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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.

Key words: *Ganoderma*, Hymenochaetaceae, indigenous forests, logging impact, macro-fungal species richness.

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 & 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 & Otrosina 1998; Garbelotto 2004; Gregory *et al.* 2010; Garbelotto & 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

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and agricultural crops as well as plantation and native forest trees (Shaw & Kile 1991; Goheen & Otrosina 1998; Coetzee *et al.* 2001; Bendz-Hellgren & Stenlid 1997; Rajchenberg & 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 & Larsson 2000; Garbelotto 2004; Shortle & 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 & 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 & 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 & 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 & 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.

METHODS

Study area

This study was conducted over a 2-year period (2014–2015) in the GRNP indigenous forests in South Africa (Fig. 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 subhumid and warm temperate climate, with precipitation ranging from 500 to 1000 mm per year and an average

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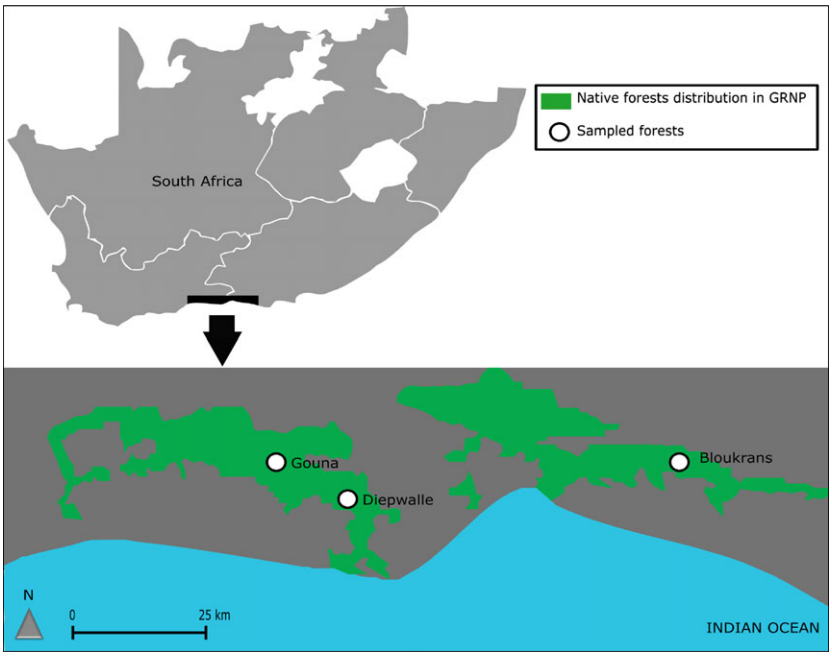

Fig. 1. Map showing the location of the three sampled timber-harvesting compartments in the GRNP indigenous forests in the Western and Eastern Cape Provinces of South Africa. [Colour figure can be viewed at wileyonlinelibrary.com]

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°56.747′ E23°35.220′), Diepwalle (S33°57.168′ E23°10.680′) and Gouna (S33°56.163′ E23°03.451′) (Fig. 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.

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.

Fungal isolation and purification

To isolate wood-rotting basidiomycetes from symptomatic plant tissues and basidiomes, four pieces of approx. 2–3 mm^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

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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.

DNA extraction, amplification and sequencing

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).

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 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.* 1990). Polymerase chain reactions (PCRs) were carried out in 25 μL reaction volumes containing 5 μL of 5 × MyTaq Reaction Buffer supplied with the enzyme, 0.5 μL (10 mmol L^{-1}) of ITS1, 0.5 μL (10 mmol L^{-1}) of ITS4, 0.5 μL (2.5 units) of MyTaq 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.

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.

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.

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) databases 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 & Michalak 2012). Ten runs were performed and 1000 bootstrap replications were used to estimate the levels of confidence at branch

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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
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
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

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Table 1. Continued

Closest Blast match taxa	MycoBank classification		Accession number	Origin	Host
	Genus	Family			
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	*Pseudolagaro basidium*	Phanerochaetaceae	DQ517883	South Africa	*Acacia cyclops*
Pseudolagarobasidium acaciicola	*Pseudolagaro basidium*	Phanerochaetaceae	DQ517882	South Africa	*Acacia cyclops*
Pseudolagarobasidium sp.	*Pseudolagaro basidium*	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
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.

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).

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.

RESULTS

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

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sap exudations from the bark (Fig. 2a), mycelial mats under the bark (Fig. 2b) and basidiomes (Fig. 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.

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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 4). *Fuscoporia* had two lineages consisting of OTUs 7 and 21, whereas the remaining genera were represented by a single lineage each (Fig. 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.

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 (Fig. 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).

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).

DISCUSSION

This study provides base-line information about the identity and species richness of the basidiomycetous

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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	–	–	–

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 & 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 & 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

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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	–	–	–	–	–	–	–

(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

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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	–	–	–	–	–	–	–

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

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Table 2. Continued

	Operational Taxonomic Units (OTUs) and their frequencies of isolation by categories						
	OTU22	OTU23	OTU24	OTU25	OTU26	Total isolates	Richness
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

these fungi (Bader *et al.* 1995; Hattori & 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 & 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 & Geldenhuys 2006). This could also be a possible explanation to the decrease in the macrofungal 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 & 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 & 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

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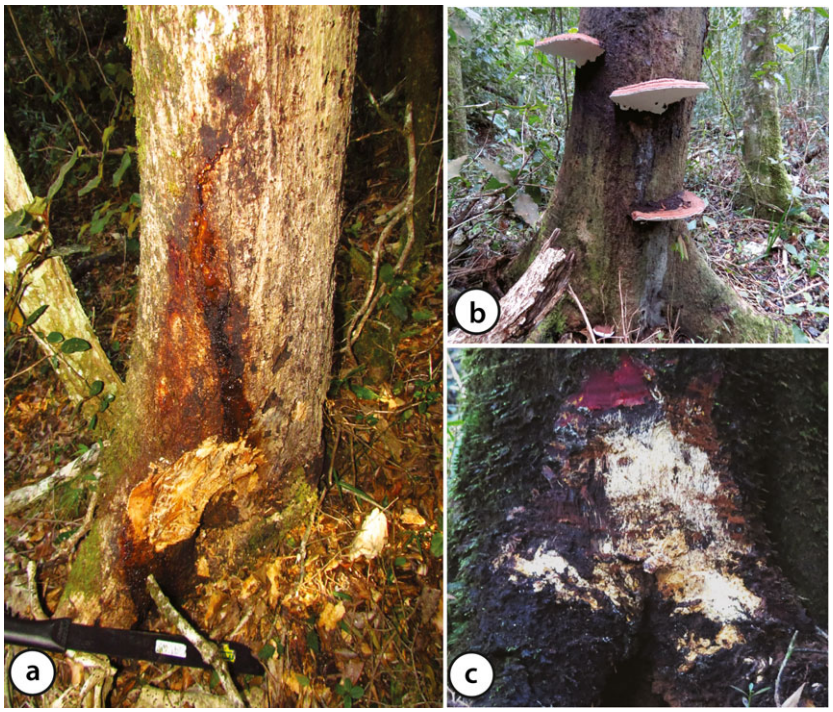

Fig. 2. Symptoms and signs observed on sampled trees in the GRNP: (a) sap exudation and basal stem rot, (b) bracket-like basidiomes of a *Ganoderma* species, (c) white mycelial mat under the bark of tree with basal stem rot. [Colour figure can be viewed at wileyonlinelibrary.com]

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

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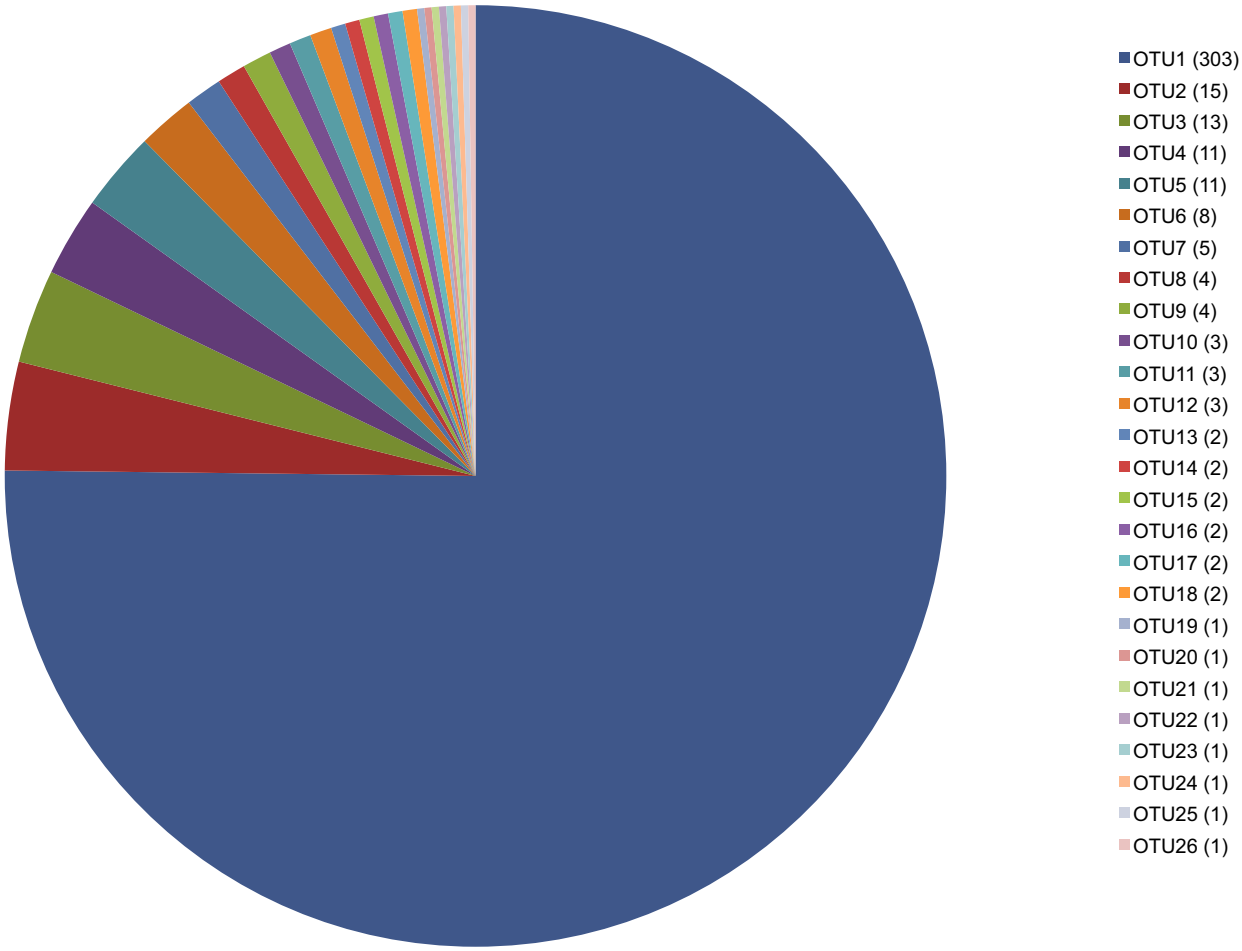

Fig. 3. Abundance of Operational Taxonomic Units (OTUs) detected (number in parenthesis) as determined in Mothur. [Colour figure can be viewed at wileyonlinelibrary.com]

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 conducted 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.

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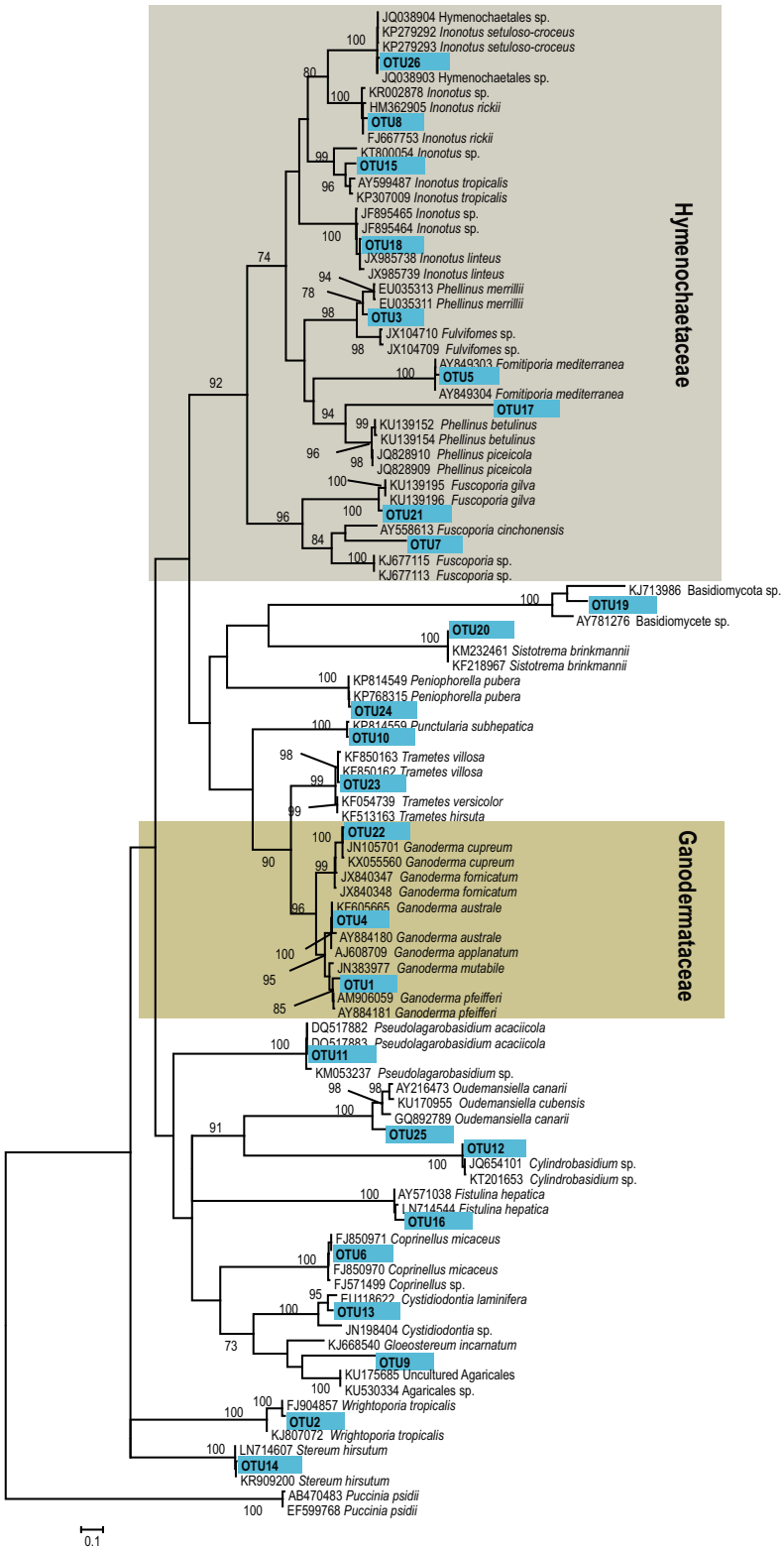

Fig. 4. Maximum Likelihood (ML) phylogenetic tree inferred from the ITS gene region showing the taxonomic affiliation between the representative sequence of each OTU from the GRNP with the best-aligned reference sequences. The data set was composed of 100 taxa and 1218 characters. The model implemented was GTR + I + G and the tree was rooted with sequences of *Puccinia psidii* (AB470483 and EF599768). Bootstrap values are shown at the nodes of the tree. Highlighted are the two dominant taxonomic groups containing species with pathogenic potential. [Colour figure can be viewed at wileyonlinelibrary.com]

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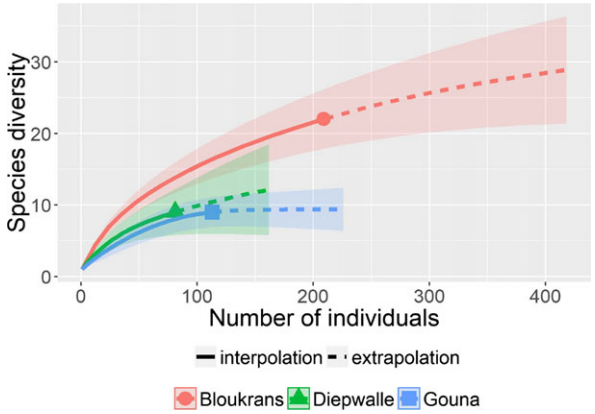

Fig. 5. Rarefaction curves of the three sampled timber-harvesting compartments (Bloukrans, Diepwalle and Gouna). Species diversity shows the OTU richness depending on the sampling intensity (number of individuals). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 3. Results of generalized linear model (GLM) analysis of presence/absence of OTU1

	d.f.	*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

Degrees of freedom (d.f.) and F-ratios, are shown. Significance (*P* value) is indicated in bold ($P < 0.05$).

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