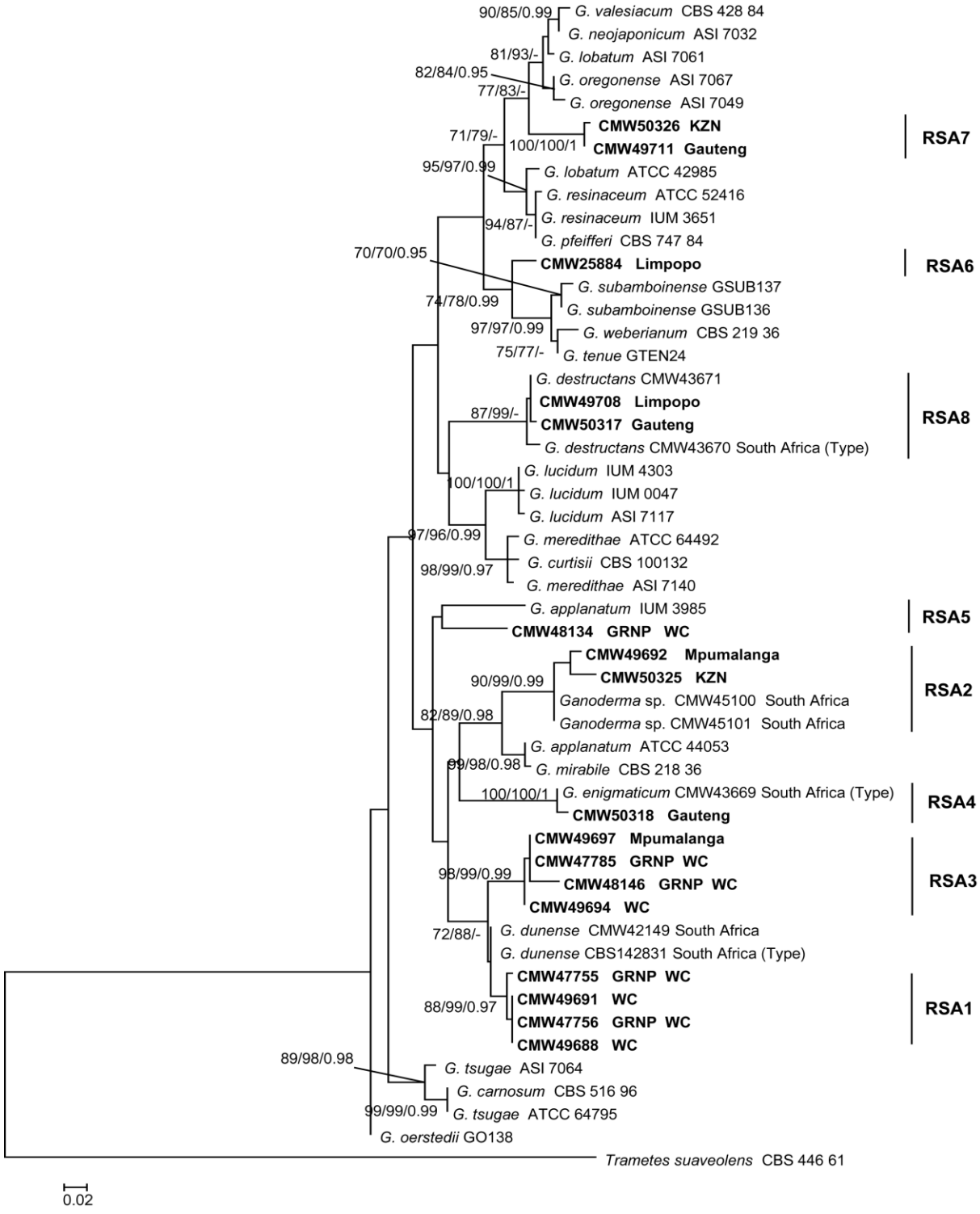


Figure 2. Phylogenetic tree derived from ML analysis of *β-Tubulin* gene sequences for *Ganoderma* from South Africa and sequences of relatives from GenBank. Bootstrap support values ≥ 70 % 1000 replicates of ML and MP analyses and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are the South African isolates analyzed in this study. Isolates with GRNP are those from the Garden Route National Park.

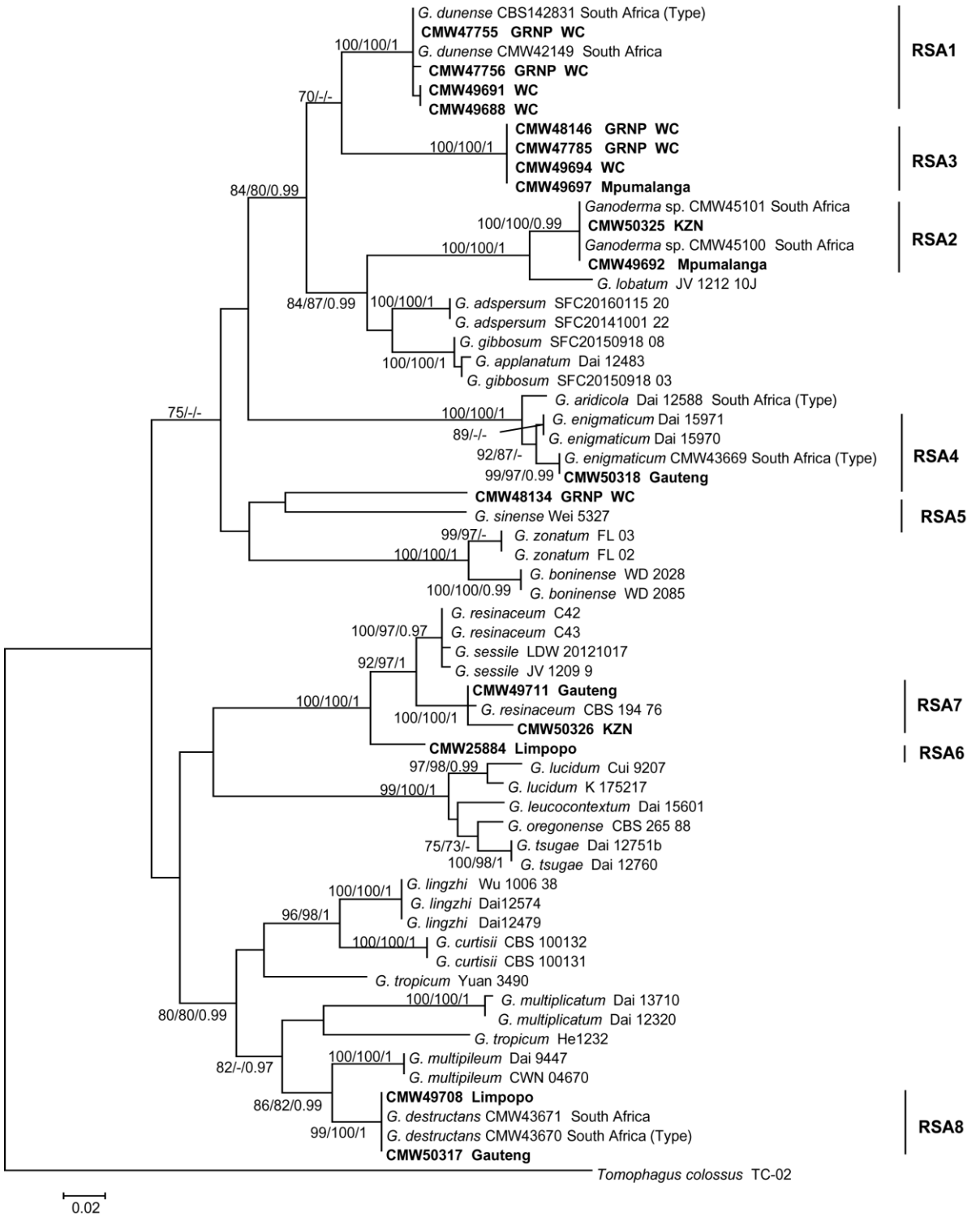


Figure 3. Phylogenetic tree derived from ML analysis of *TEF1-α* gene sequences for *Ganoderma* from South Africa and sequence of relatives from GenBank. Bootstrap support values ≥ 70 % from 1000 replicates of ML and MP analyses and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are the South African isolates analyzed in this study. Isolates with GRNP are those from the Garden Route National Park.

Figure 4. Phylogenetic tree derived from ML analysis of combined ITS, *β-tubulin* and *TEF-1α* gene sequences for *Ganoderma* from South Africa and sequences of relatives from GenBank. Bootstrap support values ≥ 70 % 1000 replicates of ML and MP analyses and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are the South African isolates analyzed in this study. Isolates with GRNP are those from the Garden Route National Park.

Figure 5. *Ganoderma knysnamense* sp. nov. (Type, PREM 62155). A. Basidiome *in situ* at the base of an unknown dead tree. B–C. Basidiome of the type specimen showing pilear and hymenial surfaces. D. Pores. E. Cross section of the basidiome showing the crust, crossed by a black melanoid line (short upward arrow) and the thin context with whitish streaks of mycelium (long downward arrow). F–G. Skeletal hyphae with knob-like protuberances (arrow). H. Shortly branched ligative hypha with knob-like protuberances. I–J. Basidiospores. Bars: A–B = 5 cm; C = 1 mm; D = 2 mm; E–I = 10 µm.

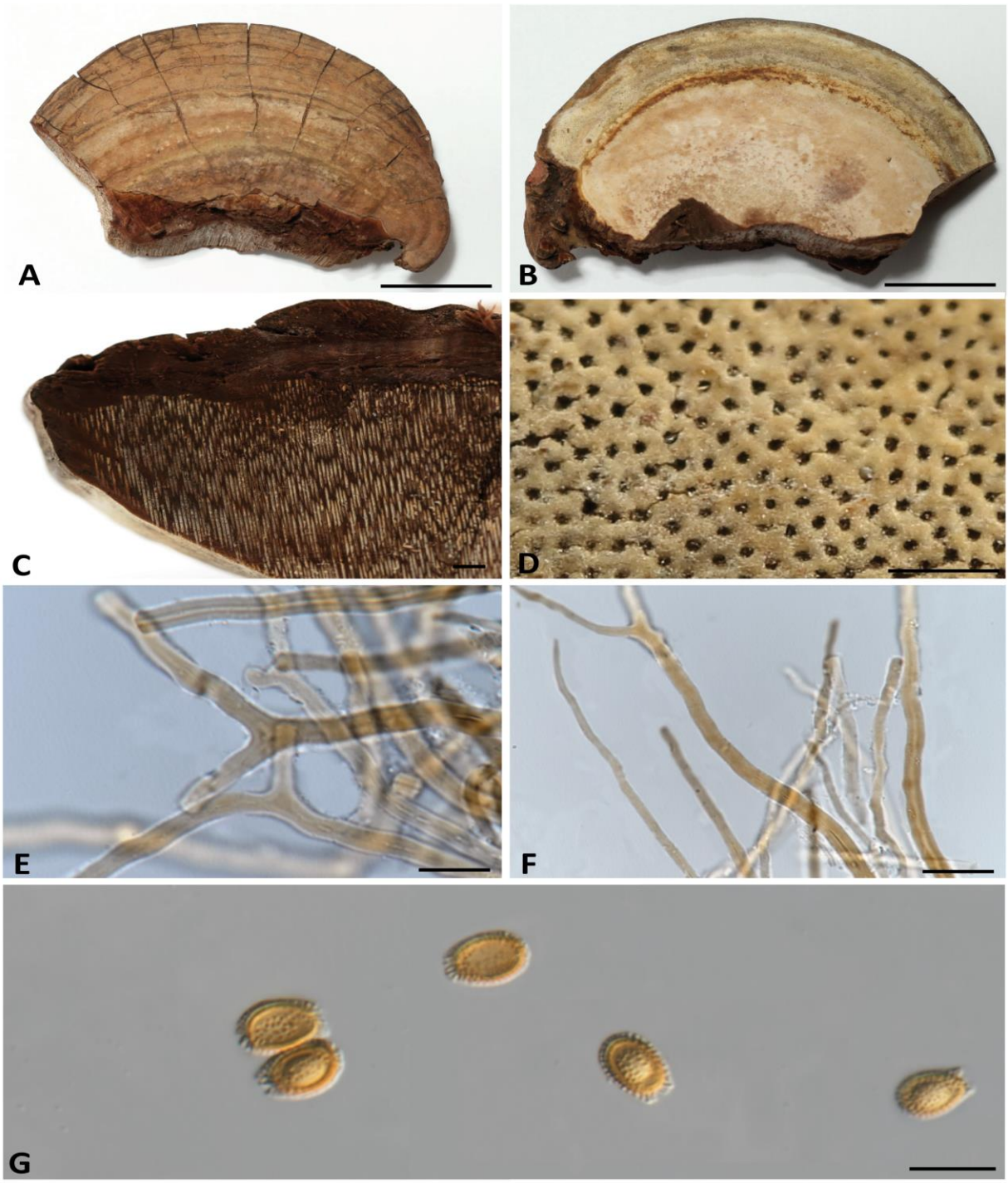


Figure 6. *Ganoderma eickeri* sp. nov. (Type, PREM 62157). A–B. Basidiome pilear and hymenial surfaces. C. Transversal section of basidiome showing context and the stratified tube layers. D. Close view of hymenial surface showing pores shape. E. Double-walled skeletal hyphae with knob-like protuberances. F. Apically branched/arboriform skeletal hypha. G. Basidiospores. Bars: A–B = 3 cm; C–D = 1 mm; E–I = 10 µm.

Supplementary Table 1. Reference isolates of *Ganoderma* species used in the phylogenetic analyses

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	***β-tubulin***	***TEF1-α***
Ganoderma adspersum	SFC20160115_20	Unknown	-	-	KY393286
G. adspersum	SFC20141001_22	Unknown	-	-	KY393285
G. australe	CTRA12	Brazil	KU569541	-	-
G. australe	CTRA10	Brazil	KU569539		
G. applanatum	Dai 12483	China	KF494999	-	KF494977
G. applanatum	ATCC 44053	Japan	JQ520161	JQ675614	-
G. applanatum	IUM 3985	Netherlands	-	JQ675615	-
G. applanatum	TH1197	South Africa	AF255150	-	-
G. applanatum	JM98/2	South Africa	AF255149	-	-
G. aridicola	Dai12588 (holotype)	South Africa	KU572491	-	KU572502
G. austroafricanum	CMW 41454	South Africa	KM507324	-	-
G. boninense	WD 2028	Japan	KJ143905	-	KJ143924
G. boninense	WD 2085	Japan	KJ143906	-	KJ143925
G. carnosum	MJ 21/08	Czech Republic	KU572492	-	-
G. carnosum	JV 8709/8	Czech Republic	KU572493	-	-
G. carnosum	CBS 516.96	Netherlands	-	JQ675616	-
G. carocalcareus	DMC 322	Cameroon	EU089969	-	-
G. carocalcareus	DMC 513	Cameroon	EU089970	-	-
G. cupreum	GanoTK4	Cameroon	JN105701	-	-
G. cupreum	GanoTK7	Cameroon	JN105702	-	-
G. curtisii	CBS 100131	United States of America (USA)	JQ781848	-	KJ143926
G. curtisii	CBS 100132	USA	JQ781849	-	KJ143927
G. curtisii	CBS 100132	Netherlands	-	JQ675617	-
G. destructans	CBS 139793 (type)	South Africa	NR132919	MG020151	MG020213
G. destructans	CMW 43671	South Africa	KR183857	MG020156	MG020220
G. dunense sp. nov	CMW42149	South Africa	MG020248	MG020153	MG020226
G. dunense sp. nov	CMW42157 (Type)	South Africa	MG020255	MG020150	MG020227
G. gibbosum	XSD-35	Unknown	EU273514	-	-
G. gibbosum	AS5.624 type 3	China	AY593856	-	-
G. gibbosum	SFC20150918-08	Unknown	-	-	KY393291
G. gibbosum	SFC20150918-03	Unknown	-	-	KY393290
G. enigmaticum	Dai 15970	Africa	KU572486	-	KU572496

Supplementary Table 1. (continued)

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	***β-tubulin***	***TEF1-α***
G. enigmaticum	Dai 15971	Africa	KU572487	-	KU572497
G. enigmaticum	CBS 139792 (type)	South Africa	NR132918	MG020157	MG020231
G. leucocontextum	Dai 15601	China	KU572485	-	KU572495
G. leucocontextum	GDGM 44489	China	KM396271	-	-
G. lingzhi	Wu 1006-38 (holotype)	China	JQ781858	-	JX029976
G. lingzhi	Dai 12574	China	KJ143908	-	JX029977
G. lingzhi	Dai 12479	China	JQ781864	-	JX029975
G. lobatum	JV 0409/13J	USA	KF605675	-	-
G. lobatum	JV 1212/10J	USA	KF605676	-	KU572501
G. lobatum	ATCC 42985	Canada	-	JQ675618	-
G. lobatum	ASI 7061	USA	-	JQ675619	-
G. lucidum	Cui 9207	China	KJ143910	-	KJ143928
G. lucidum	K 175217	UK	KJ143911	-	KJ143929
G. lucidum	ASI 7117	Korea	-	JQ675633	-
G. lucidum	IUM 4303	Bangladesh	-	JQ675635	-
G. lucidum	IUM 0047	Korea	-	JQ675627	-
G. meredithae	ATCC 64492	USA	-	JQ675643	-
G. meredithae	ASI 7140	Unknown	-	JQ675644	-
G. mirabile	CBS 218.36	Philippines	-	JQ675645	-
G. multipileum	CWN 04670	China	KJ143913	-	KJ143931
G. multipileum	Dai 9447	China	KJ143914	-	KJ143932
G. multiplicatum	Dai 12320	China	-	-	KU572500
G. multiplicatum	Dai 13710	China	-	-	KU572499
G. mutabile	Yuan 2289 (type)	China	JN383977	-	-
G. neojaponicum	ASI 7032	Unknown	-	JQ675646	-
G. oerstedii	GO138	Unknown	-	DQ288098	-
G. oregonense	CBS 265.88	USA	JQ781875	NS	KJ143933
G. oregonense	ASI 7049	USA	-	JQ675647	-
G. oregonense	ASI 7067	USA	-	JQ675650	-
G. oregonense	CBS 266.88	USA	JQ781876	-	-

Supplementary Table 1. (continued)

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	***β-tubulin***	***TEF1-α***
G. pfeifferi	CBS 747.84	Netherlands	-	JQ675651	-
G. pfeifferi	Isolate 874	Czech Republic	AM906059	-	-
G. pfeifferi	Strain GPF2	Poland	JN008874	-	-
G. philippii	E7098	Indonesia	AJ536662	-	-
G. philippii	E7092	Indonesia	AJ608710	-	-
G. resinaceum	BR 4150	France	KJ143915	-	-
G. resinaceum	CBS 194.76	Netherlands	KJ143916	-	KJ143934
G. resinaceum	C43	Unknown	-	-	KX379894
G. resinaceum	C42	Unknown	-	-	KX379893
G. resinaceum	ATCC 52416	Argentina	-	JQ675652	-
G. resinaceum	IUM 3651	Czech Republic	-	JQ675657	-
G. ryvardenii	HKAS 58055	Cameroon	HM138670	-	-
G. ryvardenii	HKAS 58053 (type)	Cameroon	HM138671	-	-
G. sessile	JV 1209/9	USA	KF605629	-	KJ143936
G. sessile	LDW 20121017	USA	KJ143917	-	KJ143935
G. sinense	Wei 5327	China	KF494998	-	KF494976
Ganoderma sp.	CMW45100	South Africa	MG020264	MG020152	MG020229
Ganoderma sp.	CMW45101	South Africa	MG020265	MG020155	MG020230
Ganoderma sp.	ASI 7150	Unknown	-	JQ675665	-
Ganoderma sp.	ASI 7151	Unknown	-	JQ675666	-
G. subamboinense	GSUB136	Unknown	-	DQ288096	-
G. subamboinense	GSUB137	Unknown	-	DQ288097	-
G. tenue	GTEN24	Unknown	-	DQ288074	-
G. tornatum	CBS 109679	Netherlands	-	JQ675670	-
G. tropicum	He 1232	China	KF495000	-	KF494975
G. tropicum	Yuan 3490	China	JQ781880	-	KJ143938
G. tsugae	Dai 12751b	USA	KJ143919	-	KJ143939
G. tsugae	Dai 12760	USA	KJ143920	-	KJ143940
G. tsugae	ATCC 64795	Canada	-	JQ675668	-
G. tsugae	ASI 7064	USA	-	JQ675669	-

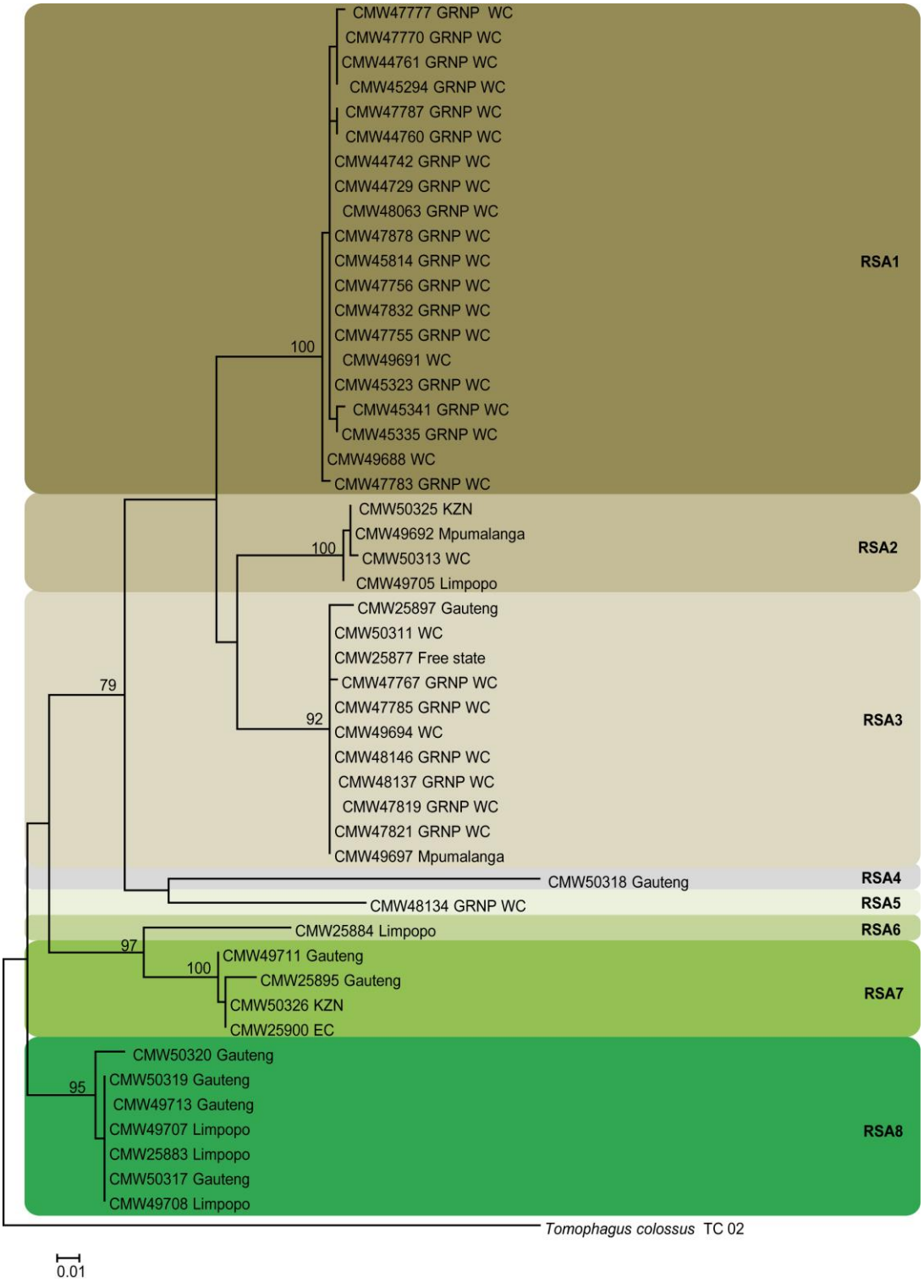
Supplementary Table 1. (continued)

Species	Voucher no.	Geographical origin	GenBank accession numbers		
			ITS	***β-tubulin***	***TEF1-α***
G. valesiacum	CBS 428.84	USA	-	JQ675671	-
G. weberianum	CBS 219.36	Philippines	-	JQ675672	-
G. zonatum	FL-02	USA	KJ143921	-	KJ143941
G. zonatum	FL-03	USA	KJ143922	-	KJ143942
Tomophagus colossus	TC-02	Vietnam	KJ143923	-	KJ143943
Trametes suaveolens	-	Unknown	-	FJ410378	-

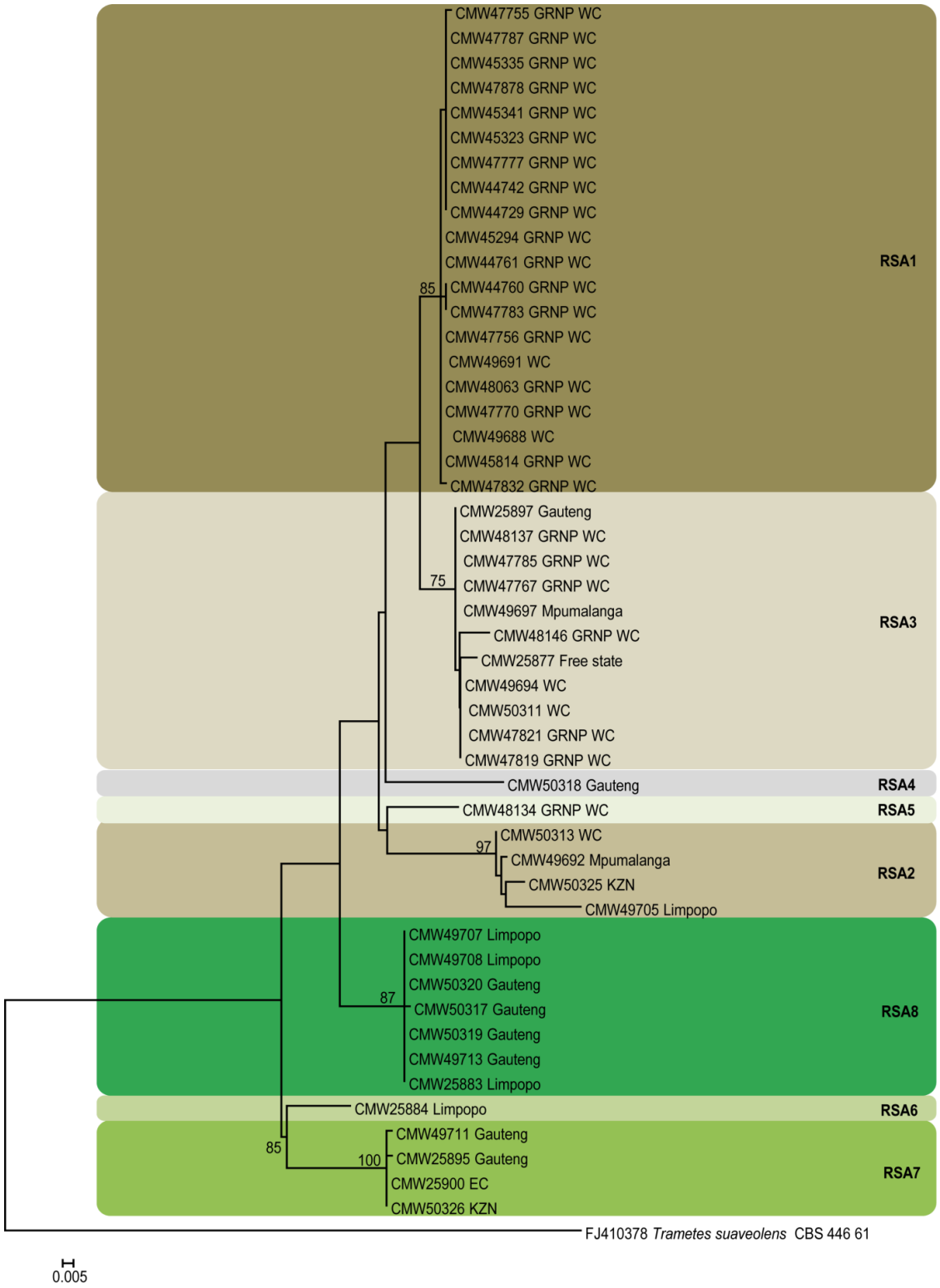
Supplementary Table 2. Summary of phylogenetic information of the sequence datasets for the three genome regions analyzed to obtain species level information on *Ganoderma* isolates from South Africa.

	Data set	**ITS**	***β-tubulin***	***TEF-1α***	**Combined**
Taxa (Nr)		78	51	61	111
Character (Nr)	Total	638	401	583	1622
	Constant	394	266	359	1119
	Variable	53	45	34	101
	PIC (Nr)	191	90	190	402
	MPT (Nr)	56	100	18	100
	TL	545	256	526	1165
	CI	0.534	0.523	0.553	0.505
	RI	0.891	0.845	0.874	0.867
	RC	0.476	0.442	0.484	0.438
	HI	0.466	0.477	0.447	0.495
ML/BI	Model	GTR+G+I	GTR+G+I	GTR+G+I	GTR+G+I

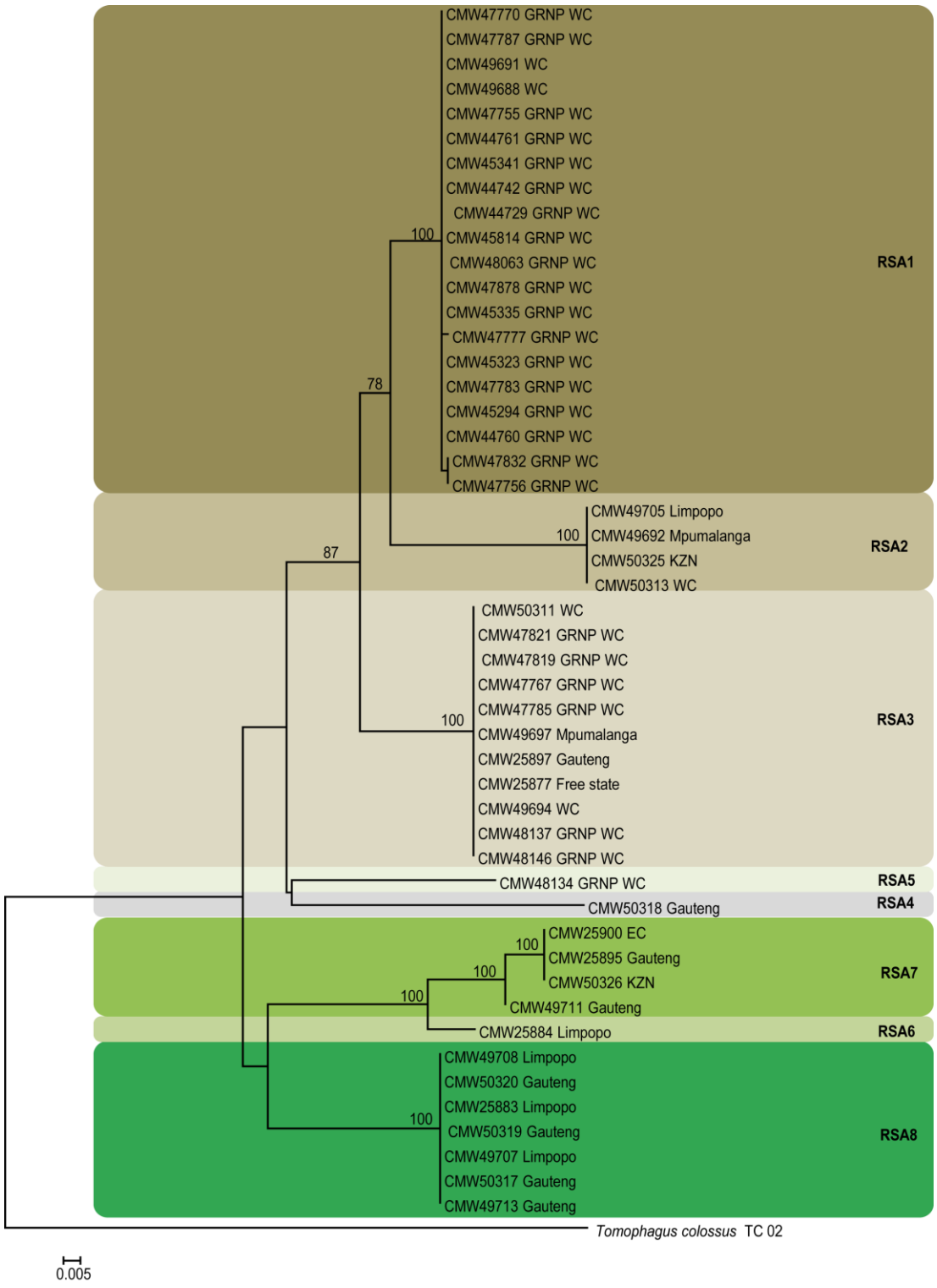
BI: Bayesian inference
ML: Maximum Likelihood
PIC: Parsimony informative character
MPT: Most parsimonious tree
TL: Tree length
CI: Consistency index
RI: Retention index
RC: rescaled retention index
HI: Homoplasy index



Supplementary Figure 1. ML phylogenetic tree based on ITS sequence data, showing the clustering of RSA isolates into eight phylogenetic groups. Bootstrap support values ≥ 70% are indicated on the branch nodes.



Supplementary Figure 2. ML phylogenetic tree based on *β-tubulin* sequence data, showing the clustering of RSA isolates into eight phylogenetic groups. Bootstrap support values ≥ 70% are indicated on the branch nodes.



Supplementary Figure 3. ML phylogenetic tree based on *TEF1-α* sequence data, showing the clustering of RSA isolates into eight phylogenetic groups. Bootstrap support values ≥ 70% are indicated on the branch nodes.

CHAPTER 5

POROID HYMENOCHAETACEAE ASSOCIATED WITH TREES SHOWING WOOD-ROT SYMPTOMS IN THE GARDEN ROUTE NATIONAL PARK OF SOUTH AFRICA

ABSTRACT

The poroid Hymenochaetaceae associated with wood-rot of trees in three timber-harvesting compartments of the Garden Route National Park (GRNP), South Africa, were investigated using phylogenetic analyses of ITS and LSU sequences, as well as the morphology of basidiomes. Results obtained revealed the presence of ten species belonging to five genera. Six of them represented known species, while three were proposed as new taxa. One species, here named as *Fuscoporia* sp. GRNP and most likely representing a new species, was not described due to the lack of fertile basidiomes. The newly proposed species include *Fomitiporia tsitsikamensis*, *Fulvifomes elaeodendri* and *Phellinus guttiformis* spp. novs. *Fomitiporia tsitsikamensis* sp. nov. was recovered from dead standing *Olea capensis* subsp. *macrocarpa* and living *Apodytes dimidiata* subsp. *dimidiata*. It is characterised by perennial, solitary or few together, resupinate to pseudo-pileate basidiomes, a dimitic hyphal system, subglobose to globose basidiospores and acuminate to ventricose setae. *Fulvifomes elaeodendri* sp. nov. was found exclusively on living trees of *Elaeodendron croceum* and is distinguished by perennial, solitary (rarely two together), broadly attached or semi-circular to dimidiate, applanate, triquetrous up to ungulate basidiomes, dimitic hyphal system and broadly ellipsoid to ellipsoid, to almost subglobose basidiospores. *Phellinus guttiformis* sp. nov. is characterised by perennial, resupinate to pseudo-pileate, drop-shaped or hoof-shaped and pendant basidiomes, dimitic hyphal system, subulate, acuminate to slightly ventricose setae and broadly ellipsoid, subglobose to globose basidiospores. It was found fruiting on branches and trunks of *Psydrax obovata* subsp. *obovata* and *O. capensis* subsp. *capensis*.

5.1. INTRODUCTION

The poroid Hymenochaetaceae (Hymenochaetales, Basidiomycota) is a group of macromycetes that encompasses important pathogenic species of forest trees worldwide. Numerous species are aggressive parasites that attack living trees, causing a white heart-rot of branches and trunks. Some species can also infect the sapwood, resulting in dieback and tree mortality, or are involved in the decomposition of fallen plants (Decock et al. 2006; Drechsler-Santos et al. 2010; Cloete et al. 2014; Cloete et al. 2016). Some species are also known to have medicinal properties (Dai et al. 2009; Wu et al. 2012). Due to their ecological and economic importance, poroid Hymenochaetaceae have been extensively studied and characterized, with most of them having been classified in the genera *Phellinus* P. Karst. and *Inonotus* Murrill (Larsen and Cobb-Poulle 1990; Ryvarden 1991; Wagner and Fischer 2001, 2002; Dai 2010; Zhou et al. 2016).

Traditionally, the genera *Phellinus* and *Inonotus* were distinguished by their basidiome consistency and hyphal systems. Based on this approach, *Phellinus* included species with hard, perennial basidiomes and a dimitic hyphal system (Larsen and Cobb-Poulle 1990), whereas taxa with soft, annual basidiomes and a monomitic hyphal system were usually placed in *Inonotus* (Ryvarden 1991, 2005). However, these criteria proved to be inconsistent due to the existence of intermediate and overlapping morphological traits. A cladistic approach integrating detailed morphological and cultural features, electrophoretic protein patterns, basidiome pigment contents, the number of nuclei in hyphal segments and the ecology of different species (Fiasson and Niemelä 1984) resulted in the separation of these two large genera into smaller ones. The advent of phylogenetic analyses based on comparison of DNA markers also showed the progressive definition of smaller and more homogeneous generic units or subgroups (Wagner and Fischer 2001, 2002; Zhou et al. 2016). Today, about 28 genera are accepted among the

poroid Hymenochaetaceae (Wu et al. 2016; Zhou et al. 2016 and Ji et al. 2017). For an overview of the taxonomic treatments see Larsen and Cobb-Poulle (1990), Fischer (1996), Wagner and Fischer (2001, 2002), Ryvarden (2004, 2005), Larsen et al. (2006), Dai (2010), Ryvarden and Melo (2014), (Wu et al. 2016), and Zhou et al. (2016, 2018) and Drechsler-Santos et al. (2016) for a general key to recognize the proposed genera.

During extensive fieldwork carried out in 2014–2015 in three timber-harvesting compartments of the indigenous forests of the Garden Route National Park (GRNP) in the southern Cape Province of South Africa, a large collection of basidiomycetous wood-rotting fungi was collected (Tchotet Tchoumi et al. 2017). Of these, numerous isolates and specimens were identified as belonging to the Hymenochaetaceae based on nuclear rDNA ITS1-5.8S-ITS2 (ITS) barcoding (Tchotet Tchoumi et al. 2017). The objective of this study was to continue the identification of these Hymenochaetaceae species by establishing their phylogenetic relationships and taxonomic status using multi-locus phylogenetic analyses and morphological characteristics.

5.2. MATERIALS AND METHODS

Twenty-five Hymenochaetaceae isolates obtained from the GRNP were selected for characterization to species level. These isolates were selected to represent each of the Hymenochaetaceae operational taxonomic units (OTUs) identified in the study by Tchotet Tchoumi et al. (2017). They were also selected to represent different host species and geographic locations within the GRNP.

5.2.1 PCR amplifications and sequencing of the nLSU gene region

The primer pair LROR/LR5 (Vilgalys and Hester 1990) was used to amplify and sequence the nLSU gene region of the selected Hymenochaetaceae isolates. PCR and sequencing reaction

mixtures as well as cycling protocols were the same as those described in Tchotet Tchoumi et al. (2018). All newly generated LSU sequences and their corresponding ITS sequences, previously generated in Tchotet Tchoumi et al. (2017), were deposited in the NCBI nucleotide sequence database (www.ncbi.nlm.nih.gov) and accession numbers are provided (Table 1).

5.2.2. Phylogenetic analyses

Phylogenetic analyses were performed in two phases. The first phase consisted in establishing the generic diversity of the GRNP isolates. This was done by conducting a phylogenetic analysis that incorporated their ITS sequences together with reference sequences retrieved from GenBank. The reference sequences represented species of genera closely related to the GRNP isolates based on BLASTn searches. For each species of the respective genera, at least two representative sequences, including type sequences when available, were extracted from GenBank and added to the global ITS sequence dataset. In the second phase, GRNP isolates were analysed separately in their respective genera. Reference sequences used in the second phase were published in studies of Yombiyeni et al. (2011), Cui and Decock (2013), Campos Santana et al. (2014), Amalfi and Decock (2014), Amalfi et al. (2014), Hattori et al. (2014), Zhou et al. (2016), Coelho et al. (2016), Cloete et al. (2016), Chen and Yuan (2017), Ji et al. (2017) and Salvador-Montoya et al. (2018) (Table 2). Sequence datasets of both phylogenetic phases were aligned using the online programme MAFFT (http://mafft.cbrc.jp/alignment/server/index.html) v.7 (Katoh and Standley 2013) and results of the alignments were manually edited in MEGA v.5.05 (Tamura et al. 2011). The appropriate nucleotide substitution model that best-fitted each sequence dataset was estimated using JModeltest v. 2.1.6 (Darriba et al. 2012) on CIPRES (Miller et al. 2010) under the Akaike Information Criterion (AIC). Maximum Likelihood (ML) phylogenetic analysis was applied to

the global ITS sequence dataset of the first phase, while ML and Bayesian inference (BI) analyses were applied to the combined ITS and LSU sequence datasets of each genus of the second phase. Maximum Likelihood and BI analyses were performed as described in Tchotet Tchoumi et al. (2018).

5.2.3. Morphological study

Macroscopic examination of the specimens included studies of the shape, size and colour of basidiomes, pilear and hymenial surfaces as well as context and tubes. Abbreviations and meaning of reagents used in microscopic analyses are as follow: CB = cotton blue, CB+ = cyanophilous, IKI = Melzer's reagent, IKI− = neither amyloid nor dextrinoid in Melzer's reagent, KOH = 5% potassium hydroxide, n = number of basidiospores measured from a given number of specimens, Q = ratio of length/width of basidiospores, and aveQ = arithmetic mean of the ratio Q. Sections stained with CB, IKI and KOH were examined under 100x with a Leica DM2500 microscope and drawings were made with a camera Lucida attached to the microscope. When presenting the size range of basidiospores, 5% of the measurements were excluded from each end of the range and are given in parentheses.

5.3. RESULTS

5.3.1. Sequence data and multi-locus phylogenetic analyses

The LSU gene region was amplified and sequenced successfully for all 25 Hymenochaetaceae isolates obtained from the GRNP. The size of the amplicons obtained varied between 865 and 935 base pairs. The GTR+G+I model was obtained as the best-fit nucleotide substitution model for the global ITS sequence dataset of the first phase. The resulting phylogeny of this dataset

revealed that GRNP isolates belonged to five taxonomic genera, namely: *Fomitiporia*, *Fulvifomes*, *Fuscoporia*, *Inonotus* and *Phellinus* (Figure 1A-C).

The concatenated dataset of ITS and LSU sequences of *Fomitiporia* consisted of 77 taxa and 1850 total characters. The GTR+G+I substitution model, as determined with jModelTest, was applied in the ML and BI phylogenetic analysis. Phylogenetic trees from both analyses resulted in almost similar topologies and, therefore, only the ML tree is presented incorporating MLB and BPP values. The phylogenetic inferences applied to the combined dataset of *Fomitiporia* resolved the GRNP isolates for this group in two clearly distinct and distant phylogenetic lineages (Figure 2). The first lineage, consisting of CMW48613, formed a strongly supported phylogenetic clade (MLB = 99%; BPP = 1) in ML and BI methods with an isolate representing *F. capensis* M. Fisch., M. Cloete, L. Mostert & F. Halleen from South Africa. This clade was closely related to another clade composed of two strains of *F. tenuis* Decock, Bitew & Castillo from Ethiopia and Gabon, respectively. Isolates of the second lineage (CMW47881, CMW48058 and CMW48621) clustered into a well-resolved monophyletic cluster, which received high statistical support (MLB = 100%; BPP = 1) in the two phylogenetic methods, and it represents a species new to science.

The *Fulvifomes* combined sequence dataset contained 54 taxa and 1774 characters in total. The best evolutionary model for nucleotide substitution obtained for this dataset was GTR+G+I. ML and BI phylogenetic methods resulted in nearly concordant topologies and, thus, only the topology of ML is presented with the statistical (MLB and BPP) values of the two methods indicated at the tree nodes. In the phylogenetic tree (Figure 3), all GRNP isolates of this genus, including CMW47808, CMW47825, CMW47909, CMW48154 and CMW48610, formed a well-resolved and strongly supported (MLB = 92%; BPP = 1) monophyletic clade. This clade was

sister to another monophyletic group consisting of two strains of *F. grenadensis* Murrill from Costa Rica and the USA, *F. hainanensis* L.W. Zhou from China and *F. thailandicus* L.W. Zhou from Thailand. The two clades, however, formed two distinct lineages, with the isolates from the GRNP representing a species new to science.

The combined *Fuscoporia* dataset included 70 taxa and 1909 total characters. The most appropriate model for nucleotide substitution estimated for this dataset was GTR+G. Only the ML tree is shown alongside with ML and BI statistical values (MLB and BPP) since both phylogenetic methods yielded almost identical tree topologies. In the combined phylogenetic tree, isolates from the GRNP separated into two groups clustering at two distant positions (Figure 4). The first group, comprising isolates CMW47749, CMW48042 and CMW48145, grouped with isolates of *F. gilva* (Schwein.) T. Wagner & M. Fisch. from China, Gabon, Puerto Rico, South Korea, Sri Lanka and the USA, into a monophyletic clade that was statistically well supported in the two phylogenetic methods (MLB = 97%; BPP = 1). This clade was closely related, but with no statistical support, to a lineage representing *F. atlantica* Motato-Vásquez, R.M. Pires & Gugliotta from Brazil. The second group, consisting of CMW45308, CMW47816, CMW48600 and CMW48060, formed a well-resolved monophyletic cluster with high bootstrap values in ML and BI phylogenetic methods (MLB = 100%; BPP = 1). This clade shared a common ancestor with a clade composed of *F. cinchonensis* (Murrill) Bondartseva & S. Herrera from South Korea and two unidentified *Fuscoporia* strains from Russia. However, both lineages were clearly distinct as they formed well-resolved sub-clusters. The lineage from the GRNP most likely represents a novel lineage.

Seventy-three taxa and 1776 total characters constituted the concatenated sequence dataset of *Inonotus*. The GTR+G+I model was estimated as the best nucleotide evolutionary model that fit

the dataset. The phylogenetic tree derived from ML analysis was chosen as the topology of this group since the two phylogenetic methods applied resulted in almost congruent topologies. The phylogenetic tree derived from this combined dataset grouped the GRNP isolates into the three main clades of this group as determined by Zhou et al. (2015), including *Sanghuangporus* Sheng H. Wu, L.W. Zhou & Y.C. Dai, *Tropicoporus* L.W. Zhou, Y.C. Dai & Sheng H. Wu and *Inonotus* s. str. (Figure 5). In *Sanghuangporus*, two GRNP isolates, CMW47748 and CMW48176, formed a strongly supported (MLB = 100%; BPP = 1) clade with an isolate representing *S. microcystideus* (Har. & Pat.) L.W. Zhou & Y.C. Dai from Tanzania. In *Tropicoporus*, GRNP isolates (CMW45333 and CMW45334) formed a highly supported sub-cluster (MLB = 100%, BPP = 1), which nested in a clade composed of three well-supported sub-clades of *T. tropicalis* (M.J. Larsen & Lombard) L.W. Zhou & Y.C. Dai from USA, China and Thailand. This large 'tropicalis clade' was strongly supported only in ML analysis (MLB = 82 %). The remaining three GRNP isolates clustered in the *Inonotus* s. str. group. Isolate CMW45823 formed a clade with high bootstrap values (MLB = 100%; BPP = 1) with two *I. rickii* (Pat.) D.A. Reid strains from Chile and China, respectively, while CMW45076 and CMW47759 clustered into a well-resolved and strongly supported monophyletic clade with two *I. setuloso-croceus* (Cleland & Rodway) P.K. Buchanan & Ryvarden strains from South Africa.

The concatenated ITS and LSU sequence dataset of *Phellinus* encompassed 67 taxa and 1705 total characters. Its best-fit evolutionary model was estimated as GTR+G+I. Because of the concordance of the phylogenies obtained from the two phylogenetic methods, only the topology resulting from ML analysis is presented with the statistical values of the two methods. The combined phylogeny of this group recovered isolates CMW45332 and CMW48059 from the GRNP as a clade with strong statistical support in ML and BI analyses (MLB = 100%; BPP = 1;

Figure 6). This clade was closely related, but with no statistical support, to a clade composed of two sub-lineages, including *Ph. bicuspidatus* Lombard & M.J. Larsen from USA and from an unknown locality and *Ph. resupinatus* M. Fisch., M. Cloete, L. Mostert & F. Halleen from South Africa. The lineage from the GRNP represents species new to science.

5.3.2. Taxonomy

Based on the phylogenetic species recognition concept, the 25 GRNP Hymenochaetaceae isolates represent 10 distinct taxa. Six of them represented previously described species, including *Fomitiporia capensis*, *Fuscoporia gilva*, *Sanghuangporus microcystideus*, *Tropicoporus tropicalis*, *Inonotus rickii* and *Inonotus setuloso-croceus*, while the remaining four are novel species. The morphological descriptions of the novel species are provided below.

Fomitiporia tsitsikamensis Tchotet, Martin P.A. Coetzee, Rajchenb. & Jol. Roux sp. nov. Figures 7–8

MycoBank: MB 829577

Typification: SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, Bloukrans (S33° 56.845' E23° 35.143'), attached to a dead standing *Olea capensis* subsp. *macrocarpa* (C. H. Wright) I. Verd. tree, July 2015, J.M. Tchotet Tchoumi, (holotype-JMT936; culture ex-type CMW47881; Fig. 8B). GenBank accession numbers: ITS = MH599111, LSU = MH599121.

Diagnosis: *Fomitiporia tsitsikamensis* is characterised by perennial, solitary or few basidiomes developing together, that are resupinate to pseudo-pileate, with light brown to brown poroid hymenial surface and a thin brown context. Hyphal system dimitic and basidiospores subglobose

to globose, thick-walled, hyaline, dextrinoid and cyanophilous (7.04–8.02 × 6.55–7.47 µm). Setae present, acuminate to ventricose and 20–30 × 4–9 µm.

Etymology: *tsitsikamensis* refers to the Tsitsikamma native forest in the Garden Route National Park (GRNP) indigenous forests, in the Southern Cape region of South Africa, where this fungus was collected.

Description: ***Basidiomes*** perennial, solitary or few together, resupinate to pseudo-pileate; when resupinate, up to 15 cm long × 9–13 cm wide, but also smaller. Pseudo-pilei drop shaped to hoof shaped, 11–17 cm long × 7–16 cm wide × 1–4 cm thick, formed from small resupinate areas growing in a more or less vertical substrate that develop an upper marginal sterile surface that hardens with age; pseudo-pilei slightly sulcate, with wide bands, smooth and very dark brown to blackish, presenting a black line 1–1.5 mm below the surface; a sterile, felty, yellowish brown to brown, up to 3 cm long margin is formed downwards towards the pores. Resupinate forms yellowish brown with a narrow felted brown margin. ***Hymenial surface*** poroid, light brown to brown, pores round to radially elongated, 4–5–6/mm; dissepiments thick. ***Context*** brown, thin (3 mm) or up to 10 mm thick; a distinct black line/cuticle develops against the substrate. ***Tubes*** light brown to brown, stratified, up to 7 mm long but generally shorter, with a contextual tissue developing in between the strata. ***Hyphal system*** dimitic. Generative hyphae simple septate 1.5–2.5–3 µm diam., thin-walled, hyaline to slightly yellowish. Skeletal hyphae unbranched, thick-walled, with a distinct lumen, 3–4 µm diam., chestnut. Hardened pseudo-pileus, when present, formed by agglutinated skeletal and generative hyphae immersed in a resinous-like matter. ***Hymenium*** sterile, with a honeycomb appearance. ***Basidiospores*** subglobose to globose, 7.04–8.02 × 6.55–7.47 µm (7.53±0.49 – 7.01±0.46), Q= 1.06–1.09, aveQ= 1.07 (n=6, N=120), thick-walled, hyaline, dextrinoid, cyanophilous. ***Setae*** present but rare and easily overlooked, mostly

acuminate but also ventricose, 20–30 × 4–9 μm, thick-walled, chestnut, either completely lacking, or solitary along the tubes, also present on the pilear surface.

Ecology: produces a white rot on standing and/or fallen trees of *Olea capensis* subsp. *macrocarpa* (C. H. Wright) I. Verd. and *Apodytes dimidiata* subsp. *dimidiata* E.Mey. ex Arn.

Other specimens examined: SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, Bloukrans (S33° 56.796' E23° 35.222'), attached to a dead standing tree of *O. capensis* subsp. *macrocarpa*, July 2015, J.M. Tchotet Tchoumi, (paratype-JMT902; culture ex-type CMW48058). GenBank accession numbers: ITS = MH599109, LSU = MH599120; SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, Bloukrans (S33° 56.796' E23° 35.222'), attached to dead standing tree of *O. capensis* subsp. *macrocarp*a, July 2015, J.M. Tchotet Tchoumi, JMT 1030 (strain CMW48621). GenBank accession numbers: ITS = MH599110, LSU = MH599123. Additional specimens examined based only on morphology: SOUTH AFRICA, Western Cape Province, Knysna, Diepwalle forest (S33° 57.230' E23° 10.728'), fruiting on a living *O. capensis* subsp. *macrocarpa* tree, May 2014, J.M. Tchotet Tchoumi, JMT333; SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, (S33° 56.839' E23° 35.205'), fruiting on a living *Apodytes dimidiata* subsp. *dimidiata*, July 2015, J.M. Tchotet Tchoumi, JMT898; SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, Bloukrans (S33° 57.672' E23° 43.528'), attached to dead standing tree of *O. capensis* subsp. *macrocarpa*, July 2015, J.M. Tchotet Tchoumi, JMT959.

Remarks: This new taxon is characterised by a pseudopileus formed by the hardened marginal areas of the basidiome when it grows on vertical/sub-vertical organs/substrates. It can be confused with *F. capensis* (Cloete et al. 2014). However, the latter species has been described

mostly as indistinctly stratified, whereas *F. tsitsikamensis* sp. nov. consistently presents a thin context between the strata. In addition, the new species consistently presents setae, though they are rare and may be overlooked at first sight; they were found in all specimens, also on the pilear surface when a pseudopileus is present. *Fomitiporia aethiopica* Decock, Adane & Castillo (Decock et al. 2005) differs from the new species by forming thin, strictly resupinate basidiomes that lack setae and contextual tissue between strata. *Fomitiporia punicata* Y.C. Dai, B.K. Cui & Decock (Dai et al. 2008) contrary to *F. tsitsikamensis* sp. nov. has effused–reflexed to pileate basidiomes with triquetrous up to ungulate pileus, shorter tube layers, lack of setae and much smaller basidiospores. Table 3 gives detailed morphological differences between *F. tsitsikamensis* sp. nov. and its closest phylogenetic neighbours. Thus, based on concordant phylogenetic and morphological results, *F. tsitsikamensis* is considered new to science.

***Fuscoporia* sp. GRNP** (Figure 9)

SOUTH AFRICA, Western Cape Province; Gouna forest, May 2014, JMT369 (strain CMW45308), fruiting on a dead standing *Olea capensis* subsp. *capensis* L. tree; Tsitsikamma forest, July 2015, fruiting respectively on: living *Psydrax obovata* subsp. *obovata* (Eckl. & Zeyh.) Bridson, JMT847 (strain CMW47816), stump of *Cunonia capensis* L., JMT918 (strain CMW48060) and a dead standing *O. capensis* subsp. *capensis* tree, JMT923 (strain CMW48600).

Basidiomes annual to perennial, solitary, resupinate, thin to distinctly pulvinate, forming ellipsoid bodies; 18–30 cm long × 4.5–10 cm wide × 0.3–4 cm thick. Margin always present and well developed, very wide when the specimen develops on a vertical tissue/organ, velutinate, becoming slightly indurated in older parts; brown to tobacco brown. ***Context*** light chocolate

brown, generally thin, 0.5–3 mm thick, woody consistency, developing a thin black line below the marginal tissue and a discontinuous to irregular black line against or near the substrate. ***Tubes*** light chocolate brown to light brown, stratified, with contextual tissue developing in between the strata, up to 4 cm for specimens developing on vertical substrates, each stratum up to 7 mm long. ***Hymenial surface*** poroid, non-cracked, light brown, pores round to ellipsoid, elongated when fruiting on vertical substrate, 5–7 per mm. ***Hyphal system*** dimitic. Generative hyphae thin to slightly thick-walled, hyaline to yellowish, simple septate, 2–4 µm diam., branched; incrusted with small rosette-like to polyhedric crystals in the hyphae protruding into the hymenium or in the pore mouth. Skeletal hyphae straight, non-ramified, 2.5–5 µm diam. ***Setae*** present in the hymenium, lanceolate, straight, numerous to very abundant, 25–45 × 5–7 µm, thick walled. ***Basidia*** and ***basidiospores*** not seen.

Remarks: repeated search to find basidiospores in all specimens examined of this taxon were unsuccessful. As a result, no name was given to it despite the fact that the concatenated phylogeny in ML and BI analyses resolved it as a distinct phylogenetic lineage. Further search is needed to obtain fertile specimens that can be used to establish its identity. Within the resupinate *Fuscoporia* species, specimens from the GRNP are distinguished by the combination of small pores, 5–7 per mm, and lack of hymenial hyphae. *Fuscoporia* sp. GRNP differs from its nearest phylogenetic neighbour, *F. cinchonensis,* as the latter has very thick and broadly attached, triquetrous basidiomes, and a pileus of woody consistency. *F. cinchonensis* also has a slightly thin context, indistinctly stratified tubes, and larger, circular pores (Bondartseva et al. 1992).

Fulvifomes elaeodendri Tchotet, Martin P.A. Coetzee, Rajchenb. & Jol. Roux sp. nov. Figures 10–11

MycoBank: MB 829578

Typification: SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, Bloukrans (S33° 56.827' E23° 35.237'), parasite at the base of a living *Elaeodendron croceum* (Thunb.) DC., July 2015, J.M. Tchotet Tchoumi, (holotype JMT1013; culture ex-type CMW47909). GenBank accession numbers: ITS = MH599096, LSU = MH599132.

Diagnosis: Fulvifomes elaeodendri is characterised by perennial, solitary, rarely two together, broadly attached or semi-circular to dimidiate, applanate to triquetrous up to ungulate basidiomes. Pilear surface generally covered with mosses; light brown; sulcate with relatively wide furrows. Context light brown and relatively thin. Hyphal system dimitic and basidiospores broadly ellipsoid to ellipsoid, some almost subglobose, with a flattened side, 5.78–6.52 × 4.76–5.40 µm, thick-walled, dull brown in KOH sol., IKI– and acyanophilous.

Etymology: elaeodendri refers to *Elaeodendron croceum*, a native tree in the Tsitsikamma forest on which all the basidiomes recovered for this fungus were fruiting.

Description: ***Basidiomes*** perennial, solitary, rarely two together, broadly attached or semi-circular to dimidiate, applanate to triquetrous up to ungulate, with a straight to roundish hymenial surface; (11–) 13–26 cm wide × (7–) 10–20 cm radius × 5–10 cm thick; ungulate specimens 11–14 cm thick. Margin regular, round to blunt. ***Pilear surface*** generally covered with mosses, light brown at the growing margin to brown or dark brown in the rest of the pileus; sulcate with relatively wide furrows (8–50 mm wide) in the margin, velutinate or smooth, then becoming indurated, glabrous and becoming cracked with age but never rimose. ***Crust*** lacking. ***Context*** light brown, relatively thin (3 mm) up to 15–20 mm thick, developing a black, continuous line below the pilear surface; woody in consistency. ***Tubes*** same colour as the context, 3–10 mm long

in each stratum; strata distinct and separated by a very thin contextual tissue. ***Hymenial surface*** poroid, golden brown to brown and in some cases dull brown; pores round or regular and entire, 5–7 per mm. ***Hyphal system*** dimitic. Upper portion of context monomitic, with generative hyphae up to 6 µm diam. and with a wide lumen, walls thickened, golden-brown to chestnut. Generative hyphae simple-septate, 2.5–4 µm diam., thin to thick-walled, yellowish to chestnut. Lower portion of context and dissepiments dimitic. Skeletal hyphae thick-walled, unbranched, 3.5–5 µm diam. Setae and basidia not seen. ***Basidiospores*** broadly ellipsoid to ellipsoid, some almost subglobose, with a flattened side, 5.78–6.52 × 4.76–5.40 µm (6.15±0.37 × 5.08±0.32 µm; n=5, N=150), Q=1.20–1.25, aveQ=1.21, thick-walled, walls yellowish in water, dull brown in KOH sol., IKI–, acyanophilous.

Ecology: all specimens of this fungal species were found producing a white stem and/or basal/butt rot on standing living *E. croceum* trees.

Other specimens examined: SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, (S33° 56.838' E23° 35.127'), fruiting at the base of a living *E. croceum* tree, July 2015, J.M. Tchotet Tchoumi, (paratype-JMT948; culture ex-type CMW48610). GenBank accession numbers: ITS = MH599095, LSU = MH599133; SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, (S33° 56.724' E23° 35.198'), fruiting at the base of a living *E. croceum* tree, July 2015, J.M. Tchotet Tchoumi, JMT838 (strain CMW47808). GenBank accession numbers: ITS = MH599093, LSU = MH599131; SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, (S33° 56.704' E23° 35.261'), fruiting at the base of a living *E. croceum* tree, July 2015, J.M. Tchotet Tchoumi, JMT859 (strain CMW47825). GenBank accession numbers: ITS = MH599094, LSU = MH599134; SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, (S33° 56.659' E23° 35.114'), fruiting at the base of a living *E. croceum* tree, July 2015, J.M.

Tchotet Tchoumi, JMT872 (strain CMW48154). GenBank accession numbers: ITS = MH599097, LSU = MH599135.

Remarks: despite some morphological similarities between *F. elaeodendri* sp. nov. and *F. hainanensis* (Zhou 2014), *F. thailandicus* (Zhou 2015) and *F. grenadensis* (Murrill 1914), there are distinct morphological traits that easily distinguish it from these species (Table 4). Unlike its pilear surface, which has wide furrows, that of the three most closely related species has relatively narrow furrows. *Fulvifomes hainanensis* is further distinguished from the new species by the uncracked nature of its pilear surface, its cinnamon-buff to clay-buff pore surface, the large size of its pores (3–4/mm) and the relatively small size of its basidiospores (5.2–6.2 × 4–4.8 µm). Similarly, *F. thailandicus* differs from *F. elaeodendri* sp. nov. by its dark grey and crusted pilear surface, the dark brown colour of its pore surface, circular pores and its slightly smaller basidiospores 5–5.8 (–6) × (4–) 4.1–4.8 (–5) µm. *Fulvifomes grenadensis*, contrary to the new species, has a dark-chestnut-brown or black pileus and small (3–4 µm) globose basidiospores. In addition to having different origins with the new species, these three fungal species were mainly found as saprophytes on decaying angiosperms, while all specimens of *F. elaeodendri* sp. nov. recovered from the GRNP were parasites of the same tree species, *E. croceum*. Their phylogenetic positions in the combined phylogeny confirm these divergences.

Fomes durissimus Lloyd (from Angola) and *Pyropolyporus pseudosenex* Murrill (from Nicaragua) resemble the new taxon. However, they present a distinct pilear crust that is lacking in *F. elaeodendri* sp. nov. and their pilear surfaces are more or less narrowly sulcate, unlike to the new species which has wide furrows. Lowe (1957) gave full descriptions of the two former taxa. *Fomes durissimus* was described from Angola and also recorded from Cuba and the East Indies. *Pyropolyporus pseudosenex* was described from Nicaragua and recorded from Mexico,

Belize and Panama. *Pyropolyporus merrillii* Murrill, described from the Philippines, recorded in Cuba and stated to be 'probably pantropical' (Ryvarden and Johansen 1980), is also morphologically close to *F. elaeodendri* sp. nov.; but the result of the combined phylogeny has shown that the strain from south-eastern China (Taiwan) representing this species is different from that of the new species.

Fomes dialeri Bres. & Torrend (Torrend 1905) from Mozambique is similar, but its pores are larger, 3–3.5/mm, and basidiospores are ellipsoid, 6.31–6.94 × 4.78–5.12 µm [Mozambique, Merurú, at the Mission, leg. L.G. Dieler (holotype at Stockholm, studied)].

Phellinus guttiformis Tchotet, Martin P.A. Coetzee, Rajchenb. & Jol. Roux sp. nov. Figures 12–13

MycoBank: MB 829579

Typification: SOUTH AFRICA, Western Cape Province, Gouna forest (S33° 56.109' E23° 03.390'), fruiting on a branch of *Psydrax obovata* subsp. *obovata*, May 2014, J.M. Tchotet Tchoumi, (holotype-JMT464; culture ex-type CMW45332). GenBank accession number: ITS = MH599107, LSU = MH599136.

Diagnosis: Phellinus guttiformis is characterised by perennial, resupinate to pseudo-pileate, drop-shaped or hoof-shaped and pendant basidiomes; chocolate brown context, poroid hymenial surface and dimitic hyphal system. Setae are subulate, acuminate to slightly ventricose and basidiospores broadly ellipsoid, subglobose to globose, thick-walled, IKI–, 5.50–6.12 × 4.79–5.39 µm.

Etymology: guttiformis refers to the drop shape of the basidiome retained as the type specimen of this novel taxon.

Description: ***Basidiomes*** perennial, resupinate to pseudo-pileate, drop-shaped or hoof-shaped and pendant, 6.5–7.6 cm long × 4–6.5 cm wide × 0.8–2.0 cm thick. Upper surface well developed, velutinate towards the margin, smooth, dull brown to dark brown, becoming glabrous, hardening, indurated and cracking with age. ***Context*** chocolate brown, very thin to thin, 2–6 mm, with woody consistency. ***Tubes*** light brown to chocolate brown, 3–6 mm long, indistinctly stratified. ***Hymenial surface*** poroid, chocolate brown, pores regularly round to ellipsoid when growing on a vertical substrate, 6–8 (–10) per mm. ***Hyphal system*** dimitic. Generative hyphae 2.2–3 µm diam, branched, thin-walled, hyaline to slightly yellowish. Skeletal hyphae straight, unbranched, narrow, 2.2–3 µm diam. ***Setae*** subulate, acuminate to slightly ventricose or with a relatively wide base, straight, generally 1-rooted, 12–25 × 4–9 µm, thick-walled, with a terminal or a lateral base, dark chestnut, variably abundant in different sections. ***Basidia*** not seen. ***Basidiospores*** broadly ellipsoid, subglobose to globose, 5.50–6.12 × 4.79–5.39 µm (5.81 ± 0.31 × 5.09 ± 0.30 µm), Q=1.13-1.15, aveQ=1.14, N=3, n=90; thick-walled, IKI–, first faintly yellowish (KOH solution) and some collapsing when not fully mature, becoming slightly brownish and with thickened walls.

Other specimen examined: SOUTH AFRICA, Western Cape Province, Tsitsikamma forest, Bloukrans (S33° 56.836' E23° 35.181'), attached to a living *Olea capensis* subsp. *capensis*, July 2015, J.M. Tchotet Tchoumi, (paratype-JMT903; culture ex-type CMW48059). GenBank accession number: ITS = MH599108, LSU = MH599137.

Remarks: *Phellinus guttiformis* sp. nov. is a new member of *Phellinus* s. str. (Cloete et al. 2016). In addition to the combined multi-locus phylogeny that placed it in this subgroup, it also showed the main morphological characteristics that define species of *Phellinus* s. str., such as resupinate basidiomes, strictly dimitic hyphal system and basidiospores of ellipsoid to subglobose shape (Sell 2008; Tomšovský et al. 2010; Cloete et al. 2016). *Phellinus guttiformis* sp. nov. is macro-morphologically similar to *Ph. gabonensis* Decock & Yombiyeni, described from the western edge of the Guineo-Congolian rainforest in Gabon, by forming resupinate basidiomes that have a marginal area that develops into a pseudopileus (Yombiyeni et al. 2011). The latter differs, though, by hymenial setae being commonly curved, by skeletal hyphae of limited growth up to 132 µm long (but 86.7 µm long in average) and by somewhat smaller, ellipsoid to broadly ellipsoid basidiospores that are hyaline and become faintly yellowish (Yombiyeni et al. 2011).

Phellinus caribeo-quercicolous Decock & S. Herrera (Decock et al. 2006) is phylogenetically close and morphologically similar to *Ph. gabonensis*, but, it differs by its hamate setae and ellipsoid to broadly ellipsoid basidiospores that are hyaline to faintly yellowish. Vlásak et al. (2011) described specimens from the southern USA as also having yellowish to brownish basidiospores (Cui and Decock 2013, and http://mykoweb.prf.jcu.cz/polypores/list_phellinus.html). *Phellinus amazonicus* Campos-Santana & Decock (Campos Santana et al. 2016) is similar in gross morphology by producing resupinate to pulvinate basidiomes that may develop a hardened pseudopileus (but that does not crack with age), but differs in hamate setae and short skeletal hyphae. Despite morphological similarities, *Ph. guttiformis* is not a member of the *Ph. caribeo-quercicolus* Decock & S. Herrera species complex formed by the aforementioned taxa, as well as the recently described *Ph. amazonicus* Camp.-Sant. & Decock (Campos Santana et al. 2016), based on the combined phylogeny of this study. Rather, it forms a

sister relationship with *Ph. bicuspidatus*, a species originally described from North America (Lombard and Larsen 1985) and with *Ph. resupinatus*, a recently described species from South Africa (Cloete et al. 2016). The two species differ morphologically from the new species in that *Ph. bicuspidatus*, exceptionally of other species of *Phellinus* s. str., has a monomitic hyphal system and short, biscuspidate setae (Lombard and Larsen 1985), while *Ph. resupinatus* has slightly wider and rarely branched generative hyphae, thick walled skeletal hyphae and smaller basidiospores. Table 5 summarizes all the morphological differences between the new species and its closest phylogenetic relatives.

5.4. DISCUSSION

This study provides novel information on the diversity and identity of poroid Hymenochaetaceae species in South Africa and specifically from one of the country's iconic native forest ecosystems, the Garden Route National Park (GRNP). Specimens examined and described were recovered from both living and dead trees, as well as from stumps and fallen branches in the forest and were obtained from a previous study which aimed to investigate the species richness and host range of wood-rotting basidiomycetes associated with trees showing wood-rot symptoms in this forest (Tchotet Tchoumi et al. 2017). Ten species belonging to five genera were identified, of which six represented known species (*Fomitiporia capensis*, *Fuscoporia gilva*, *Sanghuangporus microcystideus*, *Tropicoporus tropicalis*, *Inonotus rickii* and *Inonotus setuloso-croceus*), and three were novel taxa (*Fomitiporia tsitsikamensis*, *Fulvifomes elaeodendri* and *Phellinus guttiformis* spp. novs.). One species, here named as *Fuscoporia* sp. GRNP, most likely also represents a new species, but was not described due to the lack of fertile basidiomes.

The host and geographic distributions of the six known Hymenochaetaceae species identified from the GRNP has been extended by their discovery in this study. The host range of *Fomitiporia capensis*, whose previously known occurrence was limited to South African vineyards (*Vitis vinifera* L.) (Cloette et al. 2014), is now expanded to include a native forest host, *Gonioma kamassi*. This discovery indicates that this fungal species is likely to be found on other hosts across the country if further investigations are carried out. *Fuscoporia gilva* is a widespread species and an occasional parasite. In general, it is saprotrophic on dead trees of different species, causing white rot of the sapwood (Gilbertson 1979; Wagner and Fischer 2002). Its discovery in South Africa is a confirmation of its widespread nature, while its association with stumps and dead standing trees in the GRNP also confirms its main trophic role (saprotroph).

Sanghuangporus microcystideus and *Tropicoporus tropicalis* belong to two recently established genera from the *Inonotus linteus* complex (Zhou et al. 2015). *Sanghuangporus microcystideus* has to date only been recorded in Africa (the Republic of Congo, Ethiopia and Tanzania). Therefore, its occurrence in the GRNP of South Africa reinforces the view that it may be endemic to Africa. To the best of our knowledge, this is the first record of this species in Southern Africa. *Tropicoporus tropicalis* occurs mainly in tropical zones, where it causes white rot of trunks and branches of woody angiosperms and gymnosperms. The species has an annual habit, which could explain the absence of fruiting bodies during sampling in the GRNP. On the African continent, its presence has been reported only from East Africa (Nguyen et al. 2009; De Simone et al. 2010; Zhou et al. 2015) thus, this study represents the first report of *T. tropicalis* from Southern Africa. *Inonotus rickii* and *I. setuloso-croceus* belong to *Inonotus* s. str. as shown by the combined phylogeny of this group (Zhou et al. 2015). *Inonotus rickii* is a widespread

species that was originally described from Morocco (Malençon 1970). Several reports indicate that it causes white rot of the heartwood of branches and tree trunks, commonly in environments disturbed by human activities (Barnard 1993; Kotlaba and Pouzar 1994; Melo et al. 2002). Based on this, its presence in the GRNP is not surprising, as several compartments of these forests are subjected to logging operations that damage branches, trunks and tree roots (Tchotet Tchoumi et al. 2017). For *Inonotus setuloso-croceus* this is, as for *F. capensis,* the first record on a native host (Cloete et al. 2015), and it most likely also occur on other plant species across the country.

Four previously unknown species were identified from native trees in the GRNP. Of the four, one epithet (*Fuscoporia* sp. GRNP) has not been assigned due to the absence of important taxonomic features such as basidiospores. Thus, until further sampling provides mature and fertile material for its morphological characterisation, this taxon remains unnamed. The description of *Fomitiporia tsitsikamensis*, *Fulvifomes elaeodendri* and *Phellinus guttiformis* as novel species in the Hymenochaetaceae species were supported by both morphology and sequence data of two gene regions in which they formed well-resolved and strongly supported monophyletic clusters within their respective genera.

Trees of different biological status (living, dead, fallen and stumps) from ten different hosts were found associated with the Hymenochaetaceae reported in this study. Of these, *Olea capensis* subsp. *macrocarpa* was found to be the most commonly affected by wood rot (Tchotet Tchoumi et al. 2017). Eight species of poroid Hymenochaetaceae, including *Fomitiporia capensis*, *Fuscoporia gilva*, *Sanghuangporus microcystideus*, *Tropicoporus tropicalis*, *Inonotus rickii*, *I. setuloso-croceus* and *Fomitiporia tsitsikamensis* sp. nov. were found associated with wood-rot symptoms of this native tree. Both living and dead trees, as well as stumps of this host were affected. Based on these observations, *O. capensis* subsp. *macrocarpa* could be thus considered

especially vulnerable to wood rot and a broad range of wood rot fungi. Six of the ten identified Hymenochaetaceae species, including *Fulvifomes elaeodendri* sp. nov. *Sanghuangporus microcystideus, Tropicoporus tropicalis, Inonotus rickii, Inonotus setuloso-croceus* and *Phellinus guttiformis* sp. nov. were parasites since they were fruiting on living trees of *Elaeodendron croceum*, *O. capensis* subsp. *macrocarpa*, *O. capensis* subsp. *capensis* and *Podocarpus falcatus*. The prevalence of the parasitic life strategy of these macro-fungi reinforces the view that they are one of the main causes of the symptoms of wood rot in this native forest (Tchotet Tchoumi et al. 2017), and suggests that pathogenicity trials should be conducted in order to determine their real impact on this native forest. The presence of the remaining taxa (*Fomitiporia capensis*, *Fomitiporia tsitsikamensis* sp. nov., *Fuscoporia gilva* and *Fuscoporia* sp. GRNP) on only dead trees and stumps confirms the trophic role (saprotrophs) of these macro-fungi.

Generally, the fungi identified in this study did not show host or geographic specificity. One species, however, *Fulvifomes elaeodendri* sp. nov. showed possible host specificity. It was found only associated with basal stem rot of *Elaeodendron croceum*. However, as it was only found at one site, despite the distribution of *E. croceum* in all the compartments sampled, and given the small number of trees with which it was associated, additional investigation in other forest compartments are needed to verify this hypothesis.

The study of Tchotet Tchoumi et al. (2017) identified nine OTUs belonging to the Hymenochaetaceae from the GRNP using sequence data of the ITS gene regions. Addition of LSU sequence data for a selection of 25 isolates, representing these nine OTUs, classified them into 10 species belonging to five genera. Separate analyses of each genus allowed for more accurate species level identification of these OTUs. However, increasing numbers of studies of

wood-rotting and other Basidiomycete species globally, using additional gene regions such at the TEF, BT and others, have identified cryptic species among these fungi (Amalfi and Decock , 2014, Brazee 2015, Zhou et al. 2016, Zhou et al. 2018). Further studies of the material in the current study, as well as additional isolates from the study of Tchotet Tchoumi et al. (2017), may reveal additional species of these fungi from South Africa. The current study, however, lays a solid foundation for further studies and significantly increased our understanding of the diversity of Hymenochaetaceae in South Africa.

5.5. REFERENCES

Amalfi M, Robledo G, Decock C, 2014. *Fomitiporia baccharidis* comb. nov., a little known species from high elevation Andean forests and its affinities within the neotropical *Fomitiporia* lineages. *Mycological Progress*13, 1075–1087.

Amalfi Mario, Decock C, 2014. *Fomitiporia expansa*, an undescribed species from French Guiana. *Cryptogamie, Mycologie* 35, 72–86.

Brazee NJ, 2015. Phylogenetic relationships among species of Phellinus sensu stricto, cause of white trunk rot of hardwoods, from Northern North America. Forests, 6, 4191–4211.

Barnard EL, 1993. *Inonotus rickii* (anamorph: *Ptychogaster cubensis*): A wood- decaying fungus of importance to Florida hardwoods. Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Plant Pathology circular nº 357, 3 pp.

Bondartseva MA; Herrera Figueroa S; Sandoval D; Cejas F, 1992. Taxonomical problems of the Cuban Hymenochaetaceous fungi. *Mikologiya i Fitopatologiya* 26, 3-14.

Buchanan PK, Ryvarden L, 1993. Type studies in the Polyporaceae 24. Species described by Cleland, Rodway and Cheel. *Australian Systematic Botany* 6, 215–235.

Campos Santana M, Amalfi M, Robledo G, Borges Da Silveira RM, Decock C, 2014. *Fomitiporia neotropica*, an undescribed species from South America evidenced by multi-locus phylogenetic analyses. *Mycological Progress* 13, 601–615.

Campos-Santana M, Amalfi M, Castillo G, Decock C, 2016. Multilocus, DNA-based phylogenetic analyses reveal three new species lineages in the *Phellinus gabonensis–P. caribaeo-quercicola* species complex, including *P. amazonicus* sp. nov. *Mycologia* 108, 939–953.

Chen Q, Yuan Y, 2017. A new species of *Fuscoporia* (Hymenochaetales, Basidiomycota) from southern China. *Mycosphere* 8, 1238–1245.

Cloete M, Fischer M, Du Plessis IL, Mostert L, Halleen F, 2016. A new species of *Phellinus sensu stricto* associated with esca on grapevine in South Africa. *Mycological Progress* 15, 1–9.

Cloete M, Fischer M, Mostert L, Halleen F, 2014. A novel *Fomitiporia* species associated with esca on grapevine in South Africa. *Mycological Progress* 13, 303–311.

Cloete M, Fischer M, Mostert L, Halleen F, 2015. Hymenochaetales associated with esca-related wood rots on grapevine with a special emphasis on the status of esca in South African vineyards. *Phytopathologia Mediterranea* 54, 299–312.

Cloete M, Mostert L, Fischer M, Halleen F, 2015. Pathogenicity of South African Hymenochaetales taxa isolated from esca-infected grapevines. *Phytopathologia Mediterranea* 54, 368–379.

Coelho G, Silveira A De Oliveira, Antoniolli ZI, Yurchenko E, 2016. *Tropicoporus stratificans* sp. nov. (Hymenochaetales, Basidiomycota) from southern Brazil. *Phytotaxa*, 245. 144–152.

Cui BK, Decock C, 2013. *Phellinus castanopsidis* sp. nov. (Hymenochaetaceae) from southern China, with preliminary phylogeny based on rDNA sequences. *Mycological Progress* 12, 341–351.

Dai YC, 2010. Hymenochaetaceae (Basidiomycota) in China. *Fungal Diversity* 45, 131–343.

Dai YC, Yang ZL, Cui BK, Yu CJ, Zhou LW, 2009. Species diversity and utilization of medicinal mushrooms and fungi in China (review). *International Journal of Medicinal Mushrooms* 11, 287–302.

Darriba D, Taboada GL, Doallo R, Posada D, 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9, 772.

De Simone D, D'Amico L, Bressanin D, Motta E, Annesi T, 2010. "Molecular characterization of *Inonotus rickii* /*Ptychogaster cubensis* isolates from different geographic provenances". *Mycological Progress* 10, 301–306.

Decock C, Bitew A, Castillo G, 2005. *Fomitiporia tenuis* and *Fomitiporia aethiopica* (Basidiomycetes, Hymenochaetales), two undescribed species from the Ethiopian highlands: taxonomy and phylogeny. *Mycologia* 97, 121–129.

Decock C, Figueroa SH, Robledo G, Castillo G, 2006. *Phellinus caribaeo–quercicolus* sp. nov., parasitic on *Quercus cubana*: taxonomy and preliminary phylogenetic relationships. *Mycologia* 98, 265–274.

Douanla-Meli C, 2007. Fungi of Cameroon. Ecological diversity with emphasis on the taxonomy of non-gilled hymenomycetes from the Mbalmayo forest reserve. *Bibliotheca Mycologica* 202, 1–410.

Drechsler-Santos ER, Robledo GL, Lima-Júnior NC, Malosso E, Reck MA, Gibertoni TB, De Queiroz CMA, Rajchenberg M 2016. *Phellinotus*, a new neotropical genus in the Hymenochaetaceae (Basidiomycota, Hymenochaetales). *Phytotaxa* 261, 218–239.

Drechsler-Santos ER, Santos PJP, Gibertoni TB, Cavalcanti MAQ, 2010. Ecological aspects of Hymenochaetaceae in an area of Caatinga (semi-arid) in Northeast Brazil. *Fungal Diversity* 42, 71–78.

Fiasson JL, Niemelä T, 1984. The Hymenochaetales: a revision of the European poroid taxa. *Karstenia* 24, 14–28.

Fischer M 2009. *Fomitiporia mediterranea* as a white rotter in esca-diseased grapevine: spores are produced in relation to temperature and humidity and are able to colonize young wood. *Phytopathologia Mediterranea* 48, 174.

Fischer M, 1996. On the species complexes within *Phellinus*: *Fomitiporia* revisited. *Mycological Research* 100, 1459–1467.

Fischer M, 2002. A new wood–decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycological Progress* 1, 315–324.

Gilbertson RL, 1979. The genus *Phellinus* (Aphyllophorales: Hymenochaetaceae) in western North America. *Mycotaxon* 9, 51–89.

Góes-Neto A, Loguercio-Leite C, Guerrero RT, 2000. Taxonomy and qualitative ecological aspects of poroid Hymenchaetales in a Brazilian seasonal tropical forest. *Mycotaxon* 76, 197–211.

Hattori T, Sakayaroj J, Jones EBG, Suetrong S, Preedanon S, Klaysuban A, 2014. Three species of *Fulvifomes* (Basidiomycota, Hymenochaetales) associated with rots on mangrove tree *Xylocarpus granatum* in Thailand. *Mycoscience* 55, 344–354.

http://mykoweb.prf.jcu.cz/polypores/ list_phellinus.html). Collection of Polypores, Dr. Josef Vlasák, Hluboká nad Vltavou, Czech Republic. Genus *Phellinus* s.l.

Ji XH, He SH, Chen JJ, Si J, Wu F, Zhou LW, Vlasák J, Tian XM, Dai YC, 2017. Global diversity and phylogeny of *Onnia* (Hymenochaetaceae) species on gymnosperms. *Mycologia* 109, 27–34.

Ji XH, Wu F, Dai YC, Vlasák J, 2017. Two new species of *Fulvifomes* (Hymenochaetales, Basidiomycota) from America. *Mycokeys* 22, 1–13.

Katoh K, Standley DM, 2013. MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30, 772–780.

Kotlaba F, Pouzar Z, 1994. Two new localities of *Inonotus rickii* in Europe. *Czech Mycology* 47, 159–161.

Larsen MJ, Cobb-Poulle LA, 1990. The genus *Phellinus* (Hymenochaetaceae): a survey of the world taxa. *Fungiflora*, Oslo, Norway, 206 pp.

Larsson KH, Parmasto E, Fischer M, Langer E, Nakasone KK, Redhead SA, 2006. Hymenochaetales: a molecular phylogeny for the hymenochaetoid clade. *Mycologia* 98, 926–936.

Lindblad I, 2001. Diversity of poroid and some corticoid wood- inhabiting fungi along the rainfall gradient in tropical forests, Costa Rica. *Journal of Tropical Ecology* 17, 353–369.

Lombard F, Larsen M, 1985. *Phellinus bicuspidatus* (Hymenochaetales, Hymenochaetaceae), a new species associated with a white sap rot of oak in Louisiana. *Mycologia* 77, 55–61.

Lowe JL, 1957. Polyporaceae of North America. The genus *Fomes*. State University College of Forestry at Syracuse University, Technical Publication nº 80, 97 p.

Malençon G, 1970. *Ptychogaster cubensis* Pat. et *Xanthochrous rickii* Pat., parasites du Faux-Poivrier (Schinus molle L.) du Maroc. *Bulletin de la Société mycologique de France* 86, 693-697.

Melo I, Ramos AP, Frazano Caetano MF, 2002. First Record of *Inonotus Rickii* (Basidiomycetes, Hymenochaetaceae) in Portugal. *Portugaliae Acta Biologica* 20, 265–269.

Miller MA, Pfeiffer W, Schwartz T, 2010. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1–8.

Miller RNG, Holderness M, Bridge PD, Chung GF, Zakaria MH, 2000. Genetic diversity of Ganoderma in oil palm plantings. Plant Pathology 48, 595–603.

Murrill WA, 1914. Northern polypores. Privately printed, New York.

Nguyen DK, Davis CM, Chinen J, Vallejo JG, Noroski LM, 2009. "Basidiomycetous *Inonotus* (*Phellinus*) *tropicalis* Osteomyelitis in Pediatric and Adult X-linked Chronic Granulomatous Disease". *Journal of Allergy and Clinical Immunology* 123, S13–S13.

Núñez M, Ryvarden L, 2000. East Asian Polypores Vol. 1. Ganodermataceae and Hymenochaetaceae. *Synopsis Fungorum* 13, 1–168.

Parmasto E, Saar I, Larsson E, Rummo S, 2014. Phylogenetic taxonomy of Hymenochaete and related genera (Hymenochaetales). *Mycological Progress* 13, 55–64.

Rajchenberg M, Pildain MB, Bianchinotti MV, Barroetaveña C, 2015. The phylogenetic position of poroid Hymenochaetaceae (Hymenochaetales, Basidiomycota) from Patagonia, Argentina. *Mycologia* 107, 754–767.

Robledo G, Urcelay C, Rajchenberg M, 2003. New species decaying living *Polylepis australis* in Córdoba, central Argentina. *Mycologia* 95, 347–352.

Ronquist F, Teslenko M, van derMark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP, 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539–542.

Ryvarden L, 1991. Genera of polypores. Nomenclature and taxonomy. *Fungiflora*, Oslo, Norway, 363 pp.

Ryvarden L, 2004. Neotropical polypores I. *Syn Fung* 19, 1–227.

Ryvarden L, 2005. The genus *Inonotus*, a synopsis. *Synopsis Fungorum*. 21, 1–149.

Ryvarden L, Melo I, 2014. Poroid fungi of Europe. *Syn Fung* 31, 1–455.

Salvador-Montoya CA, Popoff OF, Reck M, Drechsler-Santos ER, 2018. Taxonomic delimitation of *Fulvifomes robiniae* (Hymenochaetales, Basidiomycota) and related species in America: *F. squamosus* sp. nov. *Plant Systematics and Evolution* 304, 445–459.

Sell I, 2008. Taxonomy of the species in the *Phellinus igniarius* group. *Mycotaxon* 104, 337–347.

Stenlid J 2008. Population biology of forest decomposer basidiomycetes. In: Boddy L, Frankland JC, van West P, (eds) Ecology of Saprotrophic Basidiomycetes. London: Academic Press/Elsevier.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.

Tchotet Tchoumi JM, Coetzee MPA, Rajchenberg M, Wingfield MJ, Roux J, 2018. Three *Ganoderma* species, including *Ganoderma dunense* sp. nov., associated with dying *Acacia cyclops* trees in South Africa. *Australasian Plant Pathology* 47, 431–447.

Tchotet Tchoumi JM, Coetzee MPA, Vivas M, Rajchenberg M, Roux J, 2017. Wood-rotting basidiomycetes associated with declining native trees in timber-harvesting compartments of the Garden Route National Park of South Africa. *Austral Ecology* 42, 947–963.

Tomšovský M, Vampola P, Sedlák P, Byrtusová Z, Jankovský L, 2010. Delimitation of central and northern European species of the *Phellinus igniarius* group (Basidiomycota, Hymenochaetales) based on analysis of ITS and translation elongation factor 1 alpha DNA sequences. *Mycological Progress* 9, 431–445.

Torrend C, 1905. Primeira contribuçao para p estudio da flora Mycologica da provincia de Moçambique. *Broteria,Rev. scienc. nat. Colleg. S. Fiel* 4, 212 – 221.

Vilgalys R, Hester M, 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *Journal of Bacteriology* 172, 4239–4246.

Vlasák J, Kout J, Vlasák JJr, Ryvarden L, 2011. New records of polypores from southern Florida. *Mycotaxon* 118, 159–176.

Wagner T, Fischer M, 2001. Natural groups and a revised system for the European poroid Hymenochaetales (Basidiomycota) supported by nLSU rDNA sequence data. *Mycological Research* 105, 773–782.

Wagner T, Fischer M, 2002. Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. *Mycologia* 94, 998–1016.

Wu F, Zhou L.W, Dai YC, 2016. *Neomensularia duplicata* gen. et sp. nov. (Hymenochaetales, Basidiomycota) and two new combinations. *Mycologia* 108, 891–898.

Wu SH, Dai YC, Hattori T, Yu TW, Wang DM, Parmasto E, Chang HY, Shih SY, 2012. Species clarification for the medicinally valuable sanghuang mushroom. *Botanical Studies* 53, 135–149.

Yombiyeni P, Douanla-Meli C, Amalfi M, Decock C, 2011. Poroid Hymenochaetaceae from Guineo-Congolian rainforest: *Phellinus gabonensis* sp. nov. from Gabon—taxonomy and phylogenetic relationships. *Mycological Progress* 10, 351–362.

Zhou LW, 2014. *Fulvifomes hainanensis* sp. nov. and *F. indicus* comb. nov. (Hymenochaetales, Basidiomycota) evidenced by a combination of morphology and phylogeny. *Mycoscience* 55, 70–77.

Zhou LW, 2015. *Fulvifomes imbricatus* and *F. thailandicus* (Hymenochaetales, Basidiomycota): two new species from Thailand based on morphological and molecular evidence. *Mycological Progress* 14, 1–8.

Zhou LW, Ji XH, Vlasák J, Dai YCh (in press) Taxonomy and phylogeny of *Pyrrhoderma*: a redefinition, the segregation of *Fulvoderma*, gen. nov., and identifying four new species. *Mycologia* https://doi.org/10.1080/00275514.2018.1474326

Zhou LW, Vlasák J, Dai YC, 2016. Taxonomy and phylogeny of *Phellinidium* (Hymenochaetales, Basidiomycota): A redefinition and the segregation of *Coniferiporia* gen. nov. for forest pathogens. *Fungal Biology* 120, 988–1001.

Zhou LW, Vlasák J, Decock C, Assefa A, Stenlid J, Abate D, Wu SH, Dai YC, 2016. Global diversity and taxonomy of the *Inonotus linteus* complex (Hymenochaetales, Basidiomycota): *Sanghuangporus* gen. nov., *Tropicoporus excentrodendri* and *T. guanacastensis* gen. et spp. nov., and 17 new combinations. *Fungal Diversity* 77, 335–347.

Zhou LW, Vlasák J, Qin WM, Dai YC, 2016. Global diversity and phylogeny of the *Phellinus igniarius* complex (Hymenochaetales, Basidiomycota) with the description of five new species. *Mycologia* 108, 192–204.

Table 1. Hymenochaetaceae isolates from the GRNP indigenous forest (Tchotet Tchoumi et al. 2017) used for DNA sequencing of LSU gene region.

					GenBank accessions	
Genera	**Isolates**	**Host**	**Source**	**Host status**	**ITS**	**LSU**
Fomitiporia	CMW47881	*Olea capensis* subsp. *macrocarpa*	Basidiocarp	Dead standing	MH599111	MH599121
	CMW48058	*O. capensis* subsp. *macrocarpa*	Basidiocarp	Dead standing	MH599109	MH599120
	CMW48613	*Gonioma kamassi*	Basidiocarp	Stump	MH599112	MH599122
	CMW48621	*O. capensis* subsp. *macrocarpa*	Basidiocarp	Dead standing	MH599110	MH599123
Fuscoporia	CMW45308	*O. capensis* subsp. *macrocarpa*	Basidiocarp	Dead standing	MH599100	MH599124
	CMW47749	*Apodytes dimidiata* subsp. *dimidiata*	Basidiocarp	Dead standing	MH599106	MH599129
	CMW47816	*Psydrax obovata* subsp. *obovata*	Basidiocarp	Alive	MH599101	MH599125
	CMW48042	*Nuxia floribunda*	Basidiocarp	Dead standing	MH599104	MH599128
	CMW48060	*Cunonia capensis*	Basidiocarp	Stump	MH599103	MH599126
	CMW48145	*Olea capensis* subsp. *macrocarpa*	Basidiocarp	Stump	MH599105	MH599130
	CMW48600	*Olea capensis* subsp. *capensis*	Basidiocarp	Dead standing	MH599102	MH599127
Fulvifomes	CMW47808	*Elaeodendron croceum*	Basidiocarp	Alive	MH599093	MH599131
	CMW47825	*E. croceum*	Basidiocarp	Alive	MH599094	MH599134
	CMW47909	*E. croceum*	Basidiocarp	Alive	MH599096	MH599132
	CMW48154	*E. croceum*	Basidiocarp	Alive	MH599097	MH599135
	CMW48610	*E. croceum*	Basidiocarp	Alive	MH599095	MH599133
Inonotus	CMW45076	*O. capensis* subsp. *macrocarpa*	Infected wood	Alive	MH599089	MH599114
	CMW45333	*Podocarpus falcatus*	Infected wood	Alive	MH599091	MH599116
	CMW45334	*Podocarpus falcatus*	Infected wood	Alive	MH599092	MH599117
	CMW45823	*Olea capensis* subsp. *capensis*	Infected wood	Alive	MH599088	MH599113
	CMW47748	*O. capensis* subsp. *macrocarpa*	Basidiocarp	Alive	MH599099	MH599119
	CMW47759	*O. capensis* subsp. *macrocarpa*	Infected wood	Alive	MH599090	MH599115
	CMW48176	*O. capensis* subsp. *macrocarpa*	Basidiocarp	Alive	MH599098	MH599118
Phellinus	CMW45332	*Psydrax obovata* subsp. *obovata*	Basidiocarp	Fallen branch	MH599107	MH599136
	CMW48059	*Olea capensis* subsp. *capensis*	Basidiocarp	Alive	MH599108	MH599137

Table 2. List of reference taxa used in the second phase of phylogenetic analyses

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	LSU
Fomitiporia aethiopica	MUCL 44777	Ethiopia	GU478341	AY618204
F. aethiopica	MUCL 44806	Ethiopia	GU461944	AY618202
F. apiahyna	MUCL 51451	Ecuador	GU461963	GU461997
F. apiahyna	MUCL 51485	Ecuador	GU461962	GU461996
F. australiensis	MUCL 49406	Australia	AY624997	GU462001
F. baccharidis	MUCL 47756	Argentina	JQ087886	JQ087913
F. baccharidis	MUCL 47757	Argentina	JQ087887	JQ087914
F. bakeri	MUCL 51098	USA	JQ087874	JQ087901
F. bannaensis	MUCL 46926	China	KF444682	KF444705
F. bannaensis	MUCL 46930	China	KF444683	KF444706
F. calkinsii	MUCL 51398	USA	KF444687	KF444710
F. calkinsii	MUCL 52346	Mexico	JQ087876	JQ087903
F. capenses	MUCL 53009	South Africa	JQ087890	JQ087917
F. castilloi	MUCL 53481 (T)	French Guiana	JQ087889	JQ087916
F. castilloi	MUCL 53980	French Guiana	JX093786	JX093830
F. cupressicola	MUCL 52486 (T)	Mexico	JQ087877	JQ087904
F. cupressicola	MUCL 52488	Mexico	JQ087878	JQ087905
F. dryophila	MUCL 46381	USA	EF429239	EF429220
F. dryophila	MUCL 51144	USA	KF444689	KF444712
F. erecta	MUCL 49871	France	GU461939	GU461976
F. erecta	MA-PA03	Italy	KF444690	KF444713
F. expansa	MUCL 55026 (T)	French Guiana	KJ401031	KJ401032
F. gabonensis	MUCL 47576 (T)	Gabon	GU461971	GU461990
F. gabonensis	MUCL 51291	Gabon	GU461967	GU461986
F. hartigii	MUCL 53550	Estonia	JX093788	JX093832
F. hartigii	MUCL 53551	Estonia	JX093789	JX093833
F. hippophaeicola	MUCL 31746	Belgium	GU461945	AY618207
F. hippophaeicola	MUCL 31747	Belgium	GU461946	GU461977
F. ivindoensis	MUCL 51311	Gabon	GU461952	GU461979
F. langloisii	MUCL 46375	USA	EF429242	EF429225

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	LSU
F. langloisii	MUCL 46373	USA	EF429243	EF429226
F. maxonii	MUCL 46017	Cuba	EF433559	EF429230
F. maxonii	MUCL 52340	Mexico	KF444694	KF444717
F. mediterranea	MUCL 38514	Italy	GU461953	AY618201
F. mediterranea	MUCL 45670	France	GU461954	GU461980
F. neotropica	MUCL 51335 (T)	Argentina	KF444698	KF444721
F. neotropica	MUCL 54196	Brazil	KF444701	KF444724
F. nobilissima	MUCL 47580	Gabon	GU461966	GU461985
F. nobilissima	MUCL 51289 (T)	Gabon	GU461965	GU461984
F. polymorpha	MUCL 46166	USA	GU461955	DQ122393
F. polymorpha	MUCL 46167	USA	GU461956	EF429233
F. pseudopunctata	MUCL 51325	Czech Rep.	GU461948	GU461981
F. pseudopunctata	MUCL 46168	France	JQ087891	JQ087918
Fomitiporia sp.	MUCL 51555	Martinique	JX093809	JX093853
Fomitiporia sp.	MUCL 53797	French Guiana	JX093810	JX093854
Fomitiporia sp.	MUCL 53798	French Guiana	JX093811	JX093855
Fomitiporia sp.	MUCL 53993	Mexico	JX093807	JX093851
Fomitiporia sp.	MUCL 53994	Mexico	JX093808	JX093852
Fomitiporia sp.	CBS 386.66	Argentina	EF433563	EF429234
Fomitiporia sp.	MUCL 53675	French Guiana	JX093791	JX093835
F. punctata	MUCL 34101	Germany	GU461947	AY618200
F. punctata	MUCL 47629	Japan	GU461950	GU461982
F. punctata	Cui 23	China	GU461974	GU461991
F. punctata	Cui 26	China	GU461975	GU461992
F. robusta	MUCL 51297	Estonia	JQ087892	JQ087919
F. robusta	MUCL 51327	Czech Rep.	–	GU461993
F. sonorae	MUCL 47689 (T)	USA	JQ087893	JQ087920
Fomitiporia sp.	MUCL 51105	USA	JQ087884	JQ087911
F. tabaquilio	MUCL 46230	Argentina	GU461940	DQ122394
F. tabaquilio	MUCL 47754	Argentina	GU461941	GU461994

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	LSU
F. tenuis	MUCL 44802 (T)	Ethiopia	GU461957	AY618206
F. tenuis	MUCL 49948	Gabon	GU461958	GU461998
F. texana	MUCL 47690	USA	JQ087894	JQ087921
F. texana	MUCL 51143	USA	JQ087895	JQ087922
F. torreyae	MUCL 47628	Japan	JQ087896	JQ087923
F. torreyae	WC31	Chine	JQ087897	JQ087924
F. tsugina	MUCL 52703	USA	JQ087899	JQ087926
F. tsugina	MUCL 51295	USA	JQ087881	JQ087908
Fulvifomes centroamericanus	JV1408/4	Costa Rica	–	KX960768
F. centroamericanus	JV0611/III	Guatemala	KX960763	KX960764
F. centroamericanus	JV0611/8P	Guatemala	KX960757	–
F. chinensis	LWZ 20130916-3	China	KJ787818	KJ787809
F. chinensis	LWZ 20130713-7	China	KJ787817	KJ787808
F. fastuosus	LWZ 20140731-13	Thailand	KR905674	KR905668
F. fastuosus	CBS 213.36	Philippines	AY558615	AY059057
F. fastuosus	LWZ 20140801-1	Thailand	KR905675	KR905669
F. grenadensis	voucher 1607-66	Costa Rica	KX960758	–
F. grenadensis	JV1212/2J	USA	KX960756	–
F. grenadensis	–	Brasil	–	AF450263
F. hainanensis	Dai 11573	China	KC879263	JX866779
F. imbricatus	LWZ 20140728-16	Thailand	KR905677	KR905670
F. imbricatus	LWZ 20140729-26	Thailand	KR905679	KR905671
F. indicus	Yuan 5932	China	KC879261	JX866777
F. inermis	LWZ 20130810-3	China	KJ787821	KJ787812
F. inermis	LWZ 20130809-5	China	KJ787819	KJ787810
F. kawakamii	CBS 428.86	USA	–	AY059028
F. krugiodendri	JV0312 24.10J	USA	KX960760	KX960766
F. krugiodendri	JV0904/1	USA	KX960762	KX960765
F. krugiodendri	JV1008/21	USA	KX960761	KX960767
F. merrillii	txid475282	Taiwan	JX484013	JX484002

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	LSU
F. nilgheriensis	CBS 209.36	USA	AY558633	AY059023
F. rimosus	–	Taiwan	JX484016	JX484003
F. robiniae	CBS 211.36	USA	AY558646	AF411825
F. robiniae	CFMR 2693	USA	KX065961	KX065995
F. siamensis	KBXG3	Thailand	JX104706	JX104753
F. siamensis	STRXG2	Thailand	JX104708	JX104755
F. squamosus	CS456	Peru	MF479269	MF479264
F. squamosus	CS385	Peru	MF479268	MF479265
F. squamosus	CS444	Peru	–	MF479263
Fulvifomes sp.	STRXM1	Thailand	JX104681	JX104728
Fulvifomes sp.	BAB-4916	India	KR155013	–
Fulvifomes sp.	BAB-4818	India	KR154999	–
Fulvifomes sp.	Yuan 5938	China	–	JX866778
F. thailandicus	LWZ 20140731-1	Thailand	KR905672	KR905665
F. xylocarpicola	KBXG5	Thailand	JX104669	JX104716
F. xylocarpicola	MU1	Thailand	JX104671	JX104718
Phellinotus piptadeniae	URM80322	Brazil	KM211290	KM211282
Phellinotus neoaridus	URM80362	Brazil	KM211294	KM211286
Phellinus populicola	BRNM 714885	Sweden	GQ383706	–
P. laevigatus	strain 83-912	–	AY340051	–
Fuscoporia atlantica	SP445618	Brazil	KP058515	KP058517
F. atlantica	SP465829	Brazil	KP058514	KP058516
F. callimorpha	JV090487	USA	JF692191	–
F. callimorpha	JV040914J	USA	JF692193	–
F. cinchonensis	CBS 427.76	Germany	–	AY059024
F. cinchonensis	CBS 447.76	South Korea	AY558613	–
F. contigua	JV0907/2a	USA	JQ794547	–
F. contigua	Dai 16045	USA	KX961105	KY189105
F. contigua	JV1204/22.3a,b-J	USA	KX961104	KY189104
F. coronadensis	RLG-9387-T	USA	JX110073	JX110117

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	LSU
F. coronadensis	RLG-9396-T	USA	JX110074	JX110118
F. ferrea	JV 1606/2.2-J	USA	KX961100	KY189100
F. ferrea	Cui 11801	China	KX961101	KY189101
F. ferrea	FP-133592-Sp	USA	KU139189	KU139259
F. ferruginosa	JV1309/4	Slovakia	KX961102	KY189102
F. ferruginosa	JV0408/28	Czech	KX961103	KY189103
F. gilva	Dai14246	China	KX961108	KY189109
F. gilva	Dai15130	China	KX961109	KY189108
F. gilva	xsd08128	China	FJ481039	–
F. palmicola	MUCL 44080	Ethiopian	–	AY618209
F. montana	–	Taiwan	JX484015	–
F. rutincta	PRM915961	USA	GU594160	–
F. rutincta	JV0610/14PK	Belize	JQ794579	–
F. senex	CBS 442.76	Korea	AY558647	–
F. senex	KUC20110922-13	Korea	JX463658	JX463652
F. senex	Q32	China	KC414230	–
F. subferrea	Dai 16326	China	KX961097	KY053472
F. subferrea	Dai 16327	China	KX961098	KY053473
F. subferrea	Cui 8173	China	KX961099	KY189111
F. torulosa	JV 1405/2	Czech	KX961106	KY189106
F. torulosa	JV 1312/19-Kout	Spain	KX961107	KY189107
Coniferiporia sulphurascens	Cui 10429	China	KR350565	KR350555
Inonotus rickii	IFP 10359	China	FJ667753	–
I. rickii	HFUTA 0009/2014	Chile	KR905931	–
I. latemarginatus	Dai 9758	China	KP030784	–
I. henanensis	Dai 12221	China	KP030782	–
I. henanensis	Dai 13157	China	KP030783	–
I. pachyphloeus	Wu 0407–6	Taiwan	KP030785	KP030770
I. chihshanyenus	TFRI 708	Germany	–	AY059039
I. tricolor	Dai 12228	China	KP030786	–

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	LSU
I. setuloso-croceus	STE-U7165	South Africa	KP279292	–
I. setuloso-croceus	STE-U7173	South Africa	KP279293	–
Sanghuangporus alpinus	Cui9666	China	JQ860311	–
S. alpinus	Cui9658	China	JQ860310	–
S. baumii	KUC20130809-20	South Korea	KJ668511	–
S. baumii	–	China	–	HQ328528
S. baumii	Dai3683	Taiwan	JN642567	–
S. baumii	Dai3684	Taiwan	JN642568	–
S. ligneus	MG12	Iran	KR073081	–
S. ligneus	MG13	Iran	KR073082	–
S. lonicericola	Dai8335	Taiwan	JN642573	–
S. lonicericola	Dai8340	Taiwan	JN642574	–
S. lonicericola	Dai 8376	China	–	KP030772
S. lonicericola	–	China	–	HQ328529
S. lonicerinus	TAA55428	Taiwan	JN642575	–
S. lonicerinus	MG280	Germany	KU213573	–
S. microcystideus	O 915609	Tanzania	KP030787	–
S. pilatii	MJ 25/08	Czech	–	KT428766
S. pilatii	BRNM 771989	Czech	KT428764	KT428765
S. sanghuang	Dai12723	China	JQ860316	–
S. sanghuang	Wu 0903–2	China	KP030773	–
S. vaninii	Dai 9061	Taiwan	–	JN169792
S. vaninii	Dai3624	Taiwan	JN642590	–
S. vaninii	Dai7011	Taiwan	JN642590	–
S. weigelae	WD 1838	Japan	–	KP030774
S. weigelae	Cui6012	China	JQ860319	–
S. weigelae	Cui7176	China	JQ860320	–
S. weigelae	WD-1838	Taiwan	JN642596	KP030774
S. weirianus	CBS 618.89	South Korea	AY558654	AY059035
S. zonatus	Cui6631	China	JQ860305	–

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	LSU
S. zonatus	Dai10841	China	JQ860306	KP030775
Tropicoporus boehmeriae	LWZ 20140729-13	China	KT223641	–
T. boehmeriae	LWZ 20140729-10	China	KT223640	–
T. cubensis	MUCL47079	Cuba	JQ860325	KP030776
T. cubensis	MUCL47113	Cuba	JQ860324	KP030777
T. dependens	JV 0409/20-J	China	KC778778	–
T. dependens	JV 1207/3.4-J	China	KC778779	–
T. excentrodendri	IFP Yuan 6232	China	NR_137949	KP030779
T. excentrodendri	Yuan 6234	China	KP030791	–
T. excentrodendri	MUCL 43130	China	–	KP030779
T. guanacastensis	JV 1408_25	Costa Rica	KP030793	KP030778
T. guanacastensis	O 19228	Costa Rica	KP030794	–
T. linteus	JV0904/140	China	JQ860323	–
T. linteus	JV0904/64	China	JQ860322	–
T. pseudolinteus	O 906288	China	KP030795	–
T. pseudolinteus	JV 0402/35-K	China	KC778781	–
T. rudis	O 915614	China	KP030796	–
T. rudis	O 915617	China	KP030797	–
T. sideroxylicola	JV 1207/4.3-J	China	KC778783	–
T. sideroxylicola	JV 0409/30-J	China	KC778782	–
T. sp.	Dai 16774	Thailand	KY053493	KY053494
T. stratificans	PHSPV3	Brazil	KM199689	–
T. stratificans	PHSPV2	Brazil	KM199688	–
T. tropicalis	UTHSC-01-1419	USA	–	AY598826
T. tropicalis	UTHSC-01-1652	USA	–	AY598827
T. tropicalis	UTHSC 02-617	USA	AY641432	–
T. tropicalis	–	USA	AY599487	–
T. cf. tropicalis	Yuan 5898	China	KP030798	–
Mensularia radiata	C38	China	KC414257	–
Phellinus amazonicus	MUCL 51487	Ecuador	KU499942	KU376307

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	LSU
P. amazonicus	MUCL 53036 (T)	French Guiana	KU499940	KU376305
P. amazonicus	MUCL 53056	French Guiana	KU499937	KU376302
P. alni	TW 162	Germany	AY340035	AF311025
P. alni	TW 322	Germany	AY340041	–
P. alni	BRNM 714880	Czech Republic	GQ383774	–
P. alni	BRNM 714864	Czech Republic	GQ383775	–
P. arctostaphyli	MF 91-329a	Germany	–	AY059026
P. baumii	TAA 82-20	Germany	–	AY059058
P. betulinus	JV 0410/19-J	China	JX069817	–
P. betulinus	JV 0809/85	China	JX069821	–
P. bicuspidatus	CBS 427.86	USA	AY189699	AY059022
P. bicuspidatus	KCTC 6651	–	AY558610	–
P. caribaeo-quercicola	MUCL 46004 (T)	Cuba	HM635698	DQ127280
P. caribaeo-quercicola	MUCL 46005	Cuba	HM635699	DQ127281
P. caribaeo-quercicola	MUCL 46003	Cuba	HM635697	DQ127279
P. chaquensis	–	Caribbean	–	DQ122396
P. castanopsidis	CUI 10153	China	JQ837944	JQ837956
P. castanopsidis	CUI 10157	China	JQ837945	JQ837957
P. cinereus	strain 89-826a	Estonia	AY340046	–
P. cinereus	strain 85-917/1	Germany	AY340048	AF311027
P. ellipsoideus	MUCL 47867	China	KU954545	KU954540
P. ellipsoideus	MUCL 47820	China	JQ837952	KU954542
P. ellipsoideus	MUCL 47822	China	JQ837954	KU954543
P. extensus	–	Cuba	–	DQ349099
P. gabonensis	MUCL 51275	Gabon	HM635720	HM635683
P. gabonensis	MUCL 51277	Gabon	HM635719	HM635684
P. gabonensis	MUCL 52025 (T)	Gabon	HM635708	HM635690
P. glaucescens	MUCL 52270	Japan	–	HM635691
P. glaucescens	MUCL 52271	Japan	–	HM635692
P. glaucescens	MUCL 52272	Japan	–	HM635693

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	**LSU**
P. igniarius	strain 83-1110a	Germany	–	AF311033
P. igniarius	FS656173	Taiwan	GU903009	–
P. igniarius	–	China	EU682413	HQ328533
P. laevigatus	Cui3710	China	–	FJ627260
P. laevigatus	TN 3260	Germany	–	AF311034
P. linteus	TAA 84-12	Germany	–	AY059018
P. lundellii	TN 5760	Finland	AY340060	AF311035
P. lundellii	Dai2684	Finland	AY340058	–
P. lundellii	strain 95-430	Germany	AY340061	–
P. monticola	Yuan 5559	China	JQ828892	–
P. monticola	voucher Li 757	China	JQ828890	–
P. nigricans	BRNM 714879	Czech Republic	GQ383721	–
P. nigricans	BRNM 714894	Czech Republic	GQ383725	–
P. nigricans	MJ 557/94	Czech Republic	GQ383724	–
P. occidentalis	CBS 196.55	South Korea	AY558634	–
P. occidentalis	CBS 169.55	Germany	–	AY059019
Phellinus sp.	MUCL 51334	Argentina	KU954539	KU954538
P. resupinatus	STE U7771	South Africa	KM523245	KM523249
P. resupinatus	STE-U7769 9 (T)	South Africa	KM523246	KM523251
P. setulosus	MUCL 54670	Australia	KU954536	KU954537
P. spiculosus	CBS 345.63	Germany	AY059055	AY189702
P. spiculosus	MUCL 53634	Mexico	KU376306	KU499941
P. pachyphloeus	CBS 446.76	Germany	–	AY059020
P. parmastoi	Dai2274	Germany	AY340052	–
P. parmastoi	TAA 7610	Germany	–	AY059017
P. parmastoi	Dai2930	Germany	AY340056	–
P. tremulae	strain 89-826c	Germany	–	AF311042
P. tremulae	–	China	–	HQ328536
P. tuberculosus	TW 114	Germany	–	AF311043
P. uncisetus	MUCL 46231	Argentina	GU461960	EF429235

Table 2. (continued)

Genus & species names	Voucher no.	Geographical origin	GenBank accession numbers	
			ITS	**LSU**
P. undulatus	MUCL 44139	Caribbean	–	DQ131561
P. vaninii	Dai 1980	Germany	–	AY059056
Stereum hirsutum	Shi5	Spain	EU851114	–
S. hirsutum	—	United Kingdom	FN539063	–

Table 3. Morphological characterisation of *Fomitiporia tsitsikamensis* sp. nov. and its closest phylogenetic relatives

Taxon	Origin	Substrate	Shape	Consistency	Pore surface	Pores/ mm	Hyphal system	Setae	Basidiospores	Reference
Fomitiporia aethiopica	Ethiopia	unidentified angiosperm	resupinate	seasonal (to perennial)	grayish orange–golden brown	5–6	dimitic	absent	6.0–8.8 × 5.5–7.2 µm; globose to subglobose	Decock et al. 2005
F. capensis	South Africa	*Vitis vinifera*	resupinate, thin	perennial	yellowish brown–rusty brown	4–6	dimitic	mostly absent	6.0–8.0 × 5.0–7.5 µm; ovoid to subglobose	Cloete et al. 2014
F. erecta	unspecified	*Quercus*	tuberculiform, claviform	perennial	golden tawny	5–6	dimitic	absent	6.0–7.5 × 5.0–6.5 µm; globose	Fiasson & Niemelä 1984
F. juniperinus	Italy	*Juniperus phoenicea*	ungulate to rounded	seasonal (to perennial)	cinnamon to umber brown	5-6	dimitic	present	6–7.5 × 5.5–6.5 µm; subglobose	Index of Fungi 6: 444
F. mediterranea	Germany	*V. vinifera*	resupinate	perennial	yellowish brown–pale brown	5–8	dimitic	very rare	5.5–7.5 × 4.5–6.5 µm; ellipsoid to subglobose	Fischer 2002
F. pseudopunctata	French mediterranean coast	Various angiosperm	resupinate	perennial	yellowish brown–greyish brown	6–8	dimitic	abundant	6.5–7.5 × 5.5–7 µm; subglobose	David et al. 1982
F. punicata	China	*Punica granatum*	effused-reflexed	perennial	yellowish brown–	4–6	dimitic	absent	5.0–7.5 × 4.0–6.5 µm; subglobose to globose	Dai et al. 2008
F. tsitsikamaensis sp. nov.	South Africa	*Olea capensis* subsp. *macrocarpa*	resupinate to pseudo-pileate	perennial	cinnamom brown	4–6	dimitic	present	7.0–8.0 × 6.5–7.5 µm; subglobose to globose	This study

Table 4. Morphological characterisation of *Fulvifomes elaeodendri* sp. nov. and its closest phylogenetic relatives

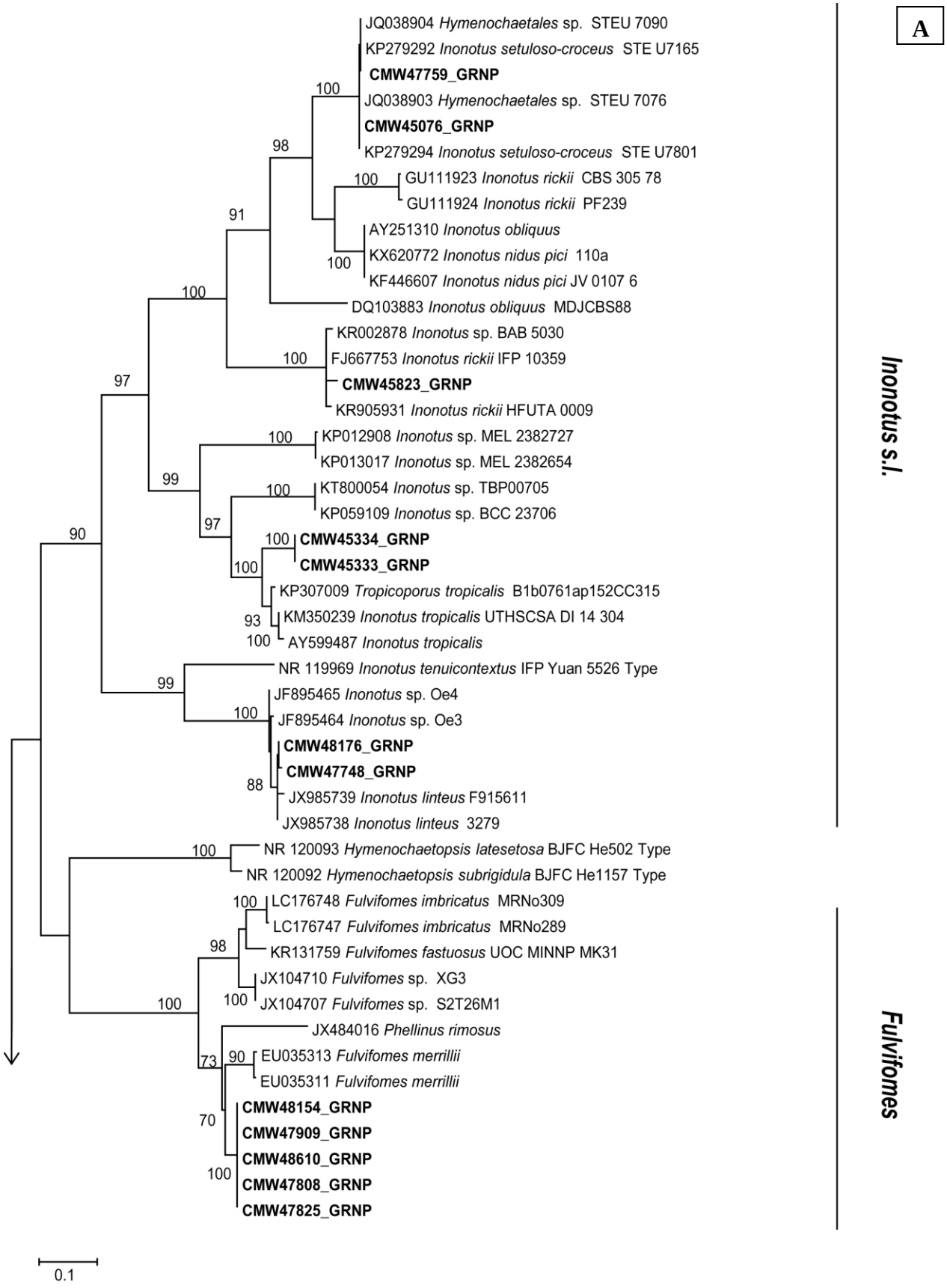
Taxon	Origin	Substrate	Shape	Consistency	Pore surface	Pores/mm	Hyphal system	Setae	Basidiospores	Reference
Fulvifomes elaeodendri sp. nov.	South Africa	*Elaeodendron croceum*	semi-circular to dimidiate, applanate to triquetrous up to ungulate	perennial	golden brown to brown; dull brown	5–7	dimitic	absent	5.7–6.5 × 4.7–5.4 µm; broadly ellipsoid to ellipsoid, some almost subglobose	This study
F. grenadensis	Grenada	dead wood	applanate to ungulate	perennial	fulvous to lustrous	5–7	unspecified	absent	3 × 4 µm; globose	Murrill 1908
F. hainanensis	China	rotten angiosperm wood	ungulate	perennial	yellowish brown	3–4	dimitic	absent	5.2–6.2 × 4–4.8 µm; ellipsoid	Zhou 2014
F. thailandicus	Thailand	living angiosperm tree	dimidiate, applanate	perennial	dark brown, shining	6–7	dimitic	absent	5.2–5.8 × 4.1–4.8 µm; ellipsoid	Zhou 2015

Table 5. Morphological characterisation of *Phellinus guttiformis* sp. nov. and its closest phylogenetic relatives

Taxon	Origin	Substrate	Shape	Consistency	Pore surface	Pores/ mm	Hyphal system	Setae	Basidiospores	Reference
Phellinus amazonicus	French Guiana	dead fallen trunk	resupinate to effused, cushion-shaped, up to nodulose to obclavate	perennial	light to dark brown, chocolate brown, glancing with light, then appearing paler, light to golden brown	7–10	dimitic	present, 15.0–23.0 × 5.0–9.5 µm	4.5–5.5 × 4.0–5.0 µm, ellipsoid to broadly ellipsoid	Campos Santana et al. 2016
Ph. bicuspidatus	United State of America	*Quercus* sp.	effuse and conforming to the surface of the substratum	perennial	dull ferruginous brown	5–7	monomitic	present, 16.0–24.0 × 8.0–14.0 µm	4–6 × 3–4 µm, broadly ellipsoid	Lombard and Larsen 1985
Ph. caribeo-quercicolous	Cuba	*Quercus cubana*	resupinate, either effused following the substrate, or cushion-shaped	perennial	shade of brown, chocolate brown, slightly glancing with light, then light brown, grayish light brown, grayish chocolate brown, weathering to more grayish	6–7	dimitic	present, 15.0–28.0 × 6.0–11.0 µm	4.5–5.7 × 3.5–4.3 µm, ellipsoid to broadly ellipsoid	Decock et al. 2006

Table 5. (continued)

Taxon	Origin	Substrate	Shape	Consistency	Pore surface	Pores/ mm	Hyphal system	Setae	Basidiospores	Reference
Ph. guttiformis sp. nov.	South Africa	*Psydrax obovata* subsp. *obovata*	resupinate to pseudo-pileate, drop-shaped or hoof-shaped	perennial	chocolate brown	6–8	dimitic	present, 12.0–25.0 × 4.0–9.0 µm	5.5–6.1 × 4.7–5.3 µm, broadly ellipsoid, subglobose to globose	This study
Ph. gabonensis	Gabon	*Sacoglottis gabonensis*	resupinate, effused–cushion-shaped	perennial	light to dark brown, glancing with light, then appearing paler, light to golden brown	6–8	dimitic	present, 14.0–28.0 × 5.5–12.5 µm	4.5–5.5 × 3.5–4.3 µm, ellipsoid to broadly ellipsoid	Yombiyeni et al. 2011
Ph. resupinatus	South Africa	*Vitis vinifera*	resupinate, cushion-shaped	perennial	dark yellowish–pale brownish; bright reddish	8–9	dimitic	present, 12.0–25.0 × 5.0–8.0 µm	4.5–5.0 × 3.0–3.5 µm, ellipsoid to broadly ellipsoid	Cloete et al. 2016


A
JQ038904 Hymenochaetales sp. STEU 7090
KP279292 Inonotus setuloso-croceus STE U7165
CMW47759_GRNP
JQ038903 Hymenochaetales sp. STEU 7076
CMW45076_GRNP
KP279294 Inonotus setuloso-croceus STE U7801
GU111923 Inonotus rickii CBS 305 78
GU111924 Inonotus rickii PF239
AY251310 Inonotus obliquus
KX620772 Inonotus nidus pici 110a
KF446607 Inonotus nidus pici JV 0107 6
DQ103883 Inonotus obliquus MDJCBS88
KR002878 Inonotus sp. BAB 5030
FJ667753 Inonotus rickii IFP 10359
CMW45823_GRNP
KR905931 Inonotus rickii HFUTA 0009
KP012908 Inonotus sp. MEL 2382727
KP013017 Inonotus sp. MEL 2382654
KT800054 Inonotus sp. TBP00705
KP059109 Inonotus sp. BCC 23706
CMW45334_GRNP
CMW45333_GRNP
KP307009 Tropicoporus tropicalis B1b0761ap152CC315
KM350239 Inonotus tropicalis UTHSCSA DI 14 304
AY599487 Inonotus tropicalis
NR 119969 Inonotus tenuicontextus IFP Yuan 5526 Type
JF895465 Inonotus sp. Oe4
JF895464 Inonotus sp. Oe3
CMW48176_GRNP
CMW47748_GRNP
JX985739 Inonotus linteus F915611
JX985738 Inonotus linteus 3279
NR 120093 Hymenochaetopsis latesetosa BJFC He502 Type
NR 120092 Hymenochaetopsis subrigidula BJFC He1157 Type
LC176748 Fulvifomes imbricatus MRNo309
LC176747 Fulvifomes imbricatus MRNo289
KR131759 Fulvifomes fastuosus UOC MINNP MK31
JX104710 Fulvifomes sp. XG3
JX104707 Fulvifomes sp. S2T26M1
JX484016 Phellinus rimosus
EU035313 Fulvifomes merrillii
EU035311 Fulvifomes merrillii
CMW48154_GRNP
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CMW48610_GRNP
CMW47808_GRNP
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Inonotus s.l.
Fulvifomes
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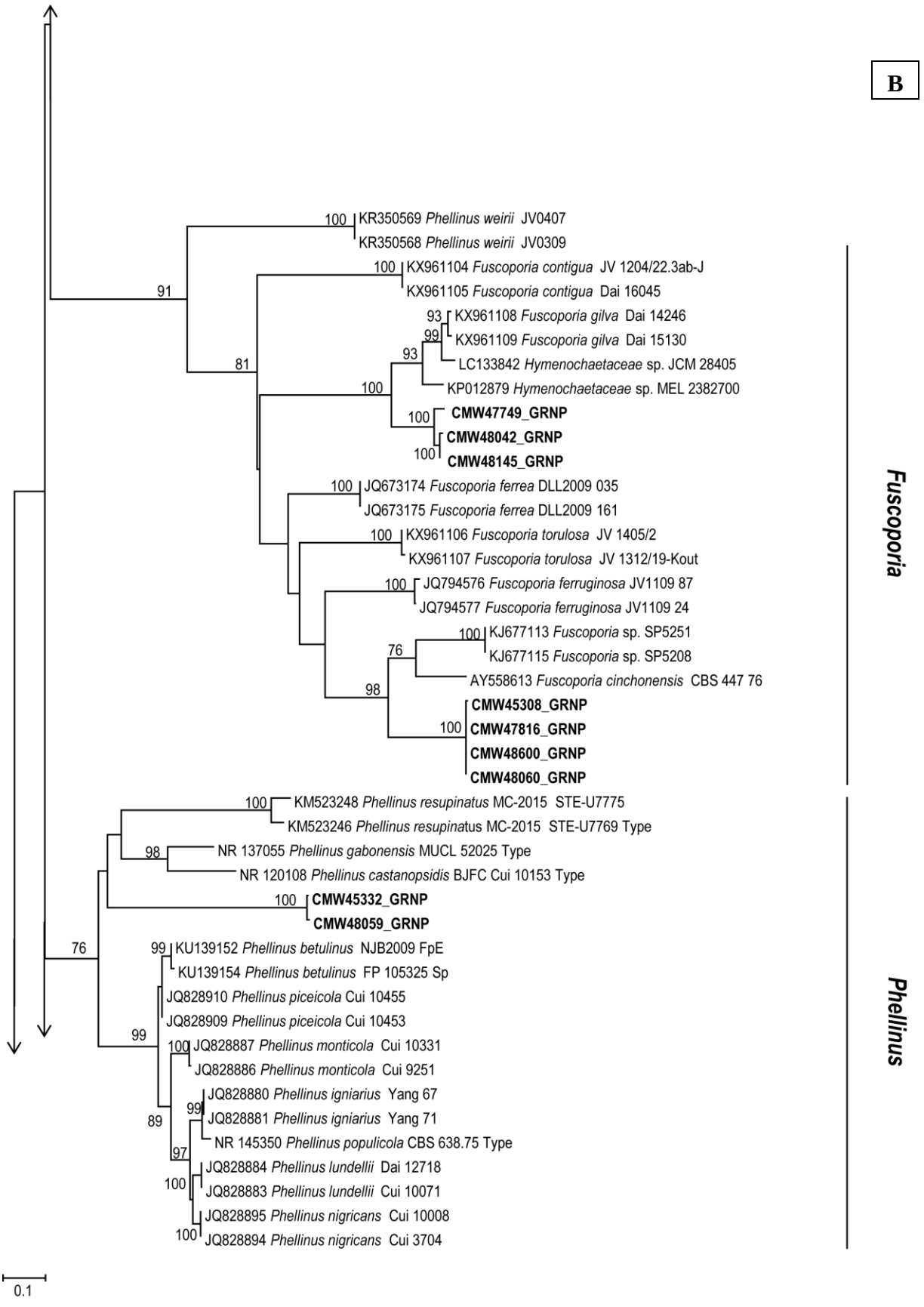
B
100 KR350569 Phellinus weirii JV0407
KR350568 Phellinus weirii JV0309
100 KX961104 Fuscoporia contigua JV 1204/22.3ab-J
91
KX961105 Fuscoporia contigua Dai 16045
93 KX961108 Fuscoporia gilva Dai 14246
99 KX961109 Fuscoporia gilva Dai 15130
93
81 LC133842 Hymenochaetaceae sp. JCM 28405
100 KP012879 Hymenochaetaceae sp. MEL 2382700
100 CMW47749_GRNP
CMW48042_GRNP
100 CMW48145_GRNP
100 JQ673174 Fuscoporia ferrea DLL2009 035
JQ673175 Fuscoporia ferrea DLL2009 161
100 KX961106 Fuscoporia torulosa JV 1405/2
KX961107 Fuscoporia torulosa JV 1312/19-Kout
100 JQ794576 Fuscoporia ferruginosa JV1109 87
JQ794577 Fuscoporia ferruginosa JV1109 24
100 KJ677113 Fuscoporia sp. SP5251
76
KJ677115 Fuscoporia sp. SP5208
AY558613 Fuscoporia cinchonensis CBS 447 76
98
CMW45308_GRNP
100 CMW47816_GRNP
CMW48600_GRNP
CMW48060_GRNP
Fuscoporia
100 KM523248 Phellinus resupinatus MC-2015 STE-U7775
KM523246 Phellinus resupinatus MC-2015 STE-U7769 Type
98 NR 137055 Phellinus gabonensis MUCL 52025 Type
NR 120108 Phellinus castanopsidis BJFC Cui 10153 Type
100 CMW45332_GRNP
CMW48059_GRNP
76
99 KU139152 Phellinus betulinus NJB2009 FpE
KU139154 Phellinus betulinus FP 105325 Sp
JQ828910 Phellinus piceicola Cui 10455
JQ828909 Phellinus piceicola Cui 10453
99
100 JQ828887 Phellinus monticola Cui 10331
JQ828886 Phellinus monticola Cui 9251
JQ828880 Phellinus igniarius Yang 67
99
89 JQ828881 Phellinus igniarius Yang 71
NR 145350 Phellinus populicola CBS 638.75 Type
97
JQ828884 Phellinus lundellii Dai 12718
100 JQ828883 Phellinus lundellii Cui 10071
JQ828895 Phellinus nigricans Cui 10008
100 JQ828894 Phellinus nigricans Cui 3704
Phellinus
0.1

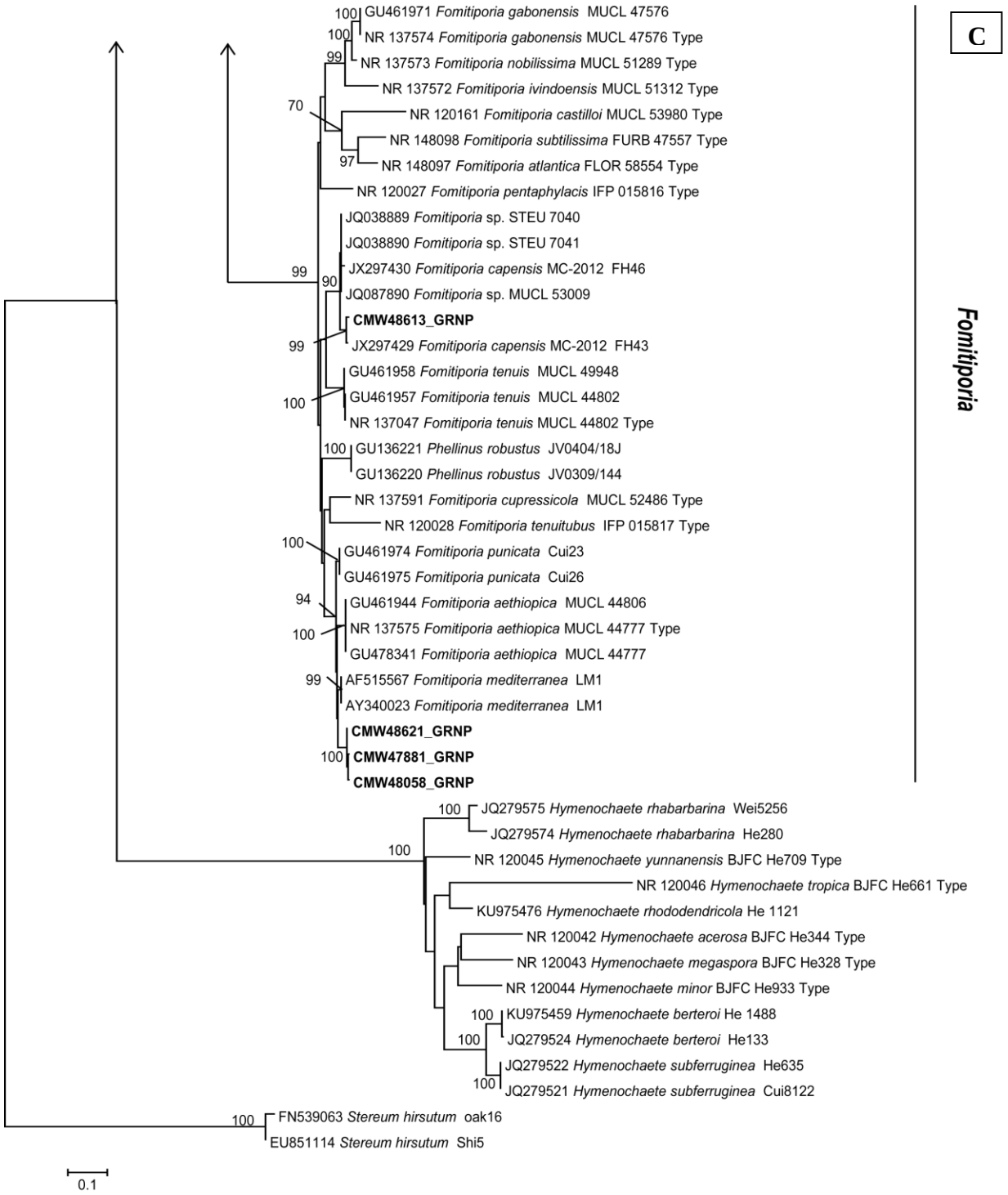

Figure 1A–C. Maximum Likelihood (ML) phylogenetic tree inferred from the ITS gene region showing the phylogenetic relationships between Hymenochaetaceae isolates from the GRNP with their closest relatives in GenBank. The data set was composed of 135 taxa and 1216 characters. The model implemented was GTR + I + G and the tree was rooted with sequences of *Stereum hirsutum* (FN539063 and EU851114). Bootstrap values ≥ 70 % are shown at the nodes of the tree. In bold are GRNP isolates.

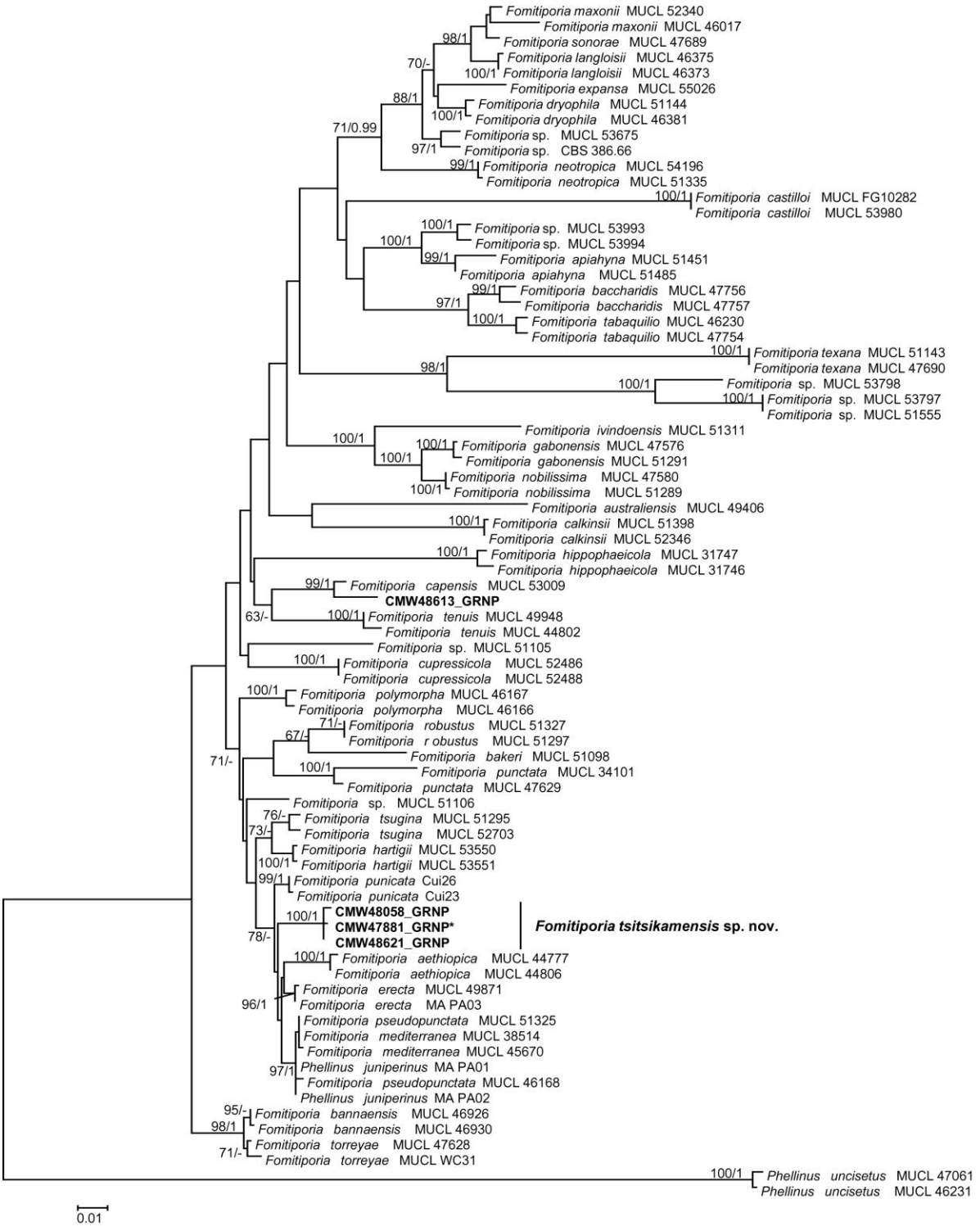

Figure 2. Phylogenetic tree derived from ML analysis of combined ITS and LSU gene sequences for *Fomitiporia* from South Africa and sequences of relatives from GenBank. Bootstrap support values ≥ 60% from 1000 replicates of ML analysis and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are those from the Garden Route National Park. Star (*) indicates the type.

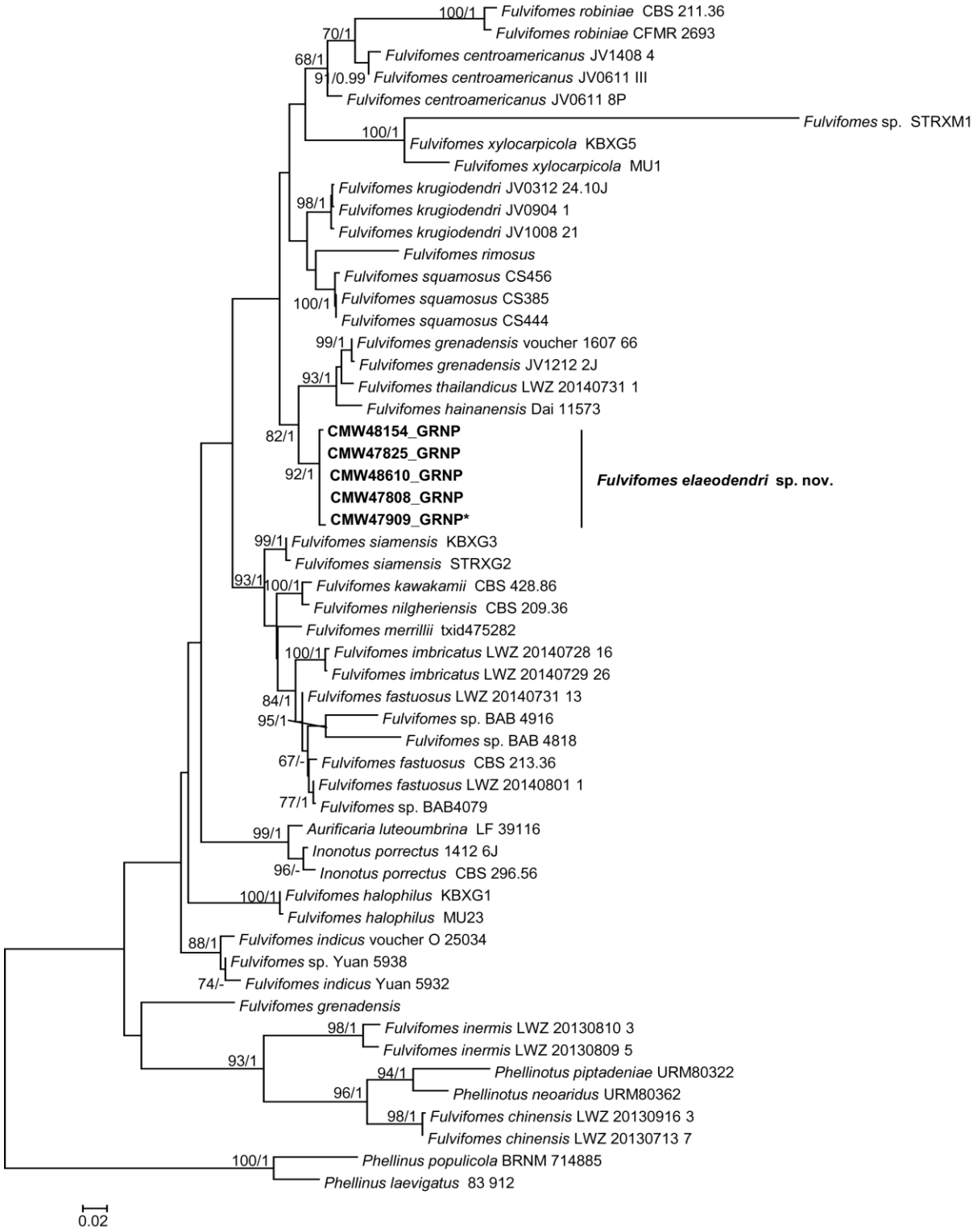

Figure 3. Combined ITS and LSU phylogenetic tree derived from ML analysis for *Fulvifomes* from South Africa. Bootstrap support values ≥ 60% from 1000 replicates of ML analysis and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are those from the Garden Route National Park. Star (*) indicates the type.

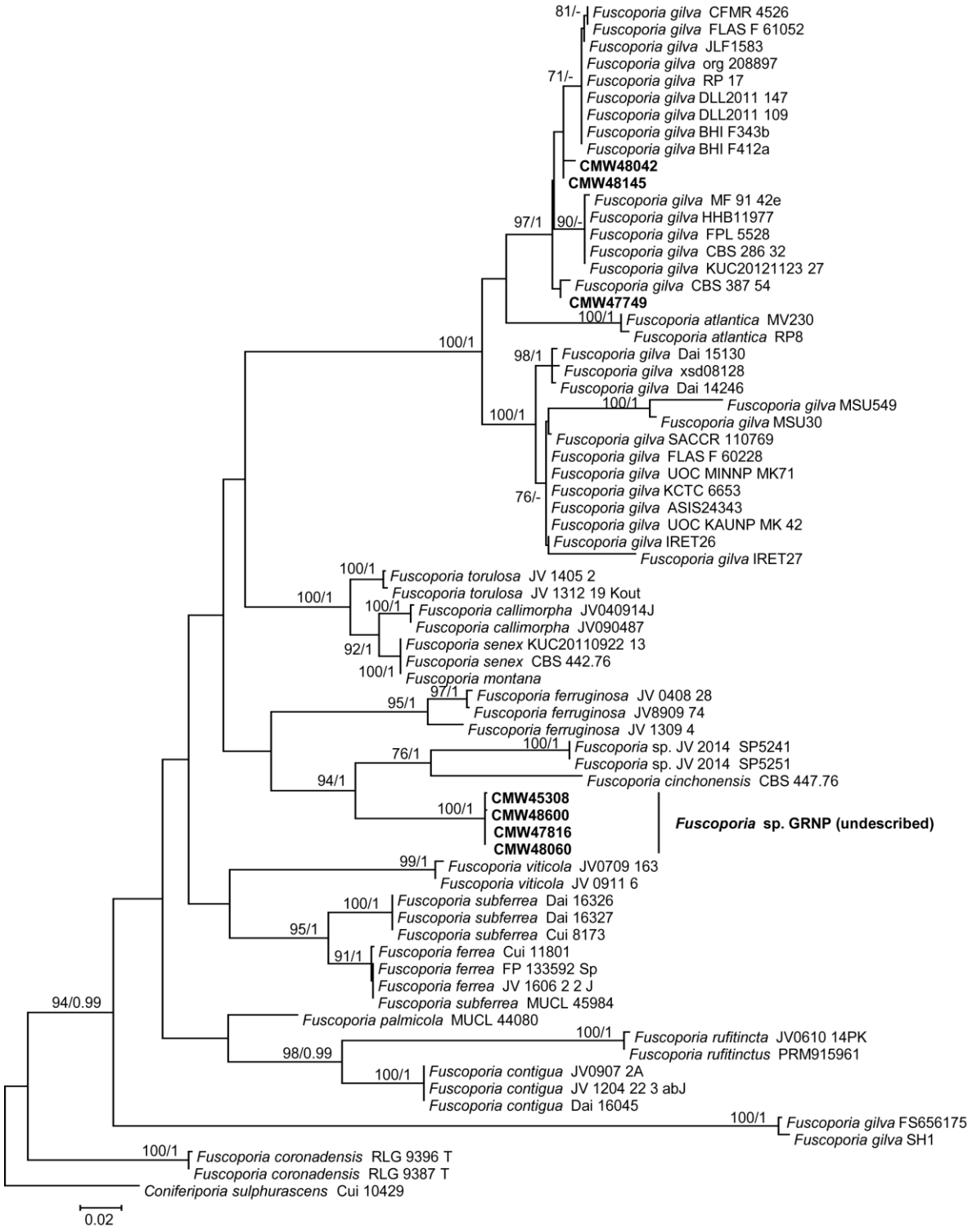

Figure 4. Combined (ITS and LSU) ML phylogenetic tree for *Fuscoporia* from South Africa and its relatives from GenBank. Bootstrap support values ≥ 60% from 1000 replicates of ML analysis and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are those from the Garden Route National Park.

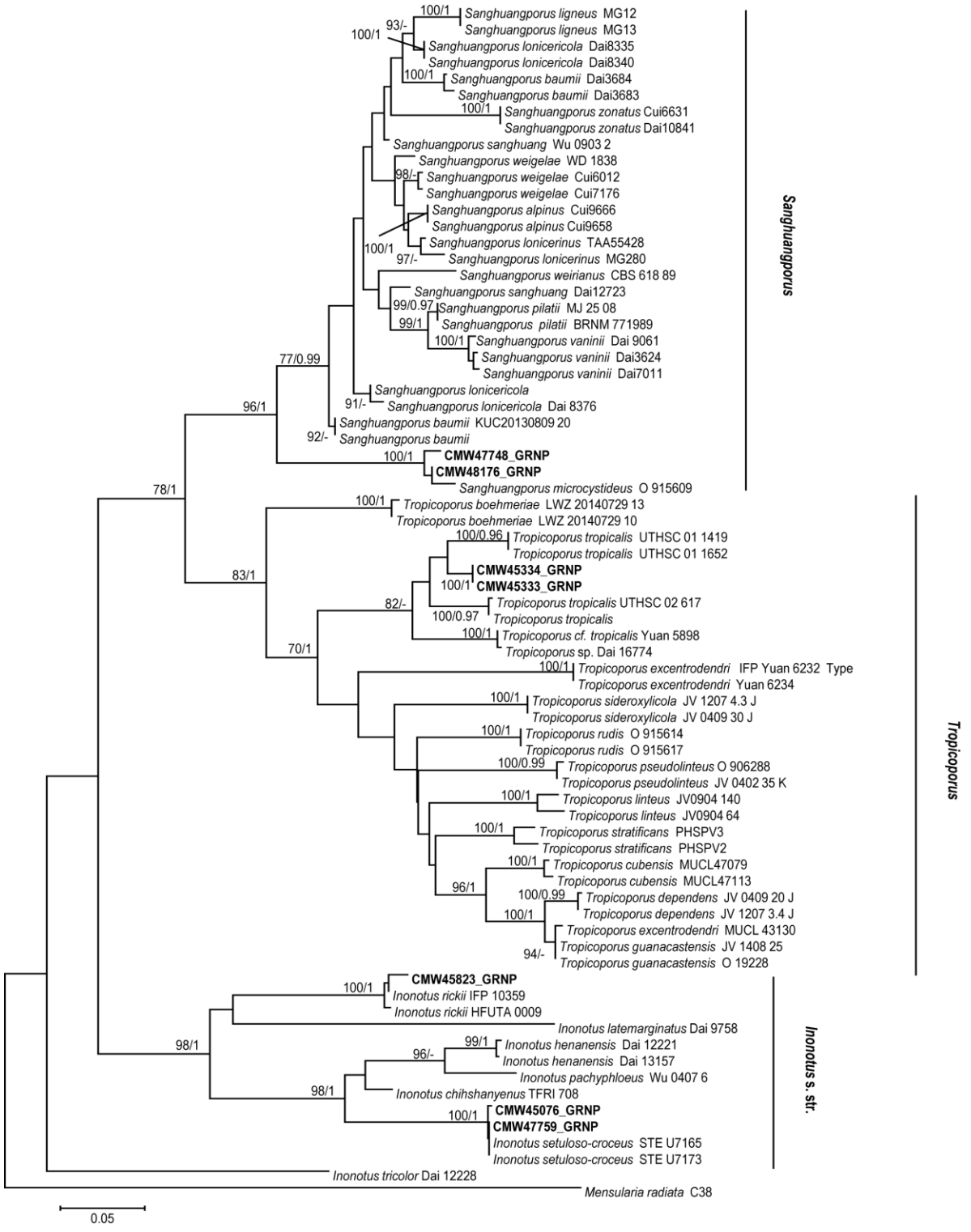

Figure 5. Combined ITS and LSU phylogeny from ML for *Inonotus* from South Africa. Bootstrap support values ≥ 60% from 1000 replicates of ML analysis and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are those from the Garden Route National Park.

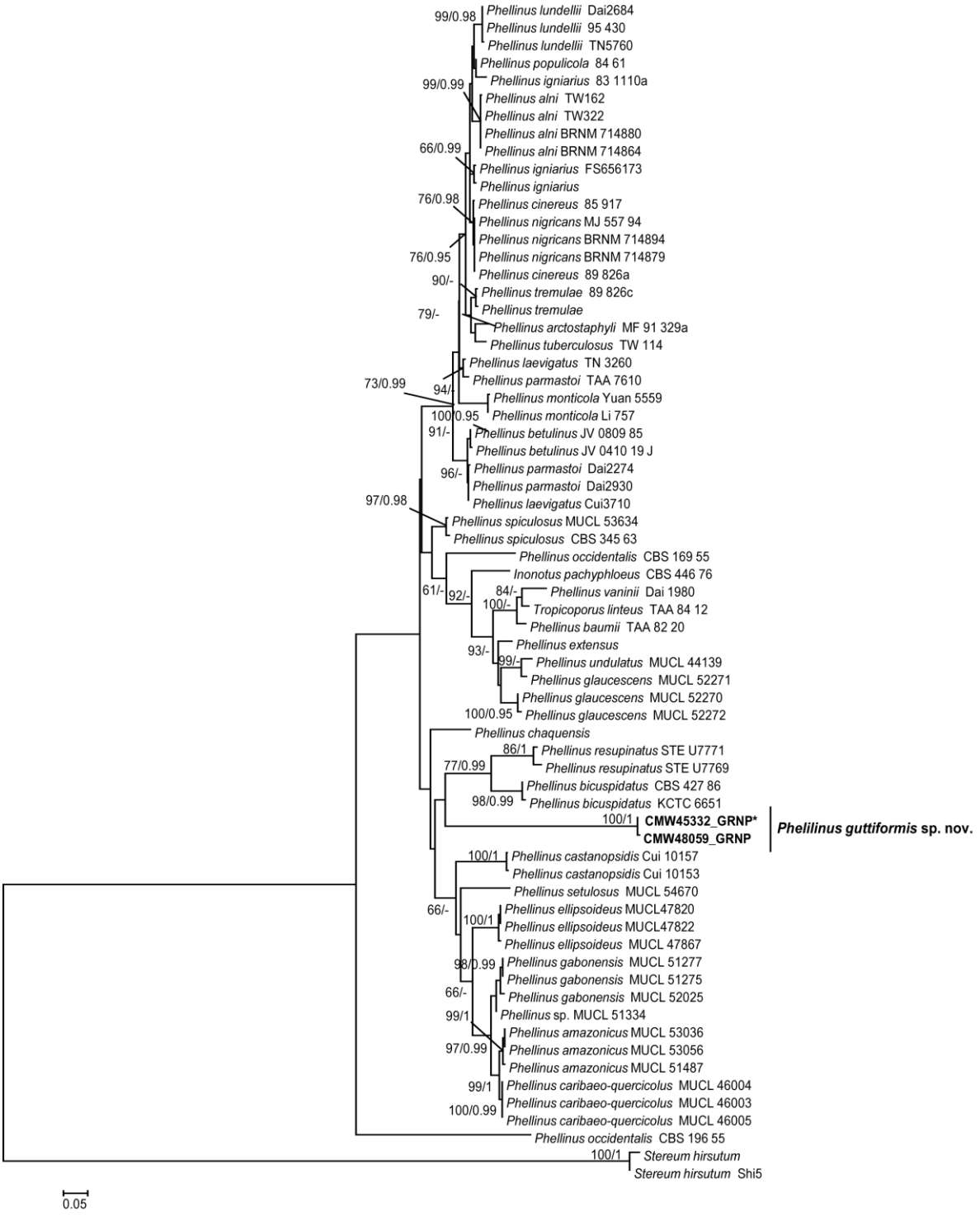

Figure 6. Maximum Likelihood combined phylogenetic tree for *Phellinus* from South Africa with its relatives from GenBank. Bootstrap support values ≥ 60% from 1000 replicates of ML analysis and posterior probability ≥ 0.95 from Bayesian Inference analysis are indicated next to branch nodes. Isolates in bold are those from the Garden Route National Park. Star (*) indicates the type.

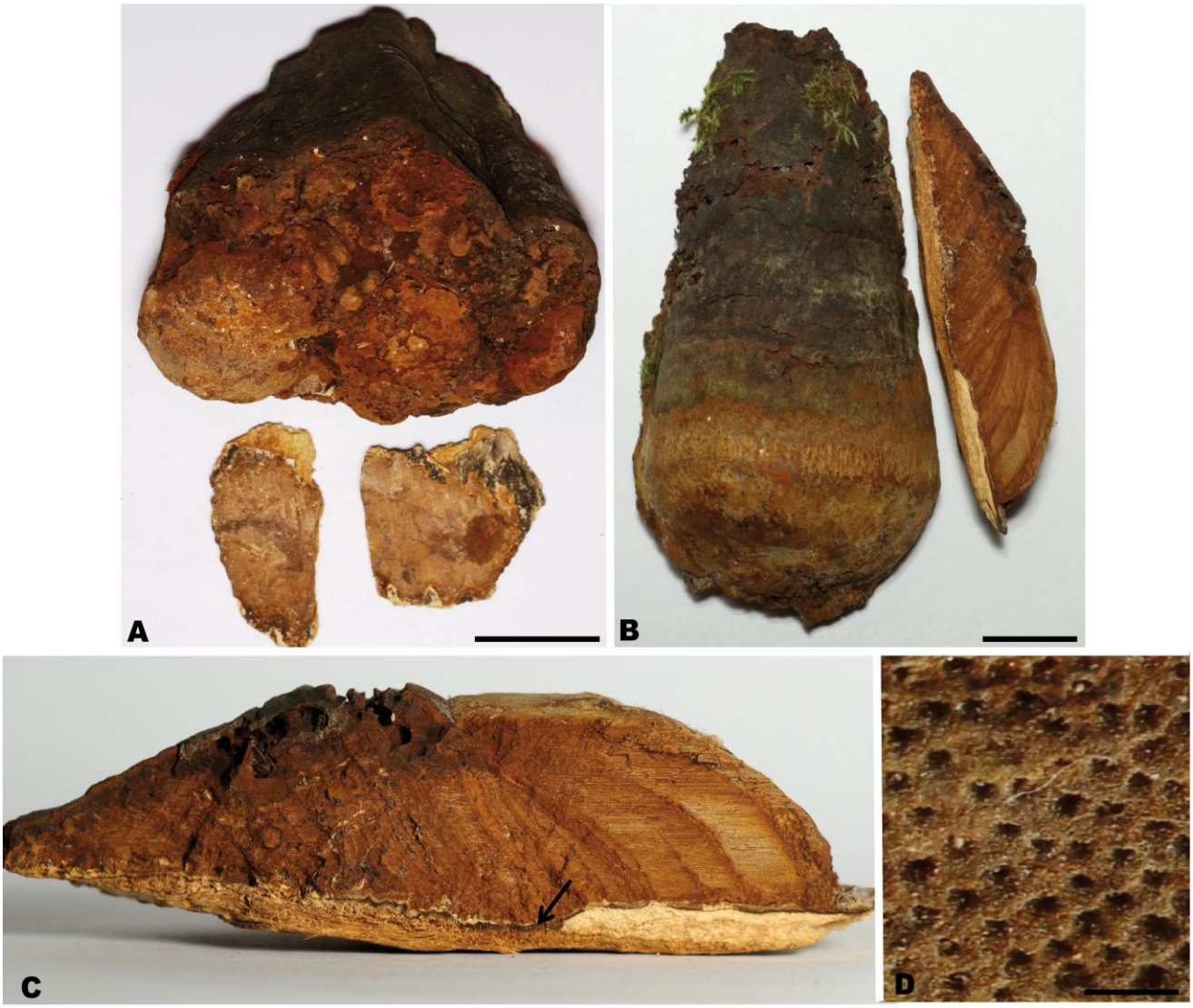

Figure 7. *Fomitiporia tsitsikamensis* sp. nov. (holotype JMT936 ; culture ex-type CMW47881 ; Fig. 8B). Macroscopic structures: A–B. Resupinate to pseudo-pileate basidiomes. C. Cross section of the pseudo-pileate basidiome showing the thin context with a distinct black line/cuticle develops against the substrate (arrow) and the stratified tubes separated by a contextual tissue developing in between the strata (arrow). D. Pores. Bars: A = 5 cm; B = 3 cm; C = 1 mm.

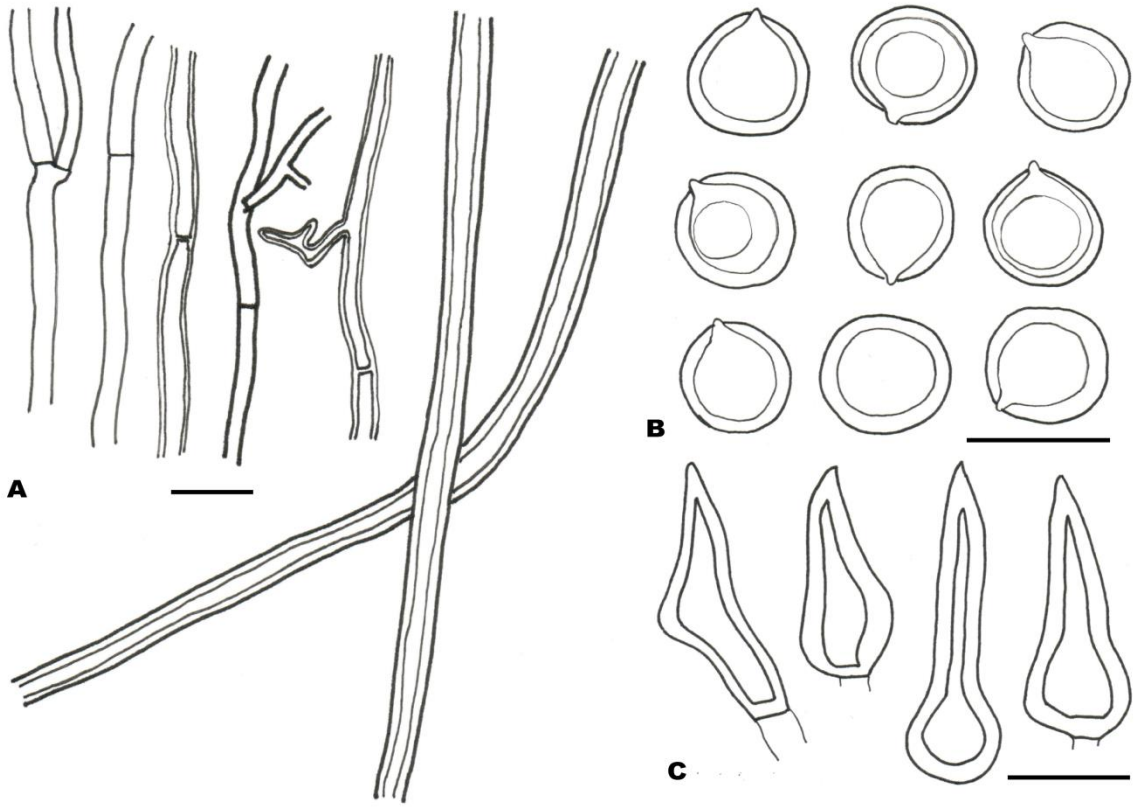

Figure 8. *Fomitiporia tsitsikamensis* sp. nov. microscopic structures: A. Generative hyphae simple septate and skeletal hyphae unbranched, thick-walled, with a distinct lumen. B. Subglobose to globose basidiospores. C. Acuminate and ventricose setae. Bars: A–B–C = 10 µm.

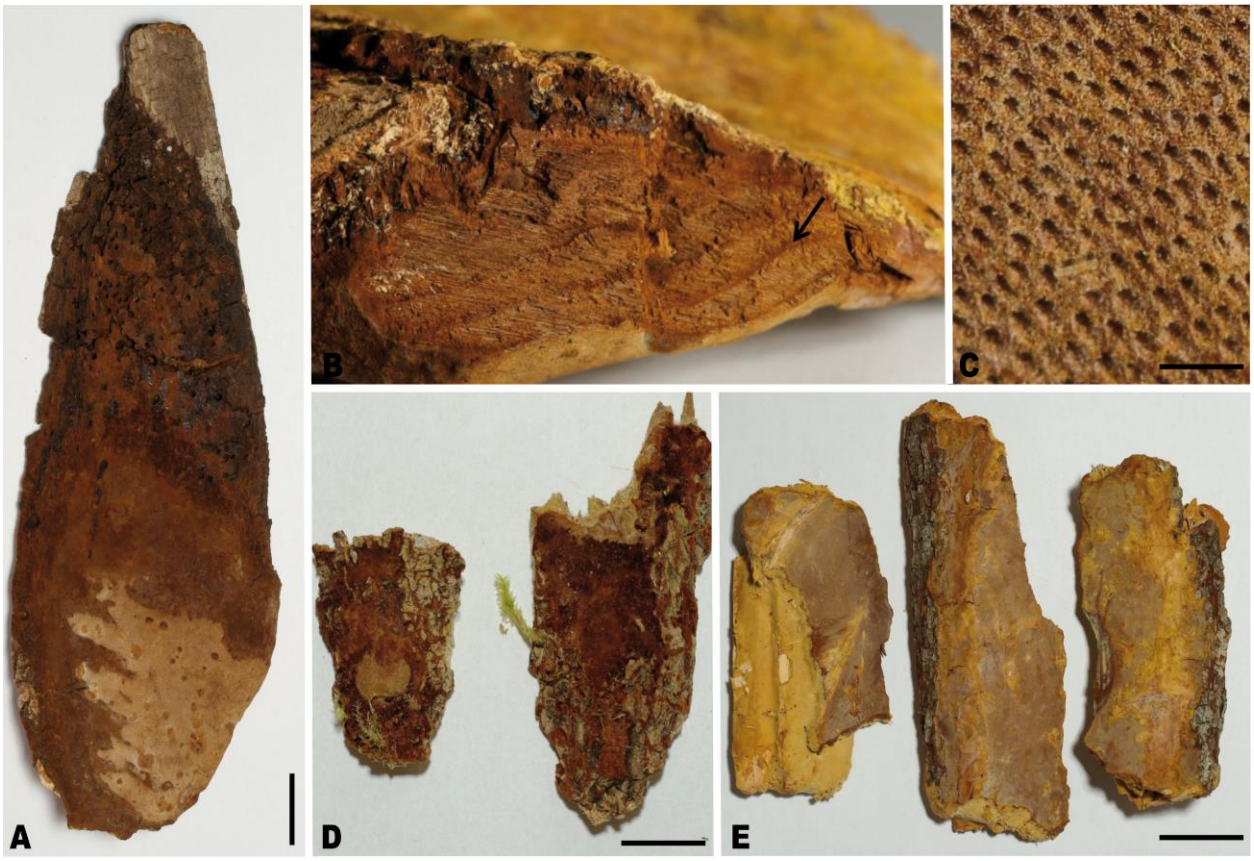

Figure 9. *Fuscoporia* sp. GRNP (undescribed). A. Basidiome with a pulvinate to ellipsoid form. B. Cross section of the pulvinate basidiome showing stratified tube layers separated by a contextual tissue (arrow). C. Pores. D–E. Resupinate basidiomes. Bars: A = 5 cm; C = 1 mm; D–E = 6 cm.

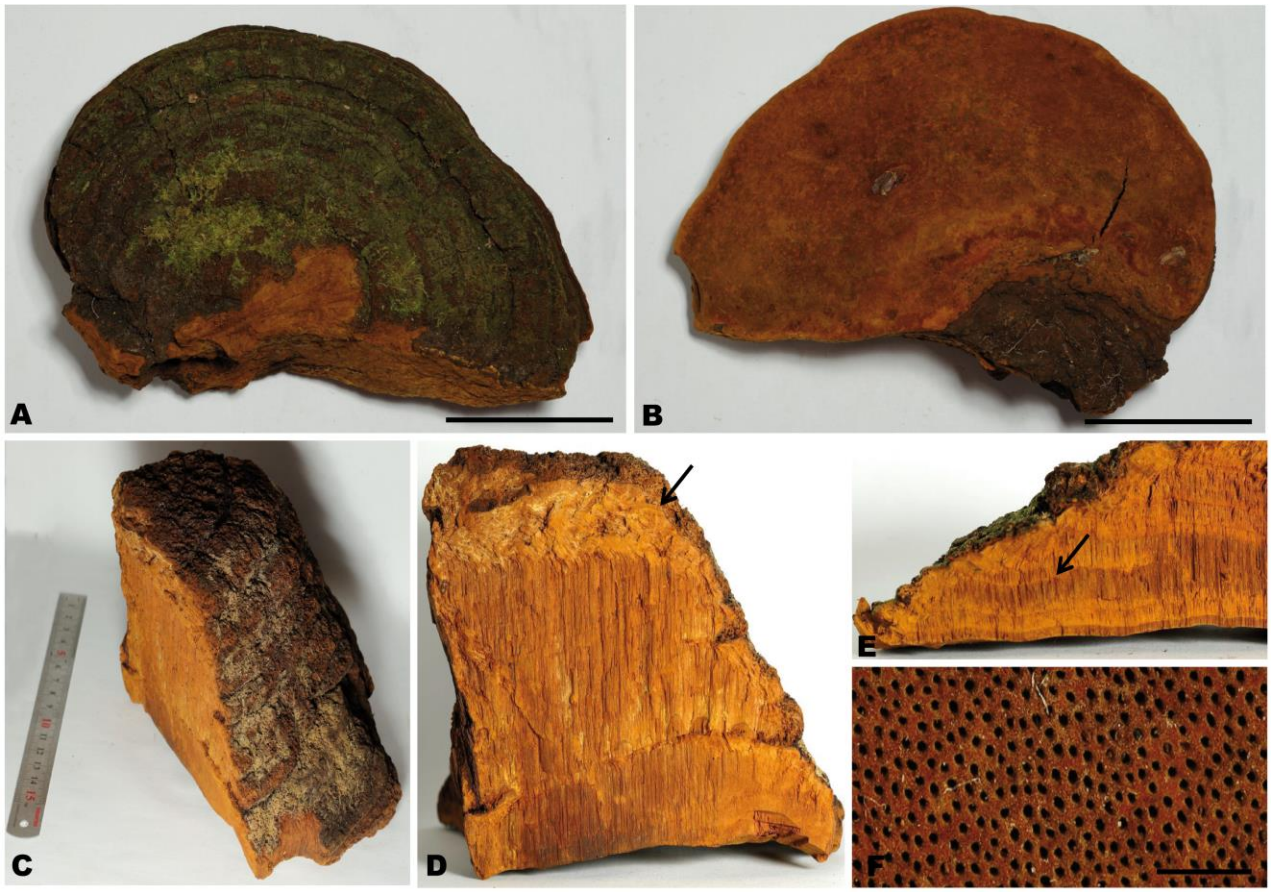

Figure 10. *Fulvifomes elaeodendri* sp. nov (holotype JMT1013; culture ex-type CMW47909; Fig. 10A–B). Macroscopic structures: A–B. Basidiome of the type specimen showing pilear and hymenial surfaces. C–D. Ttriquetrous up to ungulate basidiome (JMT948) showing thick light brown context (arrow). E. Cross section of basidiome of the type specimen showing stratified tube layers separated by a very thin contextual tissue. F. Pores. Bars: A–B = 6 cm; F = 1 mm.

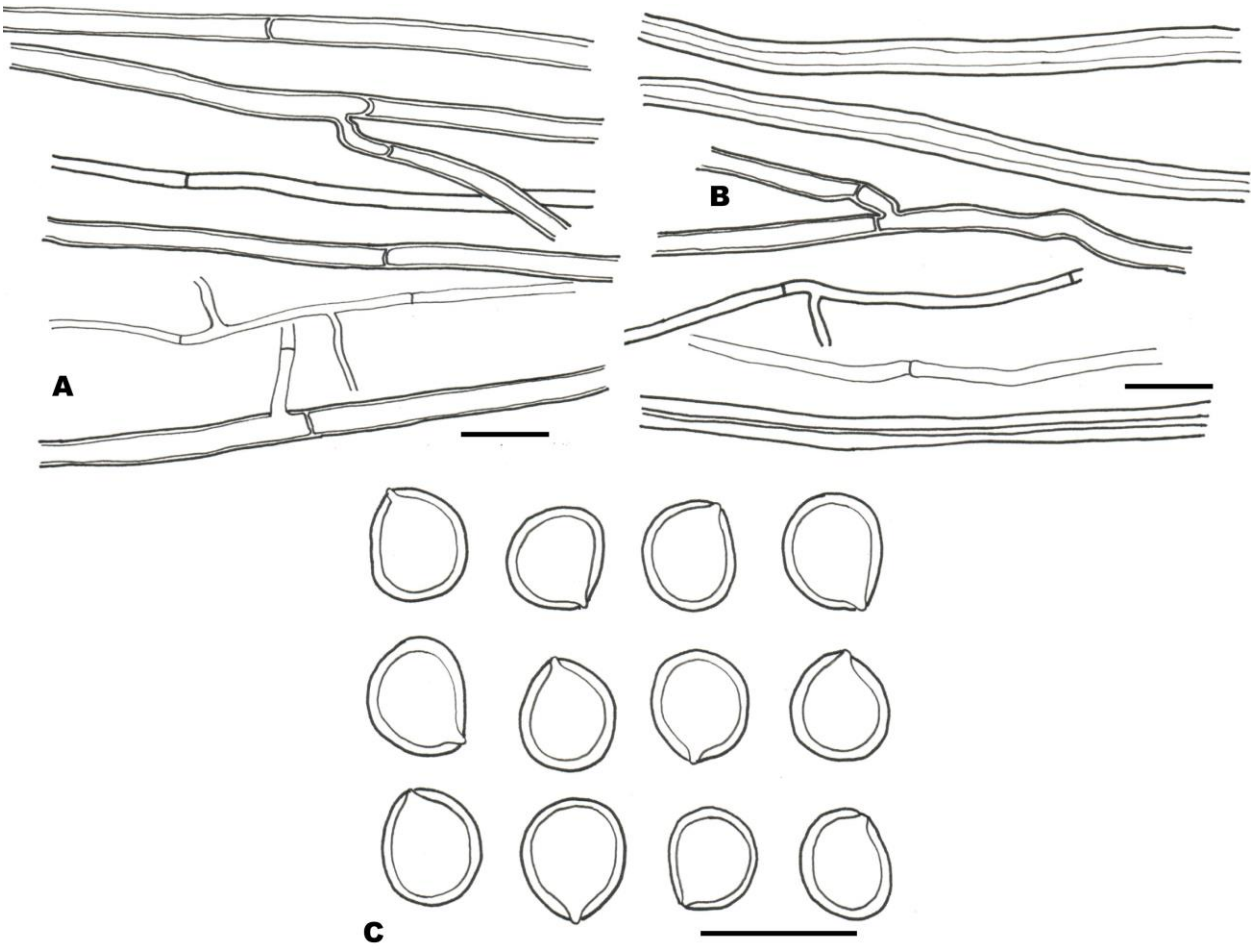

Figure 11. *Fulvifomes elaeodendri* sp. nov. microscopic structures: A–B. Generative hyphae simple septate and skeletal hyphae unbranched, thick-walled. B. broadly ellipsoid to ellipsoid basidiospores. Bars: A–B–C = 10 μm.

Figure 12. *Phellinus guttiformis* sp. nov. (holotype JMT464; culture ex-type CMW45332) macroscopic structures: A. Basidiomes attached to a branch of *Psydrax obovata* subsp. *obovata*. B. Cross section of the basidiome showing a chocolate brown context and indistinctly stratified, light brown to chocolate brown tubes. C. Pores. Bars: A = 2 cm; C = 1 mm.

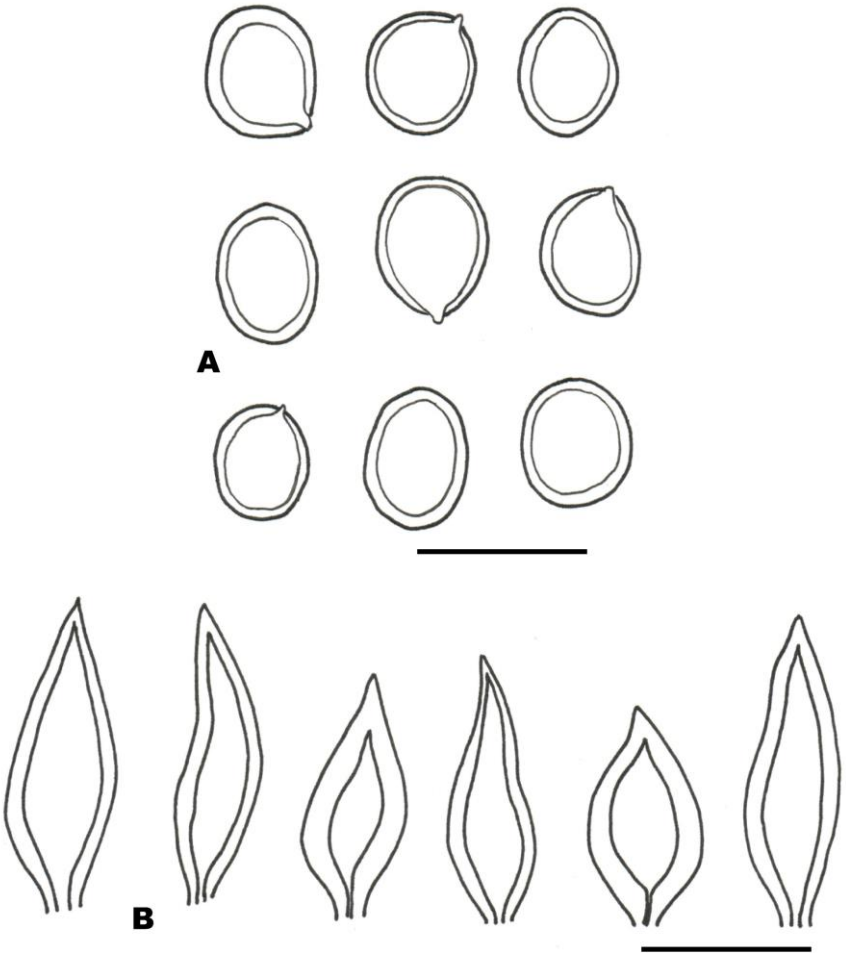

Figure 13. *Phellinus guttiformis* sp. nov. microscopic structures: A. Broadly ellipsoid, subglobose to globose basidiospores. B. Subulate, acuminate and slightly ventricose setae. Bars: A–B = 10 μm.

GENERAL CONCLUSIONS: WOOD ROT FUNGI IN THE NATIVE FORESTS OF THE GARDEN ROUTE NATIONAL PARK (GRNP), SOUTH AFRICA

Wood-rotting Basidiomycetes are essential to the sustainability of forests as they contribute to the emergence of new biological communities and nutrient cycling. Pathogenic species can, however, induce irreversible negative changes in the structure and composition of forests. Their presence and potential impact on native South African forests have, to date, received limited attention. Similarly, general knowledge regarding the diversity, taxonomy and ecology of these fungi in the country has received little attention compared to other regions globally.

Research presented in this thesis aimed at contributing to the knowledge of the diversity and taxonomy of wood-rotting macro-fungi in South Africa, particularly those occurring in the indigenous forests of the Garden Route National Park (GRNP) in the southern Cape region of South Africa. These iconic forests are selectively harvested, based on the occurrence of signs of wood-rot and crown death of trees (Seydack et al. 1995). The cause/s of this decay and death is, however, mostly unknown. Based on some preliminary studies (Roux et al. 2011; Roux et al. 2013), it was hypothesised that it may be due to attack by wood-rotting fungi, as sporocarps resembling species of the pathogenic wood-rot genera *Phellinus* sensu lato (s.l.) and *Ganoderma* s.l. were observed on dying trees and stumps.

The aim of this thesis was to establish the identity and host range of the Basidiomycetous wood-rotting fungi associated with wood-rot symptoms in the GRNP and thus to lay a foundation on which to continue studies to determine the impact of selective harvesting and other anthropogenic impacts on these forests. Three compartments (Bloukrans, Diepwalle, Gouna),

with varying levels of selective harvesting, were selected for study and the isolation frequency of macro-fungi, their identities, tree species affected and disease status, determined. In each of the three compartments, sampling was mainly focused on freshly cut stumps, standing dead trees and living trees showing wood-rot symptoms and the presence of basidiomes. A total of 120 transects were sampled at a rate of 40 per compartment. The obtained isolates and basidiomes were characterized and identified based on morphology and sequencing of multiple gene regions. Initial identification was done to the level of Operational Taxonomic Unit (OTU), where after selected isolates were further characterised using sequences of additional genome regions.

A total of 26 OTUs, belonging to 17 genera after clustering the sequences at a 97% identity threshold, was identified. Among the recorded taxa, species with pathogenic potential, *Ganoderma* and Hymenochaetaceae species were the most prevalent. These results confirm that of the preliminary study by Roux et al. (2013) and reinforce the hypothesis that these fungi are the leading causes of signs and symptoms of wood rot in this natural ecosystem. Three *Ganoderma* species were identified from the GRNP, including *G. cupreum*, *G. applanatum* and a novel taxon described as *G. knysnamense*. Ten species of Hymenochaetaceae, belonging to five genera, were identified, while *Olea capensis* subsp. *macrocarpa* (Oleaceae) was the most commonly affected tree by the wood rot. Pathogenicity trials in which these species are inoculated to their hosts is necessary to confirm their role in the observed wood-rot, particularly with regard to *G. knysnamense* sp. nov, which alone accounted for 303 isolates out of 403 obtained in the GRNP.

Wounds on trees, such as those from broken branches, falling trees and exposed upper surfaces of stumps, such as those caused during selective harvesting and other anthropogenic activities, represent ideal entry points through which wood-rotting fungi can infect and colonize trees.

Given the wide variety of wood-rotting Basidiomycetes revealed by this study and particularly the common occurrence of species with pathogenic potential, more attention should be given to better understand their ecological role in this natural ecosystem as well as the effects of logging that may enhance their dissemination, or negatively affect their diversity and the health of trees in the region.

Diseases caused by wood-rotting Basidiomycetes are in general difficult to eradicate. However, knowing their infection and dispersal mechanisms makes it possible to develop effective strategies for their management. Basidiomes of perennial species, particularly those of *G. knysnamense* sp. nov. were dominating the infected wood in the three harvesting compartments. This suggests an almost permanent production of basidiospores over time, and thus the crucial role that these structures would play in the infection and dissemination of these fungi in this forest. Indeed, the abundance of basidiomes, coupled with prolonged sporulation over time, increases the likelihood of spread and successful infection of these macro-fungi through basidiospores (Fischer 2002, Roccotelli et al. 2014). The preferential association of *G. knysnamense* sp. nov. with the most widespread and abundant host at all sites (*Olea capensis* subsp. *macocarpa*), and therefore the most heavily harvested, could also point to dissemination of this fungus through root to root contact. This, and the role that stumps left behind from the exploitation of trees could play as reservoirs of inoculum, should be considered in future. A detailed epidemiological study is necessary to reliably establish the pathway (s) of *G. knysnamense* sp. nov. and other important wood rot fungi dissemination in this forest.

The management of wood rot disease is usually based on protecting the surface of stumps and wounds caused during harvesting operations. This can be achieved through the use of physical barriers, including fungicides and biological agents, in the prevention of wound infections and

the removal of infected wood from the sites in order to reduce the amount of inoculum (Di Marco et al. 2000, Sosnowski et al. 2008; Rolshausen et al. 2010; Sosnowski et al. 2011). The sanitation approach would be limited in the context of the GRNP where basidiomes of *G. knysnamense* sp. nov. are present on almost all sampled hosts; indeed, it would imply the removal of a great diversity of tree species and thus an impoverishment of biodiversity. Current SANParks procedures of sealing wounds caused during harvesting operations should be continued and improved upon, eg. through application of sealant and chemical within hours of wounding and not days later. Chemical agents such as benomyl, carbendazim and thiophanate-methyl, as well as demethylation inhibitors such as flusilazole and tebuconazole, have been used to this effect with some success (Sosnowski et al. 2008, Rolshausen et al. 2010, Díaz and Latorre, 2013). The biological control approach utilizes the application of biological agents to exposed surfaces of stumps or wounds to prevent colonization by pathogens. Schubert et al. (2008) demonstrated, for example, using *Trichoderma* species that they had a very significant preventive effect as a protective agent against several species of *Ganoderma* and *Inonotus hispidus* on six different tree species. This method, which seems more ecological friendly, could be tested in the GRNP.

REFERENCES

Di Marco S, Mazzullo A, Calzarano F, Cesari A, 2000. The control of esca: status and perspective. *Phytopathologia Mediterranea* 39, 232–240.

Díaz GA, Latorre BA, 2013. Efficacy of paste and liquid fungicide formulations to protect pruning wounds against pathogens associated with grapevine trunk diseases in Chile. *Crop Protection* 46, 106–112.

Fischer M 2002. A new wood–decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycological Progress* 1, 315–324.

Roccotelli A, Schena L, Sanzani SM et al., 2014. Characterization of basidiomycetes associated with wood rot of citrus in southern Italy. *Phytopathology* 104, 851–858.

Rolshausen PE, Úrbez–Torres JR, Rooney–Latham S, Eskalen A, Smith R, Gubler WD, 2010. Evaluation of pruning wound susceptibility and protection against fungi associated with grapevine trunk diseases. *American Journal of Enology and Viticulture* 61, 113–119.

Roux J, Chapman D, Coetzee MPA, 2011. *Phellinus* sensu lato species associated with wood rot of native tree species in South Africa and Namibia. IUFRO Forest Protection Joint Meeting. "Pathogens, insects and their associations affecting forestry worldwide", 8-11 November 2011, Colonia del Sacramento, Uruguay.

Roux J, Wingfield MJ, Coetzee MPA, 2013. Exploratory studies of *Ganoderma* species in the Garden Route of South Africa. Proceedings of the 10th International Congress of Plant Pathology; 25–30 Aug 2013, Beijing, China.

Schubert M, Fink S, Schwarze F, 2008. Evaluation of *Trichoderma* spp. as a biocontrol agent against wood decay in urban trees. *Biological Control* 45, 111–123.

Sosnowski MR, Creaser ML, Wicks TJ, Lardner R, Scott ES, 2008. Protection of grapevine pruning wounds from infection by *Eutypa lata*. *Australian Journal of Grape and Wine Research* 14, 134–142.

Sosnowski MR, Wicks TJ, Scott ES, 2011. Control of Eutypa dieback in grapevines using remedial surgery. *Phytopathologia Mediterranea* 50, 277–284.

SUMMARY

The research presented in this thesis considered the diversity, ecology and taxonomy of wood-rotting macro-fungi associated with trees showing wood-rot symptoms in South Africa, focusing mainly on the indigenous forests of the Garden Route National Park (GRNP) in the southern Cape region of the country. Within the GRNP, samples of wood-rotting macro-fungi associated with declining native trees were collected in three timber-harvesting compartments (Bloukrans, Diepwalle and Gouna). Twenty-six basidiomycetous wood-rotting fungi were found associated with 20 different hosts, of which *Olea capensis* subsp. *macrocarpa* was most frequently colonized. Species richness was significantly higher in the Bloukrans forest compartment, which had undergone less long-term logging as compared to the other two sites. *Ganoderma* and Hymenochaetaceae species emerged as the two predominant groups of macro-fungi associated with declining trees in these native forests. Surveys conducted in dying stands of *A. cyclops*, also present in the same area, revealed that three species of *Ganoderma* were associated with the death of this tree species. Phylogenetic and taxonomic studies conducted of the two main groups of macro-fungi (*Ganoderma* and Hymenochaetaceae) found in these studies led to the recognition of several species, including six species new to science. *Ganoderma dunense*, *G. knysnamense* and *G. eickeri* were the newly described species of *Ganoderma*, while *Fomitiporia tsitsikamensis*, *Fulvifomes elaeodendri* and *Phellinus guttiformis* were the newly described species of Hymenochaetaceae. Although the studies presented in this thesis focused mainly on the GRNP, the considerable number of potential pathogenic macro-fungi identified suggests that future studies should pay more attention to a better understanding of their ecological role in natural ecosystems.