A SINGLE DOMINANT *GANODERMA* SPECIES IS RESPONSIBLE FOR ROOT ROT OF *ACACIA MANGIUM* AND *EUCALYPTUS* IN SUMATRA

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Ganoderma root rot is the most serious disease affecting commercially planted *Acacia mangium* in plantations in Indonesia. Numerous *Ganoderma* spp. have been recorded from diseased trees of this species and to a lesser extent *Eucalyptus*, causing confusion regarding the primary cause of the disease. In this study, a large collection of *Ganoderma* isolates were obtained from the roots of *A. mangium* showing early signs of root rot in disease centres in South Sumatra plantations. Isolates were also collected from *Eucalyptus* roots at Lake Toba in North Sumatra showing similar symptoms as well as from sporocarps connected to these samples. Phylogenetic analyses showed that a single *Ganoderma* sp., identified as *G. philippii,* is the major causal agent of Ganoderma root rot on *A. mangium*. Results from this study also showed that the isolates obtained for *Eucalyptus* trees in North Sumatra belong to *G. philippii*. Isolates from roots and connected fruiting bodies together with the morphology of the fruiting structures confirmed

this identification. Symptoms associated with this pathogen are obvious and it should not be confused with other diseases. Other *Ganoderma* spp. found in disease centres are considered to be of minor importance and management strategies for root rot should be focused on *G. philippii*.

KEYWORDS

Ganoderma root rot, *Ganoderma philippii*, Indonesia, *Acacia mangium*, *Eucalyptus*

INTRODUCTION

Forest products are a major source of national revenue in Indonesia. Nearly 33% (69.9 million ha) of the country's land area includes production forests (FAO 2009). By 2006, industrial forest plantations covered nearly 2.5 million ha, with various tree species being planted for wood and pulp production (Arisman and Hardiyanto 2006). Several *Acacia* species, including *A. mangium*, are planted mainly for pulp production. The total area of *Acacia* plantations established in this country amounted to approximately 1 million ha in 2006 (Arisman and Hardiyanto 2006). In addition, *Eucalyptus* spp. are increasingly planted in Indonesia with this resource currently being utilised in pulp and rayon production.

Root rot is the most important disease affecting *Acacia* trees, including *A. mangium,* in South-East Asian plantations (Lee 2000, Old et al. 2000, Sankaran *et al.* 2005, Wingfield et al. 2010b). Similar infections are also occasionally seen in *Eucalyptus* plantations, although these are relatively rare. The disease is recorded to be caused by a number of fungal genera, but *Ganoderma* spp. are most frequently associated with root rot on

tropical *Acacia* spp. (Glen et al. 2009, Lee 2000, Mohammed et al. 2006, Old et al. 2000). Various *Ganoderma* spp. have been isolated from infected *A. mangium* roots (Glen et al. 2009). However, inspection of the roots of dying trees at the periphery of disease centres during the past 10 years have suggested that a single fungus is the primary causal agent of the rot in *A. mangium* plantations in Sumatra (MJ Wingfield, pers. comm., FABI, 2011). Furthermore, the identity of the pathogen causing root rot on *Eucalyptus* has not yet been considered.

The aim of this study was to inspect a large number of dying *A. mangium* trees in South Sumatra, and to make isolations from recently infected roots. In this way, it was hoped to resolve the question of whether a single or a suite of different *Ganoderma* spp. are responsible for root rot. An additional aim of this study was to confirm the identity of the fungus causing root rot on *Eucalyptus* in Sumatra and to link isolates to fruiting bodies using DNA sequence data. For this purpose, isolates were collected from the roots of dying *Eucalyptus* trees at Lake Toba in North Sumatra showing similar symptoms to those on *Acacia*, as well as from sporocarps linked to these roots.

MATERIALS AND METHODS

Recently infected roots were collected from dying *A. mangium* at the periphery of root rot centres at 21 different localities in South Sumatra (**Figure 1**). In addition, infected *Eucalyptus* roots showing the same symptoms as those on *A. mangium* were collected from an infection centre at Lake Toba in North Sumatra and from sporocarps physically linked to the infected roots. Isolations were made from these samples on malt extract agar (MEA: 2% w/v malt extract, 1.5% w/v agar) containing streptomycin sulphate (100

mg L^{-1}). Isolates are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa.

DNA was extracted from the cultures grown on MEA (2% w/v) or directly from fruiting bodies following standard protocols (Coetzee et al. 2003a). The internally transcribed spacer (ITS) rRNA region was amplified using primers ITS1 and ITS4 (White et al. 1990). PCR mixtures and reaction conditions were as described by Coetzee et al. (2003b), with the exception that FastStart *Taq* DNA polymerase (Roche Applied Science, Randburg, South Africa) instead of *Taq* polymerase was used. ITS amplicons were purified and their DNA sequences determined using the ABI Prism® BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase, FS (Perkin Elmer, Applied BioSystems, Roosevelt Park, South Africa) following the manufacturer's protocol. Vector NTI v. 10.3 (Invitrogen) was used for editing and assembling of DNA sequences.

The ITS sequences obtained were compared with other *Ganoderma* sequences in GenBank using BLASTn searches. The ITS-2 region was utilized in BLASTn searches for isolates that showed ITS-1 intra-strain sequence heterogeneity. ITS sequences for *Ganoderma* spp. previously identified from Indonesia and Malaysia on *A. mangium*, including *G. australe, G. philippii, G. steyaertanum, G. mastoporum* and *Amauroderma subresinosumi* (Glen et al. 2009), were downloaded from GenBank for phylogenetic analysis. These sequences were generated by Glen et al. (2009) and Smith and Sivasithamparam (2000) from strains considered authentic representatives of the respective species. DNA sequences were aligned using MAFFT v. 5 (Katoh et al. 2005).

Phylogenetic analyses were conducted using MEGA v. 4 (Kumar et al. 2008). A maximum composite likelihood model with among site rate variation (the gamma parameter was set to 0.1) was applied in the analysis. A phylogenetic tree was generated using a neighbour-joining tree building algorithm (Saitou and Nei 1987) and the tree was rooted with *Am. subresinosum* as the outgroup taxon. Confidence levels of the nodes were determined by bootstrap with 1000 replicates (Felsenstein 1985), using the same substitution model.

RESULTS

Acacia mangium trees showed symptoms typical of Ganoderma root rot (Old *et al.* 2000). These included dying trees at the periphery of rot centres that had wilting leaves (**Figure 2a**). Recently infected roots were covered with red-coloured rhizomorphs (**Figure 2b**) and white mycelium mottled with yellow patches was obvious on the underside of the bark (**Figure 2b**). Fruiting bodies of a *Ganoderma* sp. were occasionally observed at the bases of dead trees (**Figure 2c**). In the case of the *Eucalyptus* infection centre examined, roots had identical signs of infection, including red rhizomorphs and the typical mottled pattern of mycelial growth below the bark. Fruiting bodies of a *Ganoderma* sp. identical to those seen in *A. mangium* infection centres were also found on a tree and these were linked to roots having these symptoms. In total, 189 isolates were obtained from the recently infected roots of *A. mangium* trees (Table 1). Six isolates (CMW36253, CMW36254, CMW36256, CMW36257, CMW 36259 and CMW36260) originated from *Eucalyptus* roots and two from fruiting bodies (CMW36252 and CMW36255) associated with the infected roots of the *Eucalyptus* trees. In culture, the majority of isolates appeared as white to

yellow coloured mycelia that were either flat or fluffy. A small number of isolates had a brown fluffy morphology.

The ITS region yielded PCR fragments of approximately 700 base pairs (bp) in length for all isolates. DNA sequences from these amplicons were obtained for all isolates. BLASTn searches revealed a high similarity with *G. philippii* (99%) for the majority of isolates, including those for which only the ITS2 region was used. The DNA sequences for one isolate (CMW30066, from *A. mangium*) had highest similarity (98%) to that of *G. mastoporum* (AJ627585, from Malaysia). Isolates CMW29740, CMW29744, CMW29789, CMW29844 and CMW29998 (from *A. mangium*) had DNA sequence similarities with other basidiomycete species, such as *Phellinus noxius* and *Tinctoporellus epimiltinus,* which are also associated with root rot disease.

Phylogenetic trees generated from the ITS sequence data supported initial sequence identifications based on BLASTn searches. Isolates from *A. mangium,* identified as *G. philippii* based on sequence similarity, grouped with a high bootstrap support with sequences AJ627584, AJ608710 and AJ608713 from GenBank that represent this species and originated from *A. mangium* in Malaysia and Indonesia (**Figure 3**). Isolate CMW30066 (from *A. mangium*) grouped together with *G. mastoporum* (AJ627585) with a 100% bootstrap support (**Figure 3**). Isolates from *Eucalyptus* grouped with a 100% bootstrap support with sequences of *G. philippii* (AJ627584, AJ608710 and AJ608713) from GenBank (**Figure 4**)

DISCUSSION

This study represents the largest single collection of isolates from recently infected *A. mangium* roots in root rot centres in Indonesia. DNA-based identification of 179 isolates showed that 97% of these represented a single species, which was identified as *G. philippii*. Although a number of root rot pathogens have been implicated in the root rot disease problem in *A. mangium* plantations (Glen et al. 2009), results of this study show that a single pathogen, rather than a suite of different pathogens, is predominantly responsible for the disease.

Eucalyptus trees showing symptoms of Ganoderma root rot were discovered in North Sumatra during this study. Ganoderma root rot is known to affect several species of *Eucalyptus* growing in South-East Asia; however, the causal agent of the disease in Sumatra and other regions has been uncertain (Old et al. 2003). DNA sequence comparisons and phylogenetic analyses in the current study showed that *G. philippii* is the causal agent of the disease on these trees. To the best of our knowledge, this is the first report of *G. philippii* causing root rot on *Eucalyptus* in Sumatra.

Very little is known regarding the biology of *G. philippii*. This presents an important impediment to developing management strategies to reduce the impact of the pathogen. Yet recognition that a single *Ganoderma* sp. is primarily responsible for the serious root disease encountered in *A. mangium* and to some extent on *Eucalyptus* implies that future studies focussed on understanding the biology of this pathogen will be useful. Furthermore, it will be possible to refine and simplify studies to consider host susceptibility and other management options including biological control.

CONCLUSIONS

Ganoderma root rot in Indonesia, as shown to be primarily caused by *G. philippii* in this study, represents the first substantially serious disease of *A. mangium* established as a non-native in plantations. Plantation forestry is relatively young in Indonesia and likewise, plantations based on non-native tropical *Acacia* spp. are relatively new, worldwide. In this regard, *A. mangium* is a species that has not been extensively exposed to pests and pathogens outside its native range (Wingfield et al. 2011). As is true for other tree species such as those of *Eucalyptus* and *Pinus* that has been exploited as non-natives for plantation development, pest and disease problems are likely to increase in number over time (Wingfield 2003, Wingfield et al. 2010a, Wingfield et al. 2010b). This and other pest and disease problems are likely to challenge plantation forestry in the future but there are also outstanding prospects for management using innovative technologies that are constantly emerging.

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Table1: List of collection sites in South Sumatra and the *Ganoderma* isolates collected from *A. mangium* at each site.

Collection site number	Culture number [a]
1	CMW29740, CMW29843, CMW29844, CMW29907, CMW29998, CMW30060, CMW30061
2	CMW29745, CMW29908, CMW30006, CMW30062
3	CMW29746, CMW29748, CMW29943, CMW29944, CMW29999, CMW30000, CMW30001
4	CMW29743, CMW29744, CMW29750, CMW29751, CMW30002, CMW30003, CMW30063
5	CMW29845, CMW29847, CMW29848, CMW30004,, CMW30005, CMW30064, CMW30065
6	CMW29788, CMW29789, CMW29790, CMW29791, CMW29792, CMW29856, CMW29857, CMW29910, CMW29950, CMW29951
7	CMW29793, CMW29794, CMW29795, CMW29796, CMW29858, CMW29911, CMW30010
8	CMW29797, CMW30066
9	CMW29802, CMW29803, CMW29804, CMW29805, CMW29860, CMW29912, CMW29952, CMW30011, CMW30024, CMW30067, CMW30068

Table 1 (continued).

Collection site number	Culture number [a]
10	CMW29806, CMW29815, CMW29861, CMW29913, CMW29953, CMW29954, CMW29955, CMW30012, CMW30013, CMW30014, CMW30015, CMW30016, CMW30017, CMW30018, CMW30069, CMW30070, CMW30071, CMW30072, CMW30074
11	CMW29807, CMW29813, CMW29814, CMW30019, CMW30020, CMW30021, CMW30022, CMW30075, CMW30076, CMW30078, CMW30079, CMW30080, CMW30081
12	CMW29808, CMW29810, CMW29811, CMW29812, CMW29863, CMW29864, CMW29865, CMW29914 CMW30023, CMW30025, CMW30026, CMW30027, CMW30082, CMW30083
13	CMW29816, CMW29817, CMW29818, CMW29820, CMW29866, CMW29867, CMW29868, CMW29915, CMW30029, CMW30030, CMW30084, CMW30085
14	CMW29782, CMW29784, CMW29788
15	CMW29859, CMW29916, CMW29945, CMW30086
16	CMW29783, CMW29786, CMW30087, CMW30088
17	CMW29780, CMW29781, CMW29785, CMW29946, CMW30009, CMW30089, CMW30090
18	CMW29755, CMW29756, CMW29761, CMW29762, CMW29763, CMW29917, CMW29947, CMW29948, CMW30007

Table 1 (continued)

Collection site number	Culture number [a]
19	CMW29754, CMW29764, CMW29765, CMW29766, CMW29767, CMW29768, CMW29769, CMW29770, CMW29771, CMW29918, CMW29919
20	CMW29753, CMW29757, CMW29758, CMW29772, CMW29773, CMW29774, CMW29849, CMW29850, CMW29851, CMW29852, CMW29920, CMW29921, CMW29922, CMW30091
21	CMW29759, CMW29775, CMW29776, CMW29777, CMW29778, CMW29779, CMW29853, CMW29854, CMW29855, CMW29949, CMW30008

[a] CMW refers to the culture collection of the FABI, University of Pretoria.

List of Figures

Figure 1

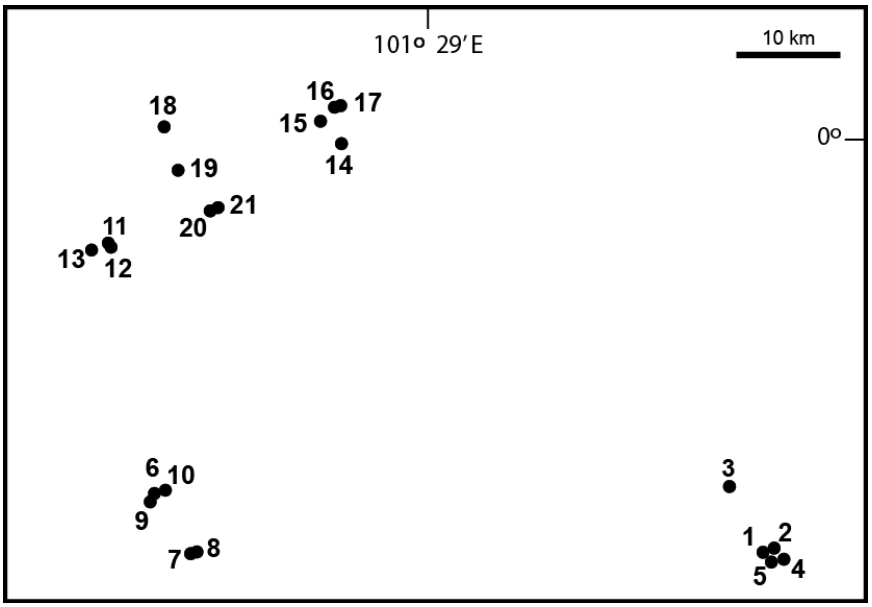


Figure 2

Figure 3

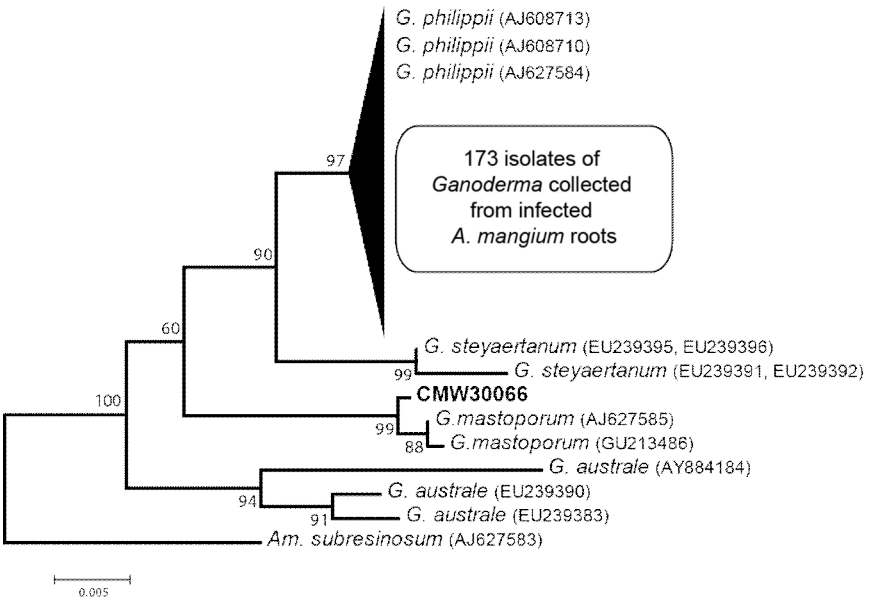
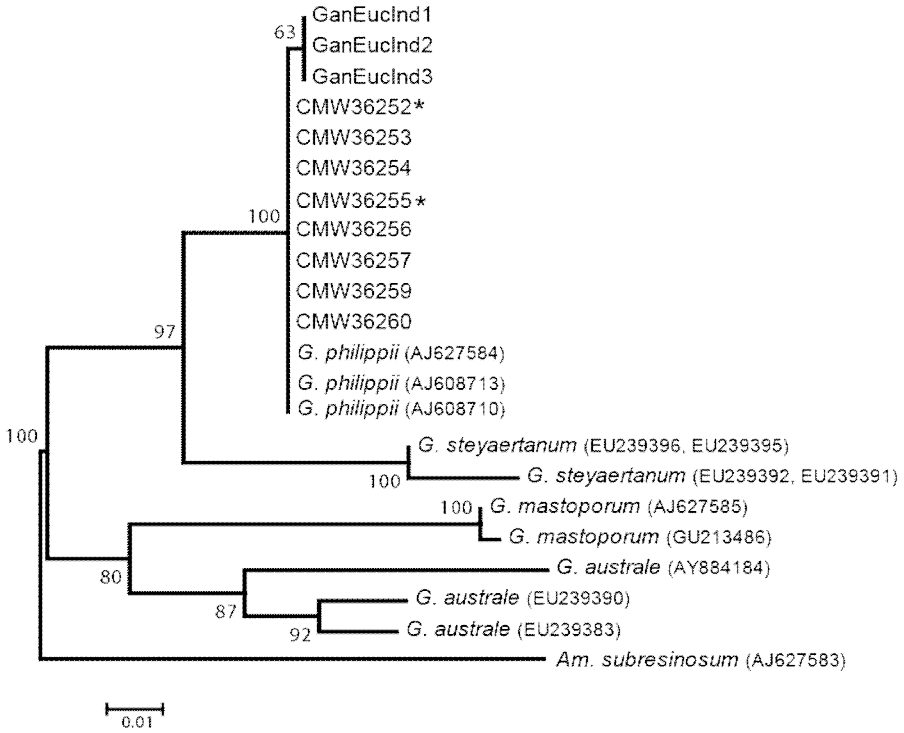

G. philippii (AJ608713)
G. philippii (AJ608710)
G. philippii (AJ627584)
173 isolates of Ganoderma collected from infected A. mangium roots
97
90
60
100
99
G. steyaertanum (EU239395, EU239396)
G. steyaertanum (EU239391, EU239392)
CMW30066
99
G.mastoporum (AJ627585)
88
G.mastoporum (GU213486)
G. australe (AY884184)
94
G. australe (EU239390)
91
G. australe (EU239383)
Am. subresinosum (AJ627583)
0.005

Figure 4


GanEucInd1
GanEucInd2
GanEucInd3
CMW36252*
CMW36253
CMW36254
CMW36255*
CMW36256
CMW36257
CMW36259
CMW36260
G. philippii (AJ627584)
G. philippii (AJ608713)
G. philippii (AJ608710)
G. steyaertanum (EU239396, EU239395)
G. steyaertanum (EU239392, EU239391)
G. mastoporum (AJ627585)
G. mastoporum (GU213486)
G. australe (AY884184)
G. australe (EU239390)
G. australe (EU239383)
Am. subresinosum (AJ627583)
63
100
97
100
100
100
80
87
92
0.01