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 australofianum was described from these trees previously (the "City of Jacarandas") 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 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 sp. nov. Interestingly, the previously described G. australofianum 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.

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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 (Gemplasm Resources Information Network; http://www.ars-grin.gov.gov/4/cgi-bin/ngp5s/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 & 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 basidiome 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 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 basidiome 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

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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 (Crouś 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 (Crouś 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 polyvinylpyrrolidone) 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 LROR 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 MgCl2, 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 PCRPac kit (STRATEGEC 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 (Husakemberg & 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 Axiosvision 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. neoajphonicum in GenBank. DNA sequences from the remainder of the isolates had the highest similarity to sequences representing G. lucidum (s. lat.), G. multipileum, G. martincense, 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 CMW 43669 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. resinacea, G. neoajphonicum, 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. martincense, 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 elliptoid 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.


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, lustuously 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 mm, round to somewhat irregular and elongated, 135–292 × 92–181 μm (av. 193.3 × 137.4 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 elliptoid, the endosporum brown, the exosporium hyaline, appearing verrucose 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, felfy, 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.
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.


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 basidiomata 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), dissepsiments 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 skeloeto-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 verrucose 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

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. Basidiospores 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. martincense*, *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 martincense* is restricted to Martinique (French West Indies) and related to *G. multipileum* (Welti & Courtecuisse 2010). The basidiospores of *G. martincense* are smaller than those of *G. destructans*. Six base pair differences were observed in the ITS sequence alignment between *G. destructans* and *G. martincense*. 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 *Ganoderma 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 colonists. 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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