

Community composition and distribution of *Phytophthora* species across adjacent native and non-native forests of South Africa

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Abstract

The diversity of *Phytophthora* species associated with various ecological niches is poorly understood. In this study, the community composition and distribution of *Phytophthora* species associated with non-native plantation trees, *Eucalyptus grandis* and *Acacia mearnsii*, was compared with adjacent natural forests in South Africa using soil baiting and metabarcoding approaches. Through soil baiting, 85 *Phytophthora* isolates were recovered representing five taxa: *P. alticola*, *P. cinnamomi*, *P. frigida*, *P. multivora* and *P. pseudocryptogea*. Metabarcoding revealed molecular operational taxonomic units corresponding to 32 *Phytophthora* taxa. Among these, 14 were new reports from South Africa, including seven undescribed taxa. The community composition of *Phytophthora* species clustered according to vegetation type. Most species in plantations were present in the natural forest sites, but few species were exclusively associated with the non-native plantations. Overall, the results revealed a substantial diversity of *Phytophthora* species that includes both described and novel phylotypes previously unknown from South Africa.

Keywords: *Acacia mearnsii*, *Eucalyptus grandis*, Internal Transcribed Spacer (ITS), metabarcoding, pyrosequencing and soil baiting

Introduction

Phytophthora species are amongst the most destructive plant pathogens (Haas *et al.* 2009; Ribeiro 2013) and yet relatively little is known regarding their global diversity (Scott *et al.* 2013). Surveys for, and discoveries of, new *Phytophthora* species have been significantly facilitated by the application of baiting techniques (Drenth and Sendall 2001) and the utilization of selective media (Erwin and Ribeiro 1996). In recent years, molecular techniques have also contributed strongly to the discovery, detection and identification of cryptic species as well as to diversity studies. For example, the *P. citricola* (Jung and Burgess 2009; Scott *et al.* 2009; Bezuidenhout *et al.* 2010) and *P. cryptogea* (Safaiefarahani *et al.* 2015) complexes have been resolved using these techniques.

The application of high throughput sequencing technology to environmental samples has the capacity to expand our knowledge of species diversity and distribution, especially as it offers the potential to detect rare or unculturable organisms missed in traditional studies. However, there are some weaknesses with these approaches such as: (i) the inability to generate pure cultures, crucial for taxonomic and genomics studies, and (ii) the amplification of relatively short fragments (~500-600bp) of DNA making it challenging to discriminate between closely related species (Huse *et al.* 2007; Burgess *et al.* 2017a). Nonetheless, the genus specific primers available for *Phytophthora* (Scibetta *et al.* 2012), based on the ITS1 gene region have provided sufficient data to be able to conduct phylogenetic analyses for identification purposes (Català *et al.* 2015; Burgess *et al.* 2017a; Català *et al.* 2017). As such, metabarcoding is an efficient tool to investigate the diversity of *Phytophthora* species in natural forests, nurseries and agricultural ecosystems.

Although *Phytophthora* species have a worldwide distribution, relatively few species have been reported from Africa, and the majority of these have been reported from South Africa (Nagel *et al.* 2013; Scott *et al.* 2013). In South Africa, *Phytophthora* species affect agricultural crops, native forests, plantations and orchards of non-native species (Nagel *et al.* 2013). The severity of *Phytophthora* diseases varies greatly within South Africa, depending on the area and crop being planted. For example, the root rot of avocado caused by *P. cinnamomi* (Milne *et al.* 1974; Milne *et al.* 1975; Kotze *et al.* 1987) and of citrus species caused by *P. citrophthora* and *P. citricola* (Doidge 1925; Von Maltitz and Von Broembsen 1985) has a severe economic impact. There are also some reports of *Phytophthora* diseases in natural ecosystems in South Africa, the best-known being those caused by *P. cinnamomi* in the Cape Floristic Region (CFR) in the Western Cape province. The CFR has received the most attention (Von Broembsen 1984; Bezuidenhout *et al.* 2010) due to its extraordinary floral diversity as well as the high levels of susceptibility of the Proteaceae in this region to *Phytophthora* infections (van Wyk 1973).

In South Africa, *Phytophthora* species cause diseases of various species of the non-native plantation tree genera *Pinus*, *Eucalyptus* and *Acacia mearnsii*. *Phytophthora cinnamomi* causes root and collar-rot of both *Pinus* and *Eucalyptus* species (Linde *et al.* 1994), and until the early 1990's *P. cinnamomi* was the only species known to cause disease on these trees. Later studies reported *P. alticola*, *P. boehmeriae*, *P. frigida*, *P. meadii* and *P. nicotianae* as pathogens of *A. mearnsii* and *Eucalyptus* species (Zeijlemaker 1967; Zeijlemaker and Margot 1970; Zeijlemaker 1971; Linde *et al.* 1994; Roux and Wingfield 1997; Maseko *et al.* 2007).

While a few studies have focused on *Phytophthora* diseases of non-native plantation trees, no studies have considered natural forests as a source of the *Phytophthora* species found in plantations of non-native trees in South Africa. Consequently, this study sought to determine the community composition of *Phytophthora* species associated with plantations of non-native *Eucalyptus grandis* and *Acacia mearnsii* and adjacent natural forests. In addition, it aimed to determine whether this community composition varies between these three very different environments. Soil baiting complemented with metabarcoding using a pyrosequencing platform was used to address the following hypotheses: (1) Community composition of *Phytophthora* species differs between the three vegetation types; (2) community composition of *Phytophthora* species differs between sites; and (3) the *Phytophthora* community is less diverse in monocultures than in the natural forests.

Materials and Methods

Collection of soil samples

Soil samples were collected from four locations in southeastern Mpumalanga and KwaZulu-Natal Provinces of South Africa in November 2014 and 2015. The four collection sites were near Howick, Melmoth, Vryheid and Comondale (Figure 1A). Howick and Comondale were sampled in 2014 and Melmoth and Vryheid in 2015. These sites were chosen where plantations of non-native *E. grandis* and *A. mearnsii* trees and native natural forests were located in close proximity (Figure 1C-D). The age of the plantations was between 10-15 y for *E. grandis* and 8-10 y for *A. mearnsii*. The natural forests were healthy protected remnants with high plant species diversity

typical of the region. Some common native trees included *Allophylus natalensis*, *Bequaertiodendron natalense*, *Celtis africana*, *Combretum krausii*, *Curtisia dentate*, *Cussonia spicata*, *Ekebergia capensis*, *Euclea natalensis*, *Heteropyxis natalensis*, *Ilex mitis*, *Kiggelaria africana*, *Millettia grandis*, *Ocotea bullata*, *Podocarpus latifolius*, *Prunus africana*, *Sideroxylon inerme*, *Vepris undulate* along with various species of *Eugenia* and *Syzygium*.

A total of 1200 soil samples were collected from these four sites (4 sites \times 3 vegetation types \times 10 plots \times 10 trees). Ten plots within each plantation as well as adjacent natural forest were selected arbitrarily (Figure 1A). Soil samples along with fine roots were arbitrarily collected from the rhizosphere of 10 trees within each 10 \times 10 m plot after removing the plant debris and 4–5 cm of topsoil. These 10 soil samples from each plot were merged together thereafter 2 kg of this composite soil mix served as one sample (Figure 1A). A portion of the 120 composite soil samples (4 sites \times 3 vegetation types \times 10 plots) was used for soil baiting, while the remaining were air-dried at room temperature (22–25°C) for metabarcoding.

Isolation of Phytophthora using soil baiting

All 120 composite soil samples were baited in a controlled environment where the temperature was kept between 22–25°C and the humidity between 70–75%. Each of the soil samples was baited separately in a 24 \times 14 \times 6 cm plastic trough using 300 g of soil following the protocol of the Centre for *Phytophthora* Science and Management (CPSM), Murdoch University. Soils were mixed thoroughly and pre-moistened overnight before flooding with water to a depth, twice that of the soil. After removing the floating debris, two leaves each of *Duranta repens*, *Hedera helix*,

Hibiscus rosa-sinensis, *Rhododendron indicum*, white rose petals and cotyledonous leaves of *Eucalyptus sieberi* were added and served as baits. The baits were monitored regularly for 10 d for signs of infection. Lesions from infected baits were plated onto *Phytophthora*-selective medium, NARPH (Masago *et al.* 1977), followed by establishment of pure cultures. Pure cultures were maintained on 10% clarified V8-Agar (10 ml clarified V8 juice, Campbell Soup Company USA; 15 g Difco™ Agar, Becton, Dickinson and Company, Sparks, USA) as well as half-strength Potato Dextrose Agar (PDA; Becton, Dickinson and Company, Sparks, USA, 19.5 g PDA powder, 7.5 g of agar and 1L of distilled water) and also as agar plugs in glass vials with sterile deionized water. Where the initial baiting did not show any signs of infection on the baits, the same soil was re-baited after drying at room temperature (22–25°C).

Identification of Phytophthora isolates recovered through baiting

Phytophthora isolates were grown on half strength PDA in Petri dishes at 20°C for 10 d. Mycelium was harvested from each isolate by scraping this from the agar surface. Thereafter, genomic DNA was extracted using ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research, USA) following the manufacturer's protocol. The region spanning the internal transcribed spacer region (ITS1-5.8S-ITS2) of ribosomal DNA was amplified using the primers ITS6 (Cooke *et al.* 2000) and ITS4 (White *et al.* 1990). Individual PCRs were performed using 5× GoTaq Flexi Buffer (Promega, MI) – 5 µl, 25 mM MgCl₂ (Promega, MI) - 2.5 µl, 0.1 mM dNTPs (Promega, MI) - 1.5 µl, BSA (Amresco, OH) – 1 µl, 1U GoTaq Hot Start Polymerase (Promega, MI), 0.5 µl of each primer and the final volume was made up to 25 µl with PCR grade

water. The PCRs were carried out with initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 secs, 55°C for 45 secs, 72°C for 1 min and final elongation at 72°C for 5 min. The PCR products were sequenced with an ABI PRISM BigDye[®] Terminator Cycle Sequencing Kit 3.1 (Life Technologies-Applied Biosystems, Foster City, CA). Electrophoresis was performed by the DNA Sequencing Facility of the University of Pretoria. Geneious R8 (Kearse *et al.* 2012) was used for assembling the amplicons. All the *Phytophthora* species were identified using BLAST available via NCBI GenBank through 100% sequence similarity. All the complete ITS sequences of the isolates obtained in this study were deposited in GenBank and cultures are maintained in the microbial culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table S1).

Metabarcoding and analysis of data

About 50 g of each of the 120 composite soil samples were pulverized using a Retsch[®] grinding jar attached to a Qiagen[®] TissueLyser II. DNA was extracted from 500 mg of each soil sample in duplicate using the Mo-Bio PowerSoil[®] DNA Isolation Kit (Carlsbad, CA). Environmental DNA (eDNA) amplifications and amplicon library generation was carried out using a nested PCR approach following Scibetta *et al.* (2012) and Català *et al.* (2015). Autoclaved fine sand served as controls. For each pyrosequencing run there were two sets of controls. These included (1) grinding controls where sterile sand was ground during the pulverization process to serve as a sample and (2) eDNA extraction controls where for each set of eDNA extractions, 0.5 g of autoclaved sand served as a control sample. Grinding and eDNA extraction

controls were assigned Multiplex Identifiers (MIDs) and processed with the same protocol as soil samples, although no product could be visualized on the gel during electrophoresis.

PCR products were visualized using 1% agarose gel electrophoresis and then pooled based on band intensity into groups of 5–6 (total volume 30 μ l). Each group was cleaned twice with Agencourt AMPure XP PCR purification beads (Beckman Coulter Genomics, USA) following the manufacturer's instructions. After cleaning, the PCR products were again visualized on an agarose gel. The samples were further pooled into a single unit based on the band intensity to standardize the DNA contribution for each sample. The final pooling was diluted to 1/5000 of the original concentration, and 50 μ l of the dilution was again cleaned with AMPure XP beads. The amplicons were sequenced at the Western Australian State Agricultural Biotechnology Centre (SABC), Murdoch University following the Roche GS Junior Sequencing Method Manual (March 2012) using GS Junior Titanium Chemistry and GS Junior Pico Titre Plates (454 Life Sciences/Roche Applied Biosystems, Nutley, NJ, USA). The reads were analyzed and clustered into molecular operational taxonomic units (MOTUs) based on 99% similarity using Geneious R8. Metabarcoding data is available at the NCBI under the accession numbers SRX3228069 and SRX3228070.

Initial species identification was carried out based on sequence similarity against a reference database containing ITS1 sequences from 192 *Phytophthora* taxa including 169 identified species and 23 designated, but as yet undescribed, *Phytophthora* species made available by the CPSM (see Burgess *et al.* 2017a). For the purpose of phylogenetic identification of the MOTUs, the database was divided into

five groups: (1) clades 1 and 2, (2) clades 3 and 4, (3) clades 5 and 6, (4) clades 7 and 8 and (5) clade 9 and 10 in order to increase resolution within a clade. All the datasets were aligned using MAFFT (Katoch *et al.* 2002) available via Geneious R8. Phylogenetic analyses using maximum likelihood (ML) approach were performed using RAxML v8 (Stamatakis 2014). The general time reversible model along with gamma distribution (GTR GAMMA) was selected using jModelTest 2.1 (Guindon and Gascuel 2003; Darriba *et al.* 2012). Fifty replicated likelihood searches were executed for each dataset followed by 1000 bootstrap replicates. The resultant trees were rooted and modified using FigTree v1.4 and Adobe Illustrator CS6.

Statistical Analyses

For isolates recovered by soil baiting, a Chi-square test was conducted to determine whether the total number of *Phytophthora* species differed between the sites and the vegetation types.

Phylotypes of *Phytophthora* species recovered through metabarcoding were analyzed after consolidating the data for each vegetation type (4 sites \times 3 vegetation types). Presence/absence data was used rather than abundance data because of sequencing bias, which has been highlighted as a problem (Catalá *et al.* 2015). To visualize variation in *Phytophthora* species community composition among the soil samples, a non-metric multidimensional scaling (NMDS) of *Phytophthora* species was conducted using Jaccard distance ($k = 3$) and the “metaMDS” function in the vegan package in R (Oksanen *et al.* 2015). Moreover, the “ordiellipse” function available in the vegan package (R core Team, 2018) was used to generate confidence ellipses ($\text{conf} = 0.95$) to cluster points based on the vegetation type. To asses

The total number of isolates of each species differed significantly ($P < 0.05$) across vegetation types (plantations of *E. grandis*, *A. mearnsii* and natural forest). Most of the isolates were recovered from plantations of non-native *A. mearnsii*, followed by natural forest and lastly plantations of non-native *E. grandis*. When the five *Phytophthora* species were taken into consideration separately there was no significant difference across the vegetation types.

Phytophthora species detected from soil eDNA

The two pyrosequencing runs collectively generated 123,459 reads (approximately 71.3% of the wells gave good quality reads), which corresponded to 314 MOTUs. The average read length was 306 bp. Approximately 98.4% of the reads corresponded to *Phytophthora* and about 0.5 – 1% of these reads were chimeras. Chimeras were discarded after making alignments of consensus MOTUs for each barcode. The MOTUs were initially identified using BLAST against a reference database with ITS1 sequences of 192 *Phytophthora* species and undescribed (but designated) taxa. After phylogenetic analysis each MOTU was assigned an identity (Figure 2). Some closely related species relevant to this study could not be separated exclusively based on ITS1 sequences: (1) *P. plurivora*, *P. acerina* and *P. pini* and (2) *P. gregata*, *P. gibbosa* and *P. taxon raspberry* (Figure 2). In order not to complicate results, these are hitherto referred to as either *P. plurivora* complex or *P. gregata* complex (Figure 2).

Clustering of the MOTUs and phylogenetic identification revealed 32 distinct *Phytophthora* phylotypes (Table S2, Figure 2). These mostly corresponded to well-defined taxa; two represented informally described species, while six were identified

as putatively new phylotypes (one each from Clade 1, 2, 3, 5, 7 and 10, Figure 2). Of the 32 *Phytophthora* species detected by metabarcoding, the greatest numbers of MOTUs were recovered for *P. frigida*, *P. alticola*, *P. parvispora*, *P. niederhauserii*, and *Phytophthora* RSA5A (Table S2). Twelve species were new reports from South Africa and these included *P. parvispora*, *P. lichii*, *P. pseudocryptogea*, *P. 'kelmania'*, *P. humicola*, *P. aff. meadii*, *P. gondwanense*, *P. asparagi*, *P. elongata*, *P. gregata* complex, *P. inundata* and *P. cambivora*. Two undescribed Australian species (Burgess *et al.* 2017a) were also identified as *Phytophthora* AUS2A and *Phytophthora* AUS9A (Figure 1C-F; Table S2).

The community composition of the *Phytophthora* species from the soil samples was different between the vegetation types, but not between the sampling sites. The NMDS plot supported the difference in *Phytophthora* species between the three vegetation types (Figure 3). PERMANOVA confirmed that vegetation type was the only factor significantly explaining the variation in *Phytophthora* species ($r^2 = 0.309$, $P < 0.01$). Moreover, *Phytophthora* species richness was influenced by site not by vegetation type ($P < 0.05$ and $P > 0.05$, respectively). The greatest species richness was recorded from the native natural forests at Melmoth where 27 species were detected. The Venn diagram shows that, of the 32 *Phytophthora* species detected, 13 were recorded from all three vegetation types (Figure 4).

Discussion

Metabarcoding using *Phytophthora* specific primers to amplify eDNA extracted from forest and plantation soils in South Africa detected 32 *Phytophthora* species across 10 clades recognized within the complete *Phytophthora* phylogeny. These

included seven undescribed phylotypes and 14 new records for South Africa. The majority of the *Phytophthora* species from natural forests were also recovered from the adjacent plantations of non-native *E. grandis* and *A. mearnsii*. Both plantations of non-native trees and natural forests had exclusive *Phytophthora* species. However, the natural forests had greater numbers of exclusive *Phytophthora* species than the plantations. *Phytophthora* species composition was influenced by vegetation type, while *Phytophthora* species richness was influenced by site.

The number of *Phytophthora* species detected in the current study was comparable to similar investigations (Vannini *et al.* 2013; Català *et al.* 2015; Prigigallo *et al.* 2016; Burgess *et al.* 2017a; Català *et al.* 2017). Of those studies, Burgess *et al.* (2017a) detected the greatest number of species (68) in a surveyed of over 500 sites across Australia. The remaining studies (Vannini *et al.* 2013 (15), Català *et al.* 2015 (36), Prigigallo *et al.* 2016 (15), and Català *et al.* 2017 (14)) were comparable in size and scope to the current study and detected a similar number of species.

Two previous studies (Català *et al.* 2015; Burgess *et al.* 2017a), and the present investigation, examined natural ecosystems with diverse habitat types. Natural ecosystems have consistently yielded the greatest number of *Phytophthora* species (Català *et al.* 2015; Burgess *et al.* 2017a). However, our findings suggest that *Phytophthora* species richness is linked to sites. This could be due to either a variation in silviculture practices or the local climate. Plantations at all sites were owned by different forestry companies. Hence, post-harvest soil treatments, sourcing of saplings as well as post-planting silviculture practices would vary between the forestry companies. These silviculture practices would most likely affect the soil microbial

elongata, *P. gondwanense* and *P. 'kelmania'* have been reported from at least one other country apart from South Africa.

In South Africa, *Phytophthora* species infect and impact both *Eucalyptus* and *Acacia mearnsii* plantations. Among the 32 *Phytophthora* species detected in the present study, 20 were either new reports or new phylotypes; their pathogenicity toward *E. grandis* and *A. mearnsii* is unknown. Both *P. nicotianae* and *P. capensis* were detected from natural forests and *A. mearnsii* plantations. The former species is a pathogen of *A. mearnsii*, while the later species infects *Curtisia dentata* (Bezuidenhout *et al.* 2010), a species commonly observed in the natural forests surveyed in the present study. *Phytophthora cinnamomi* was detected from *E. grandis* and natural forest and has been previously reported to infect *Eucalyptus* and native trees in South Africa (Nagel *et al.* 2013). Among the species shared between all three environments, *P. alticola* and *P. frigida* are known pathogens of various *Eucalyptus* species grown in South Africa (Maseko *et al.* 2007).

Several *Phytophthora* species detected in the present study were previously unknown in South Africa, but are known as pathogens elsewhere in the world. The global dispersal of *Phytophthora*, especially species known in agriculture, would have been very common in the past and continues today through the live plant trade (Eschen *et al.* 2015). This has been clearly documented for well-known pathogens such as *P. cinnamomi* (Burgess *et al.* 2017b). Thus, the *Phytophthora* species newly detected in the present study, but already known from other parts of the globe, most likely entered South Africa through trade of live plant materials and agricultural commodities as has been demonstrated for the root-rot pathogen *Armillaria mellea* (Coetzee *et al.* 2001).

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References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403-410.
- Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32-46.
- Bezuidenhout CM, Denman S, Kirk SA, Botha WJ, Mostert L, McLeod A. 2010. *Phytophthora* taxa associated with cultivated *Agathosma*, with emphasis on the *P. citricola* complex and *P. capensis* sp. nov. *Persoonia* 25:32-49.
- Bose T, Burgess TI, Roux J, Wingfield MJ. 2017. *Phytophthora alticola*; revised description based on new collections and a neotype. *Sydowia* 69:161-170.
- Burgess T, White D, McDougall KM, Garnas J, Dunstan WA, Català S, Carnegie AJ, Worboys S, Cahill D, Vettrano AM, Stukely MJC, Liew ECY, Paap T, Bose T, Migliorini D, Williams B, Brigg F, Crane C, Rudman T, Hardy GESTJ. 2017a. *Phytophthora* distribution and diversity across Australia. *Pacific Conservation Biology* 23:1-13.
- Burgess TI, Scott JK, McDougall KL, Stukely MJC, Crane C, Dunstan WA, Brigg F, Andjic V, White D, Rudman T, Arentz F. 2017b. Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. *Global Change Biology* 23:1661-1674.
- Català S, Berbegal M, Pérez-Sierra A, Abad-Campos P. 2017. Metabarcoding and development of new real - time specific assays reveal *Phytophthora* species diversity in holm oak forests in eastern Spain. *Plant Pathology* 66:115-123.
- Català S, Pérez-Sierra A, Abad-Campos P. 2015. The use of genus-specific amplicon pyrosequencing to assess *Phytophthora* species diversity using eDNA from soil and water in northern Spain. *PLoS One* 10:e0119311.
- Coetzee MP, Wingfield BD, Harrington TC, Steimel J, Coutinho TA, Wingfield MJ. 2001. The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* 10:387-396.
- Cooke DE, Drenth A, Duncan JM, Wagels G, Brasier CM. 2000. A Molecular phylogeny of *Phytophthora* and related Oomycetes. *Fungal Genetics and Biology* 30:17-32.

- Cooke DE, Schena L, Cacciola SO. 2007. Tools to detect, identify and monitor *Phytophthora* species in natural ecosystems. *Journal of Plant Pathology* 89:13-28.
- Cooke DE, Williams NA, Williamson B, Duncan JM. 2002. An ITS-based phylogenetic analysis of the relationships between *Peronospora* and *Phytophthora*. In: Spencer-Phillips PTN, Gisi U, Lebeda A, eds. *Advances in downy mildew research*. Dordrecht, Netherlands: Kluwer Academic Publishers. p. 161-165.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9:772-772.
- Doidge EM. 1925. Brown rot in citrus fruits (*Phythiacystis citrophthora* (R. and E. Sm.)). *Journal of the Department of Agriculture, Union of South Africa* 1920 10:499-503.
- Drenth A, Sendall B. 2001. Practical guide to detection and identification of *Phytophthora*, Version 1.0. Brisbane: CRC for Tropical Plant Protection. p. 1-39.
- Erwin DC, Ribeiro OK. 1996. *Phytophthora* diseases worldwide. St. Paul, Minnesota: American Phytopathological Society.
- Eschen R, Britton K, Brockerhoff E, Burgess TI, Dalley V, Epanchin-Niell RS, Gupta K, Hardy GESTJ, Huang Y, Kenis M, Kimani E. 2015. International variation in phytosanitary legislation and regulations governing importation of plants for planting. *Environmental Science and Policy* 51:228-237.
- Greslebin AG, Hansen EM, Winton LM, Rajchenberg M. 2005. *Phytophthora* species from declining *Austrocedrus chilensis* forests in Patagonia, Argentina. *Mycologia* 97:218-228.
- Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52:696-704.
- Haas BJ, Kamoun S, Zody MC, Jiang RH, Handsaker RE, Cano LM, Grabherr M, Kodira CD, Raffaele S, Torto-Alalibo T, Bozkurt TO. 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* 461:393-398.
- Hansen EM, Reeser PW, Sutton W. 2012. *Phytophthora* beyond agriculture. *Annual Review of Phytopathology* 50:359-378.
- Huse SM, Huber JA, Morrison HG, Sogin ML, Welch DM. 2007. Accuracy and quality of massively parallel DNA pyrosequencing. *Genome Biology* 8:R143.
- Jeffers SN, Martin SB. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease* 70:1038-1043.

- Nagel JH, Gryzenhout M, Slippers B, Wingfield MJ. 2013. The occurrence and impact of *Phytophthora* on the African continent In: Lamour K, ed. *Phytophthora: a global perspective*. United Kingdom: CABI International. p. 204-214.
- O'Brien PA, Williams N, Hardy GESTJ. 2009. Detecting *Phytophthora*. *Critical Reviews in Microbiology* 35:169-181.
- Oh E, Gryzenhout M, Wingfield BD, Wingfield MJ, Burgess TI. 2013. Surveys of soil and water reveal a goldmine of *Phytophthora* diversity in South African natural ecosystems. *IMA Fungus* 4:123-131.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MH, Wagner H. *Vegan: Community Ecology Package*. R package version 3.2.3:117-118.
- Prigigallo MI, Abdelfattah A, Cacciola SO, Faedda R, Sanzani SM, Cooke DE, Schena L. 2016. Metabarcoding analysis of *Phytophthora* diversity using genus-specific primers and 454 pyrosequencing. *Phytopathology* 106:305-313.
- R Core Team (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Reeser PW, Sutton W, Hansen EM, Remigi P, Adams GC. 2011. *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia* 103:22-35.
- Ribeiro OK. 2013. A historical perspective of *Phytophthora*. In: Lamour K, ed. *Phytophthora: a global perspective*. p. 1-10.
- Roux J, Wingfield MJ. 1997. Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. *Forest Ecology and Management* 99:327-336.
- Safaiefarahani B, Mostowfizadeh-Ghalmfarsa R, Hardy GESTJ, Burgess TI. 2015. Re-evaluation of the *Phytophthora cryptogea* species complex and the description of a new species, *Phytophthora pseudocryptogea* sp. nov. *Mycological Progress* 14:108.
- Scibetta S, Schena L, Chimento A, Cacciola SO, Cooke DE. 2012. A molecular method to assess *Phytophthora* diversity in environmental samples. *Journal of Microbiological Methods* 88:356-368.
- Scott P, Burgess TI, Hardy GESTJ. 2013. Globalization and *Phytophthora*. In: Lamour K, ed. *Phytophthora: a global Perspective*. United Kingdom: CABI International. p. 226-232.
- Scott PM, Burgess TI, Barber PA, Shearer BL, Stukely MJ, Hardy GESTJ, Jung T. 2009. *Phytophthora multivora* sp. nov., a new species recovered from

- declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia* 22:1-13.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312-1313.
- Van Wyk PS. 1973. Root and crown rot of silver trees. *Journal of South African Botany* 39:255-260.
- Vannini A, Bruni N, Tomassini A, Franceschini S, Vettrano AM. 2013. Pyrosequencing of environmental soil samples reveals biodiversity of the *Phytophthora* resident community in chestnut forests. *FEMS Microbiology Ecology* 85:433-442.
- Von Broembsen S. 1984. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. *Phytophylactica* 16:221-225.
- Von Maltitz PM, Von Broembsen S. 1985. Lemon shoot tip blight caused by *Phytophthora citricola* in propagation tunnel. *Phytophylactica* 17:47-48.
- White TJ, Bruns T, Lee SJ, Taylor JL. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18:315-322.
- Zeijlemaker FCJ. 1971. Black-butt disease of black wattle caused by *Phytophthora nicotianae* var. *parasitica*. *Phytopathology* 61:144-145.
- Zeijlemaker FCJ, Margot P. 1970. Black-butt disease of black wattle. Report. Wattle Research Institute, University of Natal 1971:49-50.
- Zeijlemaker FCJ. 1967. The gummosis of black wattle: a complex of disease. In: Wattle Research Institute (Pietermaritzburg, South Africa) Report. p. 40-43.

FIGURE 4

Supplementary data

TABLE S1 Details of the *Phytophthora* isolates recovered through soil baiting from the four sampling sites and the three vegetation types. EG, AM and NF are *Eucalyptus grandis*, *Acacia mearnsii* and natural forest respectively.

Taxa	CMW #	GenBank #	Site	Coordinates	Vegetation type	Baits
<i>P. alticola</i>	48711	KX247599	Commondale	S27 14.384 E31 00.904	EG	<i>E. sieberi</i>
	48712	KX247599	Melmoth	S28 39.919 E31 25.597	NF	Rose petal
	48713	KX247600	Melmoth	S28 40.505 E31 25.135	NF	<i>E. sieberi</i>
<i>P. cinnamomi</i>	48769	KU726740	Commondale	S27 14.471 E31 00.140	AM	Rose petal
	48770	KU726741	Commondale	S27 14.471 E31 00.140	AM	Rose petal
	48771	KU726742	Commondale	S27 14.503 E31 00.144	AM	<i>R. indicum</i>
	48772	KU726749	Commondale	S27 14.503 E31 00.144	AM	Rose petal
	48774	KU726765	Commondale	S27 14.381 E31 00.801	EG	Rose petal
	48775	KU726777	Commondale	S27 14.485 E31 00.141	AM	<i>H. helix</i>
	48773	KU726752	Howick	S29 18.824 E30 14.132	AM	Rose petal
	48776	KU726778	Melmoth	S28 39.004 E31 28.530	AM	Rose petal
	48777	KU726780	Melmoth	S27 39.099 E30 42.967	EG	<i>R. indicum</i>
	48778	KU726781	Melmoth	S28 39.745 E31 25.601	NF	Rose petal
	48780	KU726783	Melmoth	S28 39.936 E31 25.612	NF	<i>H. helix</i>
	48781	KU726787	Melmoth	S28 40.505 E31 25.135	NF	Rose petal
	48782	KU726789	Melmoth	S28 39.032 E31 28.511	AM	<i>E. sieberi</i>
	48783	KU726793	Melmoth	S28 39.745 E31 25.601	EG	<i>H. helix</i>
	48784	KU726794	Melmoth	S28 40.498 E31 25.084	NF	Rose petal
	48785	KU726795	Melmoth	S28 39.910 E31 25.586	NF	<i>R. indicum</i>
	48786	KU726796	Melmoth	S28 40.496 E31 25.099	NF	Rose petal
	48788	KU726799	Melmoth	S28 39.750 E31 25.636	EG	Rose petal
	48789	KU726801	Melmoth	S28 39.919 E31 25.597	NF	Rose petal
	48790	KU726802	Melmoth	S28 39.910 E31 25.586	NF	<i>H. rosa-sinensis</i>
	48791	KU726803	Melmoth	S28 39.745 E31 25.601	NF	<i>D. repens</i>
	48792	KU726804	Melmoth	S28 39.991 E31 28.507	AM	Rose petal
	48793	KU726807	Melmoth	S28 39.750 E31 25.636	EG	<i>E. sieberi</i>
	48794	KU726808	Melmoth	S28 39.991 E31 28.507	AM	Rose petal
	48795	KU726809	Melmoth	S28 39.004 E31 28.530	AM	Rose petal
	48796	KU726810	Melmoth	S28 39.032 E31 28.511	AM	<i>D. repens</i>
	48797	KU726812	Melmoth	S28 40.498 E31 25.084	NF	<i>D. repens</i>
	48799	KU726816	Melmoth	S28 39.991 E31 28.507	AM	Rose petal
	48779	KU726782	Vryheid	S27 39.061 E30 43.049	AM	<i>E. sieberi</i>
	48787	KU726798	Vryheid	S27 38.262 E30 41.352	NF	Rose petal
	48798	KU726814	Vryheid	S27 39.131 E30 43.068	AM	Rose petal
	48800	KU726818	Vryheid	S27 38.354 E30 41.358	NF	<i>E. sieberi</i>
<i>P. frigidula</i>	48726	KU726738	Commondale	S27 14.471 E31 00.140	AM	<i>D. repens</i>
	48727	KU726739	Commondale	S27 14.471 E31 00.140	AM	Rose petal
	48728	KU726743	Commondale	S27 14.471 E31 00.144	AM	Rose petal
	48729	KU726745	Commondale	S27 14.471 E31 00.144	AM	Rose petal
	48730	KU726746	Commondale	S27 14.471 E31 00.144	AM	<i>R. indicum</i>
	48731	KU726747	Commondale	S27 14.471 E31 00.144	AM	Rose petal
	48732	KU726748	Commondale	S27 14.471 E31 00.144	AM	Rose petal
	48740	KU726760	Commondale	S27 14.574 E31 00.176	AM	Rose petal
	48743	KU726767	Commondale	S29 18.868 E30 14.138	AM	<i>R. indicum</i>
	48744	KU726770	Commondale	S27 14.558 E31 00.136	AM	Rose petal
	48745	KU726772	Commondale	S27 14.068 E31 00.585	NF	<i>H. rosa-sinensis</i>
	48748	KU726779	Commondale	S27 14.485 E31 00.141	AM	Rose petal
	48733	KU726750	Howick	S29 23.193 E30 12.627	EG	Rose petal
	48734	KU726751	Howick	S29 18.818 E30 14.143	AM	Rose petal
	48736	KU726756	Howick	S29 18.831 E30 14.158	AM	<i>D. repens</i>
	48737	KU726757	Howick	S29 18.852 E30 14.138	AM	<i>D. repens</i>
	48738	KU726758	Howick	S29 18.829 E30 14.116	AM	<i>H. rosa-sinensis</i>
	48739	KU726759	Howick	S29 23.181 E30 12.602	EG	Rose petal
	48741	KU726764	Howick	S29 23.168 E30 12.641	EG	Rose petal
	48742	KU726766	Howick	S29 18.868 E30 14.120	AM	Rose petal
	48746	KU726774	Howick	S29 23.193 E30 12.627	EG	<i>R. indicum</i>
	48747	KU726776	Howick	S29 18.690 E30 14.364	NF	Rose petal

Taxa	CMW #	GenBank #	Site	Coordinates	Vegetation type	Baits
<i>P. frigida</i>	48749	KU726784	Melmoth	S28 39.032 E31 28.511	AM	Rose petal
	48751	KU726790	Melmoth	S28 40.496 E31 25.099	NF	Rose petal
	48752	KU726791	Melmoth	S28 39.997 E31 28.486	AM	<i>E. sieberi</i>
	48753	KU726797	Melmoth	S28 39.745 E31 25.601	NF	<i>H. helix</i>
	48754	KU726800	Melmoth	S28 39.804 E31 25.650	EG	<i>H. rosa-sinensis</i>
	48755	KU726806	Melmoth	S28 39.750 E31 25.636	EG	<i>D. repens</i>
	48757	KU726815	Melmoth	S28 39.804 E31 25.650	EG	Rose petal
	48758	KU726817	Melmoth	S28 39.739 E31 25.616	EG	Rose petal
	48759	KU726819	Melmoth	S28 39.745 E31 25.601	NF	<i>H. helix</i>
	48762	KU726822	Melmoth	S28 39.959 E31 25.612	NF	Rose petal
	48750	KU726785	Vryheid	S27 38.323 E30 41.353	NF	Rose petal
	48756	KU726813	Vryheid	S27 39.057 E30 42.924	EG	Rose petal
	48760	KU726820	Vryheid	S27 39.042 E30 42.981	AM	Rose petal
48761	KU726821	Vryheid	S27 39.046 E30 43.018	AM	Rose petal	
<i>P. multivora</i>	48804	KU726744	Commondale	S27 14.503 E31 00.144	AM	Rose petal
	48806	KU726762	Commondale	S27 14.558 E31 00.136	AM	Rose petal
	48807	KU726769	Commondale	S27 14.558 E31 00.136	AM	<i>R. indicum</i>
	48805	KU726761	Howick	S29 18.658 E30 14.260	NF	Rose petal
	48808	KU726773	Howick	S29 18.868 E30 14.120	AM	<i>D. repens</i>
	48810	KU726792	Howick	S28 39.739 E31 25.616	EG	Rose petal
	48811	KU726811	Howick	S28 39.959 E31 25.612	NF	Rose petal
	48809	KU726788	Vryheid	S27 39.046 E30 42.942	EG	<i>H. rosa-sinensis</i>
	<i>P. pseudocryptogea</i>	48814	KU726754	Commondale	S27 14.574 E31 00.133	AM
48815		KU726755	Commondale	S27 14.558 E31 00.136	AM	Rose petal
48816		KU726763	Commondale	S27 14.400 E31 00.849	EG	Rose petal
48817		KU726768	Commondale	S27 14.527 E31 00.144	AM	<i>E. sieberi</i>
48818		KU726771	Commondale	S27 14.034 E31 00.601	NF	Rose petal

TABLE S2 *Phytophthora* species identified from soil samples through metabarcoding. Taxon names with symbol ‘†’ indicate new report from South Africa and ‘**’ indicates new phylotypes. Acronym EG, AM and NF are *Eucalyptus grandis*, *Acacia mearnsii* and natural forest respectively.

Taxon	Total Number of reads	Sites and vegetation types											
		Commondale			Howick			Vryheid			Melmoth		
		EG	AM	NF	EG	AM	NF	EG	AM	NF	EG	AM	NF
<i>Phytophthora frigida</i>	24759	+	+	+	+	+	+	+	+	+	+	+	+
<i>Phytophthora alticola</i>	15083		+	+	+	+	+	+	+	+	+	+	+
<i>Phytophthora parvispora</i> †	11320		+		+	+	+		+			+	+
<i>Phytophthora niederhauserii</i>	9860					+		+	+	+	+	+	+
<i>Phytophthora</i> RSA5A**	8204			+		+	+	+	+	+	+	+	+
<i>Phytophthora</i> RSA7A**	6830		+	+		+		+					+
<i>Phytophthora</i> RSA1A**	6630		+				+		+		+		
<i>Phytophthora litchii</i> †	6120			+						+			+
<i>Phytophthora</i> AUS2A †	5890	+			+		+			+	+		+
<i>Phytophthora capensis</i>	4538					+	+					+	+
<i>Phytophthora multivora</i>	4530	+	+	+	+	+	+	+	+	+	+	+	+
<i>Phytophthora cinnamomi</i>	4229	+			+		+	+		+	+		+
<i>Phytophthora pseudocryptogea</i> †	1978				+			+				+	+
<i>Phytophthora</i> RSA3A**	1674		+	+		+	+			+		+	
<i>Phytophthora</i> ‘kelmania’ †	1002			+			+			+			+
<i>Phytophthora</i> RSA2A**	288					+							
<i>Phytophthora humicola</i> †	190				+								
<i>Phytophthora</i> aff. meadii †	154	+			+								+
<i>Phytophthora cryptogea</i>	133										+		
<i>Phytophthora plurivora</i> complex	123	+		+		+		+					+
<i>Phytophthora nicotianae</i>	120		+									+	+
<i>Phytophthora</i> ‘hennops’	88							+		+			+
<i>Phytophthora gondwanense</i> †	66		+										
<i>Phytophthora asparagi</i> †	55						+						+
<i>Phytophthora elongata</i> †	37				+				+	+			+
<i>Phytophthora gregata</i> complex †	22										+		+
<i>Phytophthora</i> RSA10A**	16						+						
<i>Phytophthora citricola</i>	12												+
<i>Phytophthora</i> AUS9A †	12						+						
<i>Phytophthora palmivora</i>	11												+
<i>Phytophthora inundata</i> †	6												+
<i>Phytophthora cambivora</i> †	2												+