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ARTICLE

Ganoderma species, including new taxa associated with root rot of the iconic *Jacaranda mimosifolia* in Pretoria, South Africa

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Abstract: *Jacaranda mimosifolia* trees have been progressively dying due to Ganoderma root and butt rot disease in Pretoria (the "City of Jacarandas") for many years. *Ganoderma austroafricanum* was described from these trees previously but this was based on a single collection. This study treats a substantially expanded collection of isolates of *Ganoderma* made from all dying trees where basidiomes were present in a Pretoria suburb. DNA sequences were obtained from the ITS and LSU region for the isolates and compared against sequences on GenBank. Phylogenetic analyses were used to compare sequences with those for other *Ganoderma* species. Based on sequence comparisons and morphological characters, two new *Ganoderma* species were discovered and these are described here as *G. enigmaticum* and *G. destructans* spp. nov. Interestingly, the previously described *G. austroafricanum* was not found, *G. enigmaticum* was found on only one *Ceratonia siliqua* tree and *G. destructans* was found on all other trees sampled. The latter species appears to be the primary cause of root rot of *J. mimosifolia* in the area sampled.

Key words:
Basidiomycota
Horticulture
Polyporales
Root rot

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INTRODUCTION

The city of Pretoria (South Africa) is commonly known as the "City of Jacarandas". This is because thousands of *Jacaranda mimosifolia* (*Bignoniaceae*) trees have been planted in its gardens, parks, and roadsides. In spring, the city is covered by a spectacular "blanket" of purple and this draws the interest of tourists and South African citizens alike. Any threat to these trees is thus seen as important and worthy of study.

Jacaranda mimosifolia is native to southern Bolivia and north-western Argentina where it occurs mainly along rivers in warmer temperate subhumid areas (Germplasm Resources Information Network; http://www.ars-grin.gov.4/cgi-bin/npgs/html/taxon.pl?20600). In South Africa, the first trees were introduced during the 1890s when two trees were planted at a school in Pretoria (Henderson 1990). Substantial numbers of these trees have, however, increasingly been dying since 1998 in the Pretoria suburb of Brooklyn due to a root rot disease apparently caused by a species of *Ganoderma.*

Ganoderma is a widely distributed genus (Moncalvo & Ryvarden 1997) that includes species important to forest ecosystems and in cultural traditions. Many *Ganoderma* species are important pathogens of woody plants, causing root and butt rot diseases (Old *et al.* 2000, Flood *et al*. 2001). Some species survive as saprophytes, causing white rot by decomposing lignin and cellulose (Adaskaveg *et al.* 1990). In some regions, especially East Asia, species of *Ganoderma* play an important role in cultural traditions. For example, *G. lingzhi* symbolises success and longevity, and the value of its basidiomes in traditional medicine is well known (Cao *et al*. 2012).

For many years the taxonomy of *Ganoderma* has been considered to be in a state of chaos (Ryvarden 1995). The application of the biological species concept (Adaskaveg & Gilbertson 1986) and molecular based tools has provided the necessary means to resolve the many questions pertaining to this group. Prior to the application of these methods, species delineation relied mainly on morphology but this was frustrated by the lack of useful morphological differences in the basidiomes. This has led to many currently known species being incorrectly identified or placed in species complexes (Richter *et al*. 2015). The application of molecular tools has led to many species having similar basidiodome morphology being recognized and described (e.g. Smith & Sivasithamparam 2000, Cao *et al.* 2012, Kinge *et al*. 2012). Using these methods, a concerted effort has been made to elucidate the taxonomy of *Ganoderma* species in many regions of the world. However, with the exception of the work of Ryvarden & Johansens (1980) and more contemporary research conducted in some African countries (e.g. Douanla-Meli & Langer 2009, Kinge *et al*. 2012, Kinge & Mih 2014), very little effort has been made to identify the *Ganoderma* species diversity in Africa.

Twenty morphological species of *Ganoderma* have been reported from southern Africa (Baxter & Eicker 1995). However, DNA sequences are not available for samples of

these species from this region and their phylogenetic position is unknown. As part of an effort to improve this situation, *G. austroafricanum* was recently described from *J. mimosifolia* in South Africa supported by DNA sequences for the ITS region (Crous *et al*. 2014).

Basidiomes that are similar to those of *G. lucidum* can be found at the bases of dying Jacaranda trees in Pretoria every year after the onset of rain in the Southern Hemisphere spring and early summer. The discovery of *G. austroafricanum* on *J. mimosifolia* led to an assumption that this species is the main causal agent of root rot on these trees (Crous *et al*. 2014). However, that conclusion was based on limited sampling, and so the possibility that other *Ganoderma* species could also be involved in the disease syndrome remained. The aim of this study was, therefore, to expand collections of the *Ganoderma* species associated with this root rot problem and to identify the isolates. This was achieved using morphological characteristics but more importantly nucleotide sequence data from the ITS, and LSU regions of the ribosomal RNA operon.

MATERIALS AND METHODS

Fungal isolation and cultivation

Basidiomes were collected from infected Jacaranda trees and a single *Ceratonia siliqua* (Carob) tree in the suburb of Brooklyn, Pretoria, where root rot is particularly common. Isolations were made by aseptically placing small pieces of basidiome tissue on malt extract medium (MEA: 2 % w/v malt extract, 1.5 % w/v agar; Biolab, Midrand, South Africa) supplemented with 0.1 g/L streptomycin sulphate (Sigma-Aldrich, St Louis, MO), and incubated at 22 °C in the dark for 3–5 d. Fungal colonies were aseptically transferred to fresh MEA without streptomycin and incubated for 2 wk. All the resulting isolates were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa, and the ex-type cultures of new taxa were deposited in the KNAW-CBS Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands. Dried basidiomes and cultures were also deposited in the National Collection of Fungi in South Africa (PREM), Roodeplaat, South Africa.

DNA extraction and PCR

DNA was extracted from 2-wk-old cultures grown on MEA. Cetyltrimethylammonium bromide (CTAB) extraction buffer (5 % w/v CTAB, 1.4M NaCl, 0.2 % v/v 2-mercaptoethanol, 20 mM EDTA pH 8, 10 mM Tris-HCL pH 8, and 1 % v/v polyvinylpolyrrolidone) was used to obtain DNA following the standard extraction protocol outlined in Murray & Thompson (1980), with the exception that chloroform/isoamyl alcohol (24:1) was used. DNA quantification was achieved using a NANODROP (ND-1000) spectrophotometer (Nanodrop Technologies, Wilmington, NC).

The ITS region was amplified for all isolates included in this study using primer pairs ITS1 and ITS4 (White *et al.* 1990). A portion of the LSU gene was amplified using primers LR0R and LR7 (Moncalvo *et al*. 2000) for isolates linked to basidiomes deposited in PREM. All PCR mixtures consisted of 100 ng genomic DNA, reaction buffer (10 mM Tris-HCL [pH 8.3], 3.0 mM $MgCl_2$, 50 mM KCl, Roche Diagnostics, Mannheim, Germany), 2.5 µM of each dNTP (Fermentas Life Sciences, Pretoria, South Africa), 0.4 µM of each primer, and 1 unit of FastStart *Taq* DNA polymerase (Roche Diagnostics). PCR cycles consisted of an initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 62 °C (ITS) or 60 °C (LSU) and extension at 72 °C for 30 s. A final extension at 72 °C for 7 min was included to complete the reaction. PCR products were visualised using GelRed under UV illumination after electrophoresis on agarose gels (1 % w/v).

DNA sequencing and analyses

PCR products were purified using a MSB Spin PCRapace kit (STRATEC Molecular, Berlin, Germany) following the manufacturer's instructions. Purified PCR products were sequenced in both directions using the same set of primers used for the respective PCR reactions. Sequence reactions were performed using an ABI Prism® BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase, FS (Perkin Elmer, Warrington, UK) following the protocol supplied by the manufacturer. Sequencing was done at the sequencing facility of the University of Pretoria. CLC Main Workbench (QIAGEN, Aarhus, Denmark) was used to inspect the electropherograms and assemble contigs.

ITS sequences were compared with those in GenBank using the BLASTn search algorithm. Sequences with high similarity and coverage (95–99 %) to the sequences from the Pretoria isolates were downloaded and included in the study. In addition to these sequences, the ITS data set included the sequence for *G. austroafricanum* (CMW 41454) and a sequence obtained from an isolate (CMW 43669) that originated from a basidiome collected on a *C. siliqua* tree. Sequences were aligned using the online version of MAFFT (Katoh & Standley 2013).

Phylogenetic trees were generated based on neighbour-joining (NJ) using PAUP (Swofford 2002), maximum likelihood (ML) using PHYML v. 20120412 (Guindon *et al.* 2010), and Bayesian Inference (BI) using MrBayes v. 3.2.3 (Huelsenbeck & Ronquist 2001). The nucleotide substitution model that best fit the data was determined with jModelTest v. 2.1.5 (Darriba *et al.* 2012) using the AIKE information criterion for model selection; the model was then incorporated in the phylogenetic analyses. Bootstrap analyses were performed with 1000 replicates. For BI, four Monte Carlo Markov Chains (MCMC) were run for four million generations after which the first 25 % trees were discarded as burn-in. The remaining trees from the individual runs were combined to construct a consensus tree. Effective sampling size (ESS) values, as a measure of convergence, were assessed in Tracer v. 1.5 (http://tree.bio.ed.ac.uk/software/tracer/) and the consensus tree viewed in FigTree v. 1.4. (http://tree.bio.ed.ac.uk/software/figtree/).

Morphology

The morphology of basidiomes was studied using a Zeiss Axioskop 2 Plus compound microscope and a Zeiss Discovery V12 stereomicroscope. Images were captured using an Axiocam MRc camera. All the microscopic structures were

examined on glass slides with specimens mounted in 10 % KOH and Melzer's reagent. Measurements of characteristic structures were made using the Axiovision v. 4.8 software. Twenty-five to 50 measurements were made for each structure depending on their availability, except for the basidiomes. Sizes are presented as minimum-maximum measurements.

RESULTS

Isolates

Twenty-five isolates were obtained from basidiomes collected on 29 individual *Jacaranda mimosifolia* trees. In addition, one isolate was obtained from a basidiome on *Ceratonia siliqua.*

Sequence data and analyses

BLASTn comparisons for the ITS sequences of the Pretoria isolates with those in GenBank showed a high level of similarity with other *Ganoderma* species. Isolate CMW 43669 from the single infected *Ceratonia siliqua* tree had the highest DNA sequence similarity with an unnamed *Ganoderma* sp. and *G. neojaponicum* in GenBank. DNA sequences from the remainder of the isolates had the highest similarity to sequences representing *G. lucidum* (*s. lat.*), *G. multipileum, G. martinicense, G. multiplicatum, G. perzonatum,* and *G. steyaertanum*.

Comparison of the LSU sequences between CMW 43669 from *C. siliqua*, the isolates from *Jacaranda mimosifolia* (CMW 43670, CMW 43671, CMW 43672) and *G. austroafricanum* (CMW 41454, GenBank accession no. KM507325) showed a number of nucleotide differences (including gaps). The greatest variation was observed between CMW 43669 and *G. austroafricanum* (17 differences), and CMW 43669 and the isolates from *J. mimosifolia* (18 differences). Nine differences were observed between *G. austroafricanum* and the isolates from *J. mimosifolia* collected in this study.

Phylogenetic trees (Fig. 1) generated from the ITS data matrix placed the isolates from *C. siliqua* and *J. mimosifolia* trees at different positions. Isolate CMW43669 from *C. siliqua* was distant from the isolates from *J. mimosifolia* and formed a sister group with sequences representing *G. lucidum* (*s. lat.*), *G. oregonense, G. resinaceum, G. neojaponicum,* and *G. lobatum* (ML bootstrap = 62 %). The isolate of *G. austroafricanum* (CMW 41454) previously described from *J. mimosifolia* grouped with *G. stipitatum, G. weberianum*, and *G. subamboinense,* with strong statistical support. The remainder of the isolates from *J. mimosifolia* formed a monophyletic group, although the grouping was not supported based on BI and had relatively low bootstrap support in the other analyses (ML bootstrap = 63 %, NJ bootstrap = 73 %). This group was closely related to *G. steyaertanum* and a monophyletic group that included *G. lucidum* (*s. lat.*), *G. multipileum, G. martinicense,* and *G. parvulum*.

TAXONOMY

The results of the DNA sequence comparisons showed that isolates from *Ceratonia siliqua* and *Jacaranda mimosifolia* in Pretoria represent two distinct lineages that are interpreted as two undescribed species of *Ganoderma*. They are consequently described here.

Ganoderma enigmaticum M.P.A. Coetzee, Marinc., M.J. Wingf., **sp. nov.**
MycoBank MB812509
(Fig. 2)

Etymology: The name refers to the enigmatic discovery of this fungus amongst a large number of isolates all representing a different species.

Diagnosis: Morphologically similar to species in the *G. lucidum s. lat.* complex, but with pileus covered by creamy soft non-poroid tissue, basidiospores being ellipsoid and 8–11 × 3.5–6 µm (av. 9.2 × 4. 5 µm). In culture, *G. enigmaticum* can be differentiated from *G. austroafricanum* and *G. destructans* by having an optimum growth at 30 °C on 2 % MEA. At the DNA level it differs from other *Ganoderma* species with unique nucleotide polymorphisms at ITS and LSU.

Type: **South Africa**: *Gauteng province*: Pretoria, Brooklyn (25° 45.47' S, 28° 13.87' E), on *Ceratonia siliqua*, 10 Jan. 2015, *M.J. Wingfield* (PREM 61264 – holotype; CBS 139792 = CMW 43669 – ex-holotype cultures). ITS sequence GenBank KR183855, LSU sequence GenBank KR183859.

Description: *Basidiomes* perennial, stipitate with a globular upper pileus, 10–11 × 10–13 × 15–16 cm; pilear surface covered by creamy soft non-poroid tissue showing obvious continuity to hymenophore beneath the pileus; the tissue covering pileus down in 12–30 mm becoming hymenophore, thickened at the border with the pileus; hymenophore white to creamy turning pale brown; stipe columnar, solitary, laccate, lustrously reddish to dark brown, sulcate; borders with hymenophore thickened, yellowish brown, blunt, undulate to lobate; young basidiomes bulbous, covered with creamy soft non-poroid tissue and reduced pileal surface; pores 3–5 per mm, round to somewhat irregular and elongated, 135–292 × 92–181 µm (av. 193.3 × 137.7 µm), dissepiments 48–121 µm wide (av. 82.2 µm); context soft, corky becoming woody, zonate, homogenous, dark brown, darker near the tubes; tubes 0.5–1.5 mm long, dark brown. *Hyphal system* trimitic; generative hyphae not easily observed, hyaline, thin-walled, 2–3.5 µm diam, clamped; skeletal hyphae branched, pale brown when young becoming dark brown, 3.5–7.5 µm thick; binding and skeleto-binding hyphae hyaline, branched, tapering towards the end, 1–2.5 µm thick. *Cutis* consisted of a palisade of vertical, cylindrical to narrowly clavate, and thick-walled elements, amyloid, 20–46 × 5.5–9 µm. *Basidia* not seen. *Basidiospores* ellipsoid, the endosporium brown, the exosporium hyaline, appearing verruculose with inter-wall pillars, 8–11 × 3.5–6 µm (av. 9.2 × 4. 5 µm). *Colonies* on 2 % MEA showing optimum growth at 30 °C reaching 85 mm in the dark in 7 d, followed by at 35 °C reaching 75 mm, at 25 °C reaching 73 mm, at 20 °C reaching 32 mm, at 15 °C reaching 9 mm and no growth at 10 °C; mats circular and edge flat, white above and creamy reverse at all temperatures, felty, mycelium superficial with medium density, chlamydospores not seen.

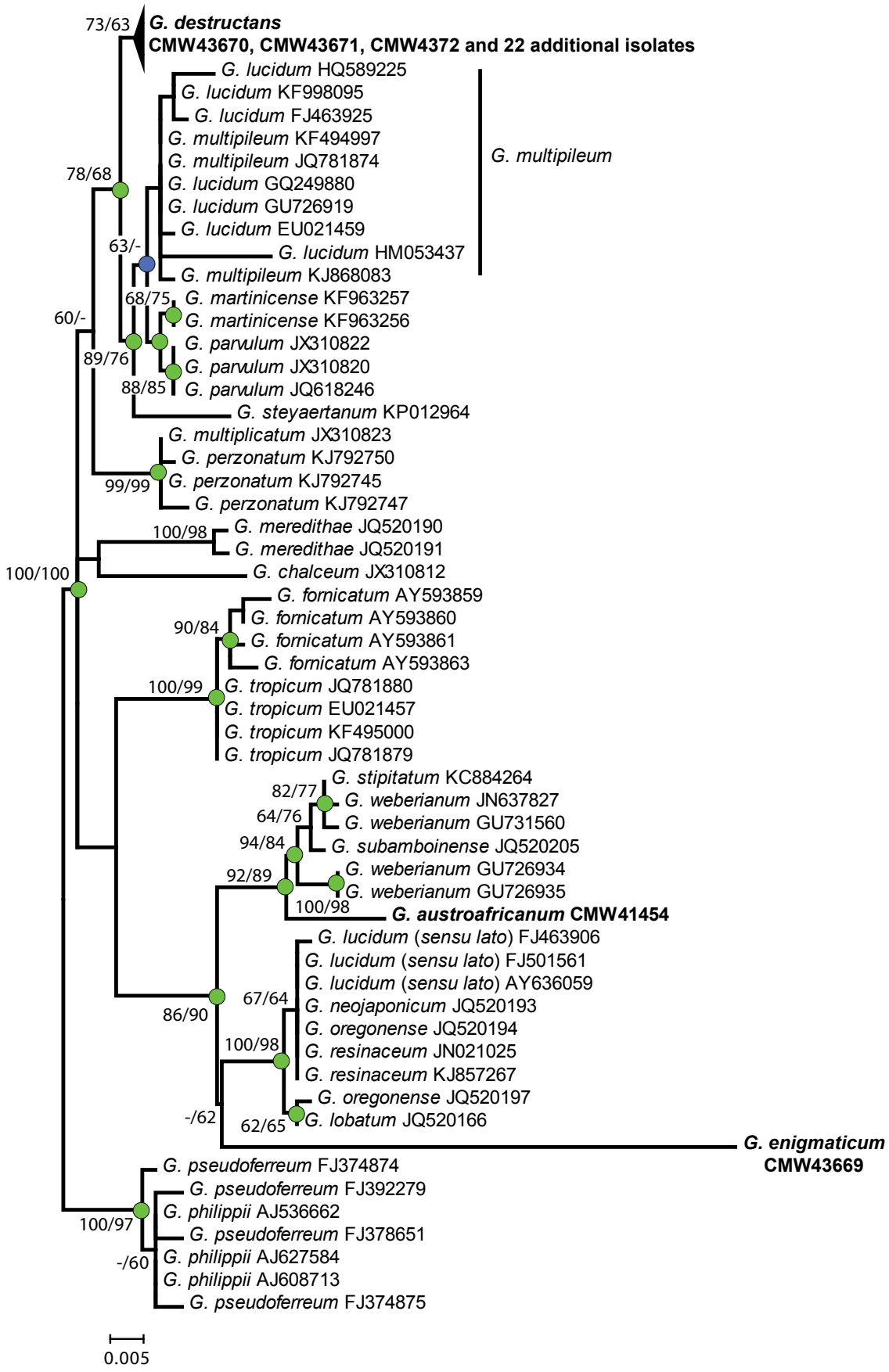

Fig. 1. Maximum likelihood tree based on ITS sequences showing the relationship of isolates included in this study and different *Ganoderma* species. GenBank accession numbers are shown next to the species names. Bootstrap values (≥ 60 %) from neighbour-joining and maximum likelihood analyses are shown at the nodes. Posterior probability values are indicated with circles at the nodes (green ≥ 0.95, blue ≥ 0.90). Scale bar indicates the number of nucleotide substitutions per site.

Fig. 2. *Ganoderma enigmaticum*. **A.** Young basidiome growing from the trunk of *Ceratonia siliqua* (Carob tree). **B.** Basidiome collected (PREM 61264). **C.** Hymenophore. **D–E.** Skeletal hyphae. **F.** Basidiospores. Bars: C = 1 mm, D = 20 µm, E = 50 µm, F = 10 µm.

Ganoderma destructans M.P.A. Coetzee, Marinc, M.J. Wingf, **sp. nov.**
MycoBank MB812511
(Fig. 3)

Etymology: The name reflects the fungus being found associated with many trees dying of root rot. It appears to be the most important tree pathogen in the area where it was collected.

Diagnosis: Morphologically similar to species in the *G. lucidum s. lat.* complex, but with pileus covered by creamy soft non-poroid tissue and basidiospores 11–14 × 7–9 µm (av. 12.3 × 8.0 µm), ovoid. In culture, *G. destructans* can be differentiated from *G. austroafricanum* and *G. enigmaticum* by having an optimum growth at 25 °C on 2 % MEA. At the DNA level it differs from other *Ganoderma* species with unique nucleotide polymorphisms at ITS and LSU.

Type: **South Africa**: *Gauteng province*: Pretoria, Brooklyn (25° 45.65' S, 28° 14.50' E), on *Jacaranda mimosifolia,* 10 Jan. 2015, *M.J. Wingfield* (PREM 61265 – holotype; CBS 139793 = CMW 43670 – ex-holotype cultures). GenBank accession numbers: ITS KR183856, LSU KR183860.

Description: *Basidiomes* perennial, stipitate with a globular upper pileus, 30–50 cm in diam; pilear surface covered by creamy soft non-poroid tissue showing obvious continuity to hymenophore; tissue covering pileus downy, becoming the hymenophore, thickened at the border with the pileus; hymenophore white to creamy turning brown when old or upon bruising; stipe columnar, solitary, laccate, lustrously reddish to dark brown, sulcate; borders with hymenophore thickened, yellowish brown, blunt, undulate to lobate; young basidiomes bulbous, covered with creamy soft non-poroid tissue and reduced pileal surface; pores 3–5 per mm, round to somewhat irregular and elongated, 172–349 × 121–250 µm (av. 260.8 × 193.0 µm), dissepiments 28–92 µm wide (av. 62.3 µm); context soft, corky becoming woody, zonate, homogenous, dark brown, darker near the tubes; tubes 0.5–1.5 mm long, dark brown. *Hyphal system* trimitic; generative hyphae not easily observed, hyaline, thin-walled, 1.5–2.5 µm diam, clamped; skeletal hyphae branched, pale brown when young becoming dark brown, 2.5–5.5 µm thick; binding and skeleto-binding hyphae hyaline, branched, tapering towards the end, 1–2.5 µm thick. *Basidia* not seen. *Basidiospores* ovoid, the endosporium brown, the exosporium hyaline, appearing verruculose with inter-wall pillars, 11–14 × 7–9 µm (av. 12.3 × 8.0 µm). *Cutis* consisted of a palisade of vertical, narrowly clavate to cylindrical with inflated apex, and thick-walled elements, amyloid, 13–35 × 4.5– 7.5 µm. *Colonies* on 2 % MEA showing optimum growth at 25 °C reaching 85 mm in the dark in 7 d, followed by at 30 °C reaching 72 mm, at 20 °C reaching 42 mm, at 15 °C reaching 15 mm, no growth at

Fig. 3. *Ganoderma destructans*. **A.** Flowers of *Jacaranda mimosiflora* (Jacaranda tree). **B.** Die-back of a tree as a symptom of root rot. **C.** Fallen tree. **D.** Young basidiomes (arrows) growing along the roots of Jacaranda tree. **E.** Mature basidiome covered with creamy soft non-poroid tissue showing obvious continuity to hymenophore (PREM 61265). **F.** Basidiomes growing at the basal trunk. **G.** Hymenophore. **H.** Skeletal hyphae. **I.** Basidiospores. Bars: G = 1 mm, H–I = 20 µm.

35 °C; mats circular and edge flat, white above and creamy reverse at all temperatures, felty, mycelium superficial with medium density, chlamydospores not seen.

Other material examined: **South Africa**: *Gauteng province*: Pretoria, Brooklyn (25° 45.58' S, 28° 14.21' E), on *Jacaranda mimosifolia*, 24 Jan. 2015, *M.J. Wingfield* (PREM 61266; CBS 139794 = CMW 43671 – living cultures; GenBank accession numbers: ITS KR183857, LSU KR183861); Brooklyn, on *J. mimosifolia*, 24 Jan. 2015, *M.J. Wingfield* (PREM 61267; CBS 139795 = CMW 43672 – living cultures; GenBank accession numbers: ITS KR183858, LSU KR183862); Brooklyn, on *J. mimosifolia*, Feb. 2007, *V.G. Muthel* (CMW 29570–29591 – living cultures).

Notes: Both *Ganoderma enigmaticum* and *G. destructans* have atypical basidiomes with the pileus covered by creamy soft non-poroid tissue showing obvious continuity to hymenophore. This distinguishes them from *G. austroafricanum* that shares the same host trees as *G. destructans*. Additionally, the basidiospores of *G. austroafricanum* (8–11 × 5.5–7 µm) are smaller than those of *G. destructans* (11–14 × 7–9 µm). *Ganoderma enigmaticum* can be distinguished from *G. destructans* and *G. austroafricanum* based on growth at 35 °C: *G. destructans* and *G. austroafricanum* did not show any visible growth whereas *G. enigmaticum* grew well at this temperature. Basidiomes of *G. enigmaticum* and *G. destructans* are indistinguishable but microscopically the former can be distinguished from the latter by having ellipsoid instead of ovoid basidiospores that are also smaller (8–11 × 3.5–6 µm).

DISCUSSION

At the onset of this study, it was expected that the *Ganoderma* isolates collected would be those of *G. austroafricanum*. This would be consistent with the isolates having been collected in the general area where *G. austroafricanum* was first found, and the primary aim of our investigation was to obtain a larger collection of that fungus for possible use in a population genetics study. Our results, however, revealed that all the newly collected isolates represented two new species, which was surprising. That all but one of the fresh isolates were of a single species, described here as *G. destructans*, was also unusual.

This study shows that there are three species of *Ganoderma* associated with root rot of trees, especially *Jacaranda mimosifolia,* in a relatively small area of Pretoria. This suggests that there are likely to be many other species of *Ganoderma* in South Africa, and perhaps even in the Pretoria area, that has only been very superficially surveyed. Clearly one species (*G. destructans*) is dominant in the area studied, and this appears to be the most important pathogen resulting in the death of *J. mimosifolia.* It is strange that the two other species known have been found on only two trees, one of *J. mimosifolia* and one of *Ceratonia siliqua*. It seems likely that they will be found again and on other trees as surveys for these fungi are expanded in the future.

Morphological and DNA sequence comparisons with other *Ganoderma* species revealed that the basidiomes collected on most Jacaranda trees in this study belong to the new species *G. destructans*. The morphology of *G. destructans* differs from the other *Ganoderma* species listed by Baxter & Eicker (1995) as occurring in South Africa. Phylogenetic trees generated in this study showed that the isolates of *G. destructans* are closely related to *G. steyaertanum, G. parvulum, G. martinicense, G. multipileum,* and *G. lucidum* (*s. lat.*). *Ganoderma steyaertanum* was described from Australia and Indonesia and it was suggested that the distribution of this species may not extend further north than Indonesia (Smith & Sivasithamparam 2003). The basidiomes of *G. destructans* and *G. steyaertanum* display some similarity, but the basidiospores of *G. destructans* are slightly larger than those described for *G. steyaertanum* (7.3–12.7 × 5.0–9.5 µm; Smith & Sivasithamparam 2003). At the molecular level, analysis of ITS sequence alignments revealed 10 nucleotide differences between *G. destructans* and *G. steyaertanum.*

Ganoderma multipileum was described by Wang *et al.* (2009), and is considered the earliest valid name for *G. lucidum s. lat.* in tropical Asia. Morphological comparisons showed that this species and *G. steyaertanum* are very similar (Wang *et al.* 2009). Analysis of ITS sequence alignments between *G. multipileum* and *G. destructans* in the present study revealed five base pair differences between the two species. *Ganoderma martinicense* is restricted to Martinique (French West Indies) and related to *G. multipileum* (Welti & Courtecuisse 2010). The basidiospores of *G. martinicense* are smaller than those of *G. destructans*. Six base pair differences were observed in the ITS sequence alignment between *G. destructans* and *G. martinicense.* The greatest sequence variation was observed between *G. destructans* and *G. parvulum* (11 nucleotide differences) and the spores of *G. destructans* are longer than the 8–10 × 5–6 µm reported for *G. parvulum* from Brazil (de Lima *et al.* 2014).

The basidiome resembling *G. lucidum s. lat.* collected from a single *C. siliqua* tree was also shown in this study to represent a new species, described here as *G. enigmaticum*. Based on initial observations of the basidiome, the fungus was thought to be either *G. destructans* or *G. austroafricanum.* Yet phylogenetic trees generated from the ITS sequence data showed clearly that this fungus represented a new taxon unrelated to those species. Morphological comparisons also showed that *G. enigmaticum* can be distinguished from *G. destructans* and *G. austroafricanum*.

Pathogenic *Ganoderma* species appear to have wide host ranges (e.g. Sankaran *et al*. 2005) and the same may be true for the new species collected in this study. That most isolates have been found on *J. mimosifolia* could be related to these trees being by far the most prevalent alongside roads in the area. It is also probable that these fungi, like most basidiomycete root-infecting fungi, are native to the area in which they have been found. There are, however, some notable examples of root-infecting basidiomycete pathogens that have been accidentally introduced into new areas either with wood or with potted plants. These include species of *Armillaria* (Coetzee *et al*. 2001, Coetzee *et al*. 2003) that are believed to have been introduced into South Africa by early colonialists. Similarly, *Heterobasidion irregulare* has been shown to be an alien invasive in Europe, most probably accidentally introduced with infected wood used by the military during the second world war (Gonthier *et al.* 2004, Garbelotto *et al*. 2013). Population genetic studies would be required to determine for certain whether the *Ganoderma* species causing root and butt rot of Jacaranda trees are native or not.

The Ganoderma root rot disease on the iconic Jacaranda trees in Pretoria appears to be increasing in magnitude, albeit relatively slowly. This could be attributed to a number of factors including a gradual build-up of inoculum. *Jacaranda mimosifolia* is an introduced tree in South Africa and it is possible that it has a relatively low level of resistance to infection by what we assume is a native pathogen. However, that *Ganoderma* species generally have wide host ranges makes this a less probable contributing factor. Stress on these trees and their roots are more likely to contribute to disease development. As street trees, they are commonly subjected to physical damage, soil compaction, an excess of water due to the installation of automated irrigation systems, and other possible predisposing factors (Ennos 2015). Residents where this disease is developing should be encouraged to reduce any obvious forms of stress on these trees.

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REFERENCES

Adaskaveg JE, Gilbertson RL (1986) Cultural 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, Blanchette RA (1990) Comparative studies of delignification caused by *Ganoderma* species. *Applied and Environmental Microbiology* **56**: 1932–1943.

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. Taipei: R.S. Hseu.

Cao Y, Wu S-H, Dai Y-C (2012) Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi". *Fungal Diversity* **56**: 49–62.

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

Coetzee MPA, Wingfield BD, Roux J, Crous PW, Denman S, *et al.* (2003) Discovery of two Northern Hemisphere *Armillaria* species on *Proteaceae* in South Africa. *Plant Pathology* **52**: 604–612.

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**: 772–772.

de Lima NC, Gibertoni TB, Malosso E (2014) Delimitation of some neotropical laccate *Ganoderma* (*Ganodermataceae*): molecular phylogeny and morphology. *International Journal of Tropical Biology and Conservation* **62**: 1197–1208.

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.

Ennos RA (2015) Resilience of forests to pathogens: an evolutionary ecology perspective. *Forestry* **88**: 41–52.

Flood J, Bridge PD, Holderness M (2001) *Ganoderma Diseases of Perennial Crops*. Wallingford: CABI Publishing.

Garbelotto M, Guglielmo F, Mascheretti S, Croucher PJP, Gonthier P (2013) Population genetic analyses provide insights on the introduction pathway and spread patterns of the North American forest pathogen *Heterobasidion irregulare* in Italy. *Molecular Ecology* **22**: 4855–4869.

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.

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, *et al.* (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.

Henderson L (1990) *Jacaranda*. [Weeds no. A_30/1990.] Pretoria: Department of Agricultural Development.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

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

Kinge TR, Mih AM (2014) *Ganoderma lobenense* (basidiomycetes), a new species from oil palm (*Elaeis guineensis*) in Cameroon. *Journal of Plant Sciences* **2**: 242–245.

Kinge TR, Mih AM, Coetzee MPA (2012) Phylogenetic relationships among species of *Ganoderma* (*Ganodermataceae*, *Basidiomycota*) from Cameroon. *Australian Journal of Botany* **60**: 536–538.

Moncalvo J-M, Lutzoni FM, Rhener SA, Johnson J, Vilgalys R (2000) Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* **49**: 278–305.

Moncalvo J-M, Ryvarden L (1997) *A Nomenclatural Study of the Ganodermataceae Donk.* [Synopsis fungorum no. 2.]. Oslo: Fungiflora.

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* **8**: 4321–4326.

Old KM, Lee SS, Sharma JK, Yuan ZQ (2000) *A Manual of Diseases of Tropical Acacias in Australia, South-East Asia and India*. Jakarta: Center for International Forestry Research.

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 (1995) Can we trust morphology in *Ganoderma*? In: *Ganoderma: systematics, phytopathology and pharmacology* (Buchanan RK, Hseu RS, Moncalvo JM, eds): 19–24. Taipei: R.S. Hseu.

Ryvarden L, Johansen I (1980) *A Preliminary Polypore Flora of East Africa*. Oslo: Fungiflora.

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

Smith BJ, Sivasithamparam K (2000) Isozymes of *Ganoderma* species from Australia. *Mycological Research* **104**: 952–961.

Smith BJ, Sivasithamparam K (2003) Morphological studies of *Ganoderma* (*Ganodermataceae*) from the Australasian and Pacific regions. *Australian Systematic Botany* **16**: 487–503.

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

Wang D-M, Wu S-H, Su C-H, Peng J-T, Shih Y-H, Chen L-C (2009) *Ganoderma multipileum*, the correct name for '*G. lucidum*' in tropical Asia. *Botanical Studies* **50**: 451–458.

Welti S, Courtecuisse R (2010) The *Ganodermataceae* in the French West Indies (Guadeloupe and Martinique). *Fungal Diversity* **43**: 103–126.

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* (MA Innis, DH Gelfand, JJ Sninsky &TJ White, eds): 315–322. San Diego: Academic Press.