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

Tanay Bose[a*], Michael J. Wingfield[a], Jolanda Roux[b], Maria Vivas[a], Treena I. Burgess[a,c]

[a]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa.

[b]Department of Plant and Soil Sciences, Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa.

[c]Murdoch University, Centre for Phytophthora Science and Management, School of Veterinary and Life Sciences, Murdoch, Perth, Australia.

* Corresponding author. [a]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Phone: +27124203906; Fax: +27124203960, E-mail address: Tanay.Bose@fabi.up.ac.za

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 (Zeiljemaker 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 Commondale (Figure 1A). Howick and Commondale 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 × 3 vegetation types × 10 plots × 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 × 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 × 3 vegetation types × 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 22-25ºC and the humidity between 70-75%. Each of the soil samples was baited separately in a 24 × 14 × 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 $MgCl_2$ (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 µl). 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 µl 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 (Katoh *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 × 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 (conf = 0.95) to cluster points based on the vegetation type. To asses

differences among the four sampling sites (Commondale, Howick, Melmoth and Vryheid) and the three vegetation types (plantations of *E. grandis* and *A. mearnsii* and natural forests) on community composition, a permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) was performed using the "adonis" function and the Jaccard's dissimilarity index in the vegan package (R core Team, 2018).

To analyze oomycete diversity, *Phytophthora* species richness was calculated for each of the 12 samples. The effects of the sampling site and the vegetation type were analyzed with a generalized linear model, where the dependent variable richness fitted a Poisson distribution (R Core Team, 2018). Finally, *Phytophthora* species that were shared between vegetation types were visualized using a Venn diagram constructed in R with the VennDiagram package (R Core Team, 2018).

Results

Phytophthora *isolates recovered through baiting*

In total, 85 isolates of *Phytophthora* were recovered using baiting (Figure 1C-F; Table S1). Based on the sequence similarity search using BLAST (Altschul *et al.* 1990), the isolates represented five taxa: *P. alticola, P. cinnamomi, P. frigida, P. multivora* and *P. pseudocryptogea*. Most isolates were identified as *P. frigida* (33) and *P. cinnamomi* (32) (Table S1). Among the baits used white rose petal was the most efficient followed by *D. repens*, *E. sieberi*, *R. indicum*, *H. helix* and *H. rosa-sinensis* (Table S1).

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 influence 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

community. The sampling sites also stretched across three different climatic zones that would certainly have influenced the *Phytophthora* species richness at different sites.

Community composition of *Phytophthora* species in the present study differed among vegetation types. In particular, the community composition in the *A. mearnsii* plantations was significantly different from the natural forest and *E. grandis*. The sampled plantations were 10-15 y old and had been established on areas of cleared native vegetation. The *Phytophthora* community composition was most likely the same in both the planted forest environments (*A. mearnsii* or *E. grandis*) originally, but would have been altered over time due to difference in host plant. The differences in *Phytophthora* species found in the *A. mearnsii* plantations and in the adjacent native forest could have been due *Phytophthora* species introduced into the plantations from nurseries during the establishment phase, as commonly occurs in Europe (Jung *et al.* 2016).

The rare or new *Phytophthora* species detected in the present study were not isolated using soil-baiting, a finding echoed in other studies considering both direct baiting and metabarcoding (Vannini *et al.* 2013; Khaliq *et al.* 2018). The discrepancy between isolation success and molecular detection could be due to several factors. Metabarcoding would detect a species even if it was dead. Some species, especially those unknown in culture, could be obligate biotrophs and not culturable, as has been found for the related genus *Peronospora* (Cooke *et al.* 2002). Efficacy of baits can also influence the variety of *Phytophthora* species recovered (Cooke *et al.* 2007; O'Brien *et al.* 2009), and this might have been a factor in the present study. However, Reeser *et al.* (2011) concluded that the type of bait was not important, but rather how

it was handled. Likewise, antibiotics used in the selective media, low inoculum levels and dormant propagules could also have affected isolation success (Jeffers and Martin 1986; Drenth and Sendall 2001).

Metabarcoding allows identification of several *Phytophthora* species without isolation into culture, but it also has various limitations (Huse *et al.* 2007). The ITS1 gene region is highly variable in *Phytophthora* but it cannot separate some closely related species (Català *et al.* 2015) including, for example, *P. plurivora, P. acerina* and *P. pini* in the present study. The key limitation here is the lack of living cultures to allow for the inclusion of data for other gene regions. Additionally, the 454-platform has sequencing bias using these *Phytophthora* specific primers (Català *et al.* 2015) and thus interpretation of results such as those in the present study must be predominantly qualitative.

The known *Phytophthora* diversity in South Africa, including those revealed in this study, most likely includes both native and introduced species. This has been shown for many countries where biodiversity studies have used traditional isolation methods, including Europe and North America (Hansen *et al.* 2012), Argentina (Greslebin *et al.* 2005) and South Africa (Oh *et al.* 2013). It is also true for investigations including the present study, applying high-throughput sequencing platforms (Vannini *et al.* 2013; Català *et al.* 2015; Prigigallo *et al.* 2016; Burgess *et al.* 2017a; Català *et al.* 2017). Among the 32 *Phytophthora* species detected in the present study, *P. frigida, P. capensis, P.* 'hennops', and *P. alticola* and the newly identified species *Phytophthora* RSA1A, RSA2A, RSA3A, RSA5A, RSA7A and RSA10A are known only from South Africa (Maseko *et al.* 2002; Oh *et al.* 2013; Bose *et al.* 2017), and they could be native to the country. *Phytophthora* AUS2A, *P.*

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).

In conclusion, and in response to the proposed hypotheses, community composition of *Phytophthora* species differed significantly between the three vegetation types but not across sites. High-throughput sequencing platforms have positively influenced studies focused on species discovery and distribution of *Phytophthora* species globally. Results of the present study contribute to our knowledge of the community composition of *Phytophthora* species in South Africa. Future surveys should include many other areas of the country, such as the Cape Floristic Region in the Western Cape province, the Garden Route National Park in the Eastern Cape province and the Soutpansberg Afromontane region in the Limpopo province, where some of the world's most diverse flora occur.

Acknowledgements

We are grateful to the University of Pretoria, the members of the Tree Protection Co-operative Programme and the Department of Science and Technology - National Research Foundation Centre of Excellence in Tree Health Biotechnology for financial support. We thank Mrs. Diane White from Centre for *Phytophthora* Science and Management (CPSM), Murdoch University and Mrs. Frances Brigg from Western Australian State Agricultural Biotechnology Centre for invaluable assistance with the 454 sequencing. We are thankful to Ms. Sarah Sapford from CPSM, Murdoch University and Dr. Sarai Oliver-Espejel from FABI for their assistance with statistical analyses. We also acknowledge the assistance from Dr. Stephen Taerum, Dr. Alistair McTaggart, Mr. Jan Nagel, Mr. Conrad Trollip, Mr. Joey Hulbert and Ms. Mandy Messal with the fieldwork. Foresters from NCT Forestry Co-operative Limited and TWK-Agri provided substantial assistance with the survey work as well as selection

and access to the plantations. Dr. Alistair McTaggart, Dr. Almuth Hammerbacher and

Dr. Casper Crous kindly provided assistance in preparing this manuscript.

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, Vettraino 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.

Jung T, Burgess TI. 2009. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora sp. nov.* Persoonia 22:95.

Jung T, Orlikowski L, Henricot B, Abad-Campos P, Aday AG, Aguín Casal O, Bakonyi J, Cacciola SO, Cech T, Chavarriaga D, Corcobado T. 2016. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases. Forest Pathology 46:134-163.

Katoh K, Misawa K, Kuma KI, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059-3066.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649.

Khaliq I, Hardy GEStJ, White D, Burgess TI. 2018. eDNA from roots: a robust tool for determining *Phytophthora* communities in natural ecosystems. FEMS Microbial Ecology 94: fiy048.

Kotze JM, Moll JN, Darvas JM. 1987. Root rot control in South Africa: past, present and future. South Africa Avocado Growers' Association Yearbook 11:89-91.

Linde C, Kemp GH, Wingfleld MJ. 1994. *Pythium* and *Phytophthora* species associated with eucalypts and pines in South Africa. European Journal of Forest Pathology 24:345-356.

Masago H, Yoshikawa M, Fukada M, Nakanishi N. 1977. Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soils and plants. Phytopathology 67:425-428.

Maseko BO, Burgess TI, Coutinho TA, Wingfield MJ. 2007. Two new *Phytophthora* species from South African *Eucalyptus* plantations. Mycological Research 111:1321-1338.

Maseko BO, Coutinho TA, van Staden J. 2002. Pathogenicity of *Phytophthora* and *Pythium* species associated with citrus root rot in South Africa. South African Journal of Botany 68:327-332.

Milne DL, Brodrick HT, Hughes JP. 1975. Progress in solving the *Phytophthora* problem when replanting avocados. Citrus and Sub-tropical Fruit Journal 502:22-24.

Milne DL, Hughes JP, Brodrick HT, de Villiers EA, Frean RT. 1974. Attempts to control *Phytophthora* root rot on established avocado trees. Fruit World and Market Grower 478:5-7.

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-Ghalamfarsa 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, Vettraino 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.

Zeiljemaker FCJ. 1967. The gummosis of black wattle: a complex of disease. In: Wattle Research Institute (Pietermaritzburg, South Africa) Report. p. 40-43.

Figure legends

FIGURE 1 (A) Soil sampling sites and strategy across Mpumalanga and KwaZulu-Natal provinces of South Africa. Sampling strategy at Commondale is illustrated here as an example. List of *Phytophthora* species detected at each environment is indicated in close-up maps for each site (C) Howick, (D) Commondale, (E) Melmoth, and (F) Vryheid. In C-F, taxa names in blue bold font were recovered through both soil baiting and metabarcoding. Taxa names in green bold font were recovered only through soil baiting. Numerical within parenthesis indicates the number of isolates recovered through soil baiting. The three vegetation types are indicated as ■ =*Acacia mearnsii*, ● = *Eucalyptus grandis* and ▲=Natural forests.

FIGURE 2 Internal Transcribed Spacer 1 (ITS1) phylogenies of *Phytophthora* species recovered through metabarcoding. MOTUs that were designated as new phylotypes are highlighted in blue. MOTUs that clustered with well-defined *Phytophthora* species are highlighted in grey. Suffix HTRSA indicates MOTUs recovered through high throughput sequencing from South Africa. Although ITS1 is highly variable still some species could not be separated based on it alone. Hence, these species are grouped within the same coloured block and has been referred to as a complex throughout the article.

FIGURE 3 Non-metric multidimensional scaling (nMDS) of *Phytophthora* species identified through metabarcoding (presence-absence data), among the four sites (red=Howick, black=Melmoth, yellow=Vryheid and blue=Commondale) and the three vegetation types (■ =*Acacia mearnsii*, ● =*Eucalyptus grandis*, ▲=Natural forests). Confidence ellipses (conf=0.95) were drawn to cluster the data points based on vegetation types.

FIGURE 4 Venn diagram showing the distribution of *Phytophthora* species identified through metabarcoding among the three vegetation types.

FIGURE 1

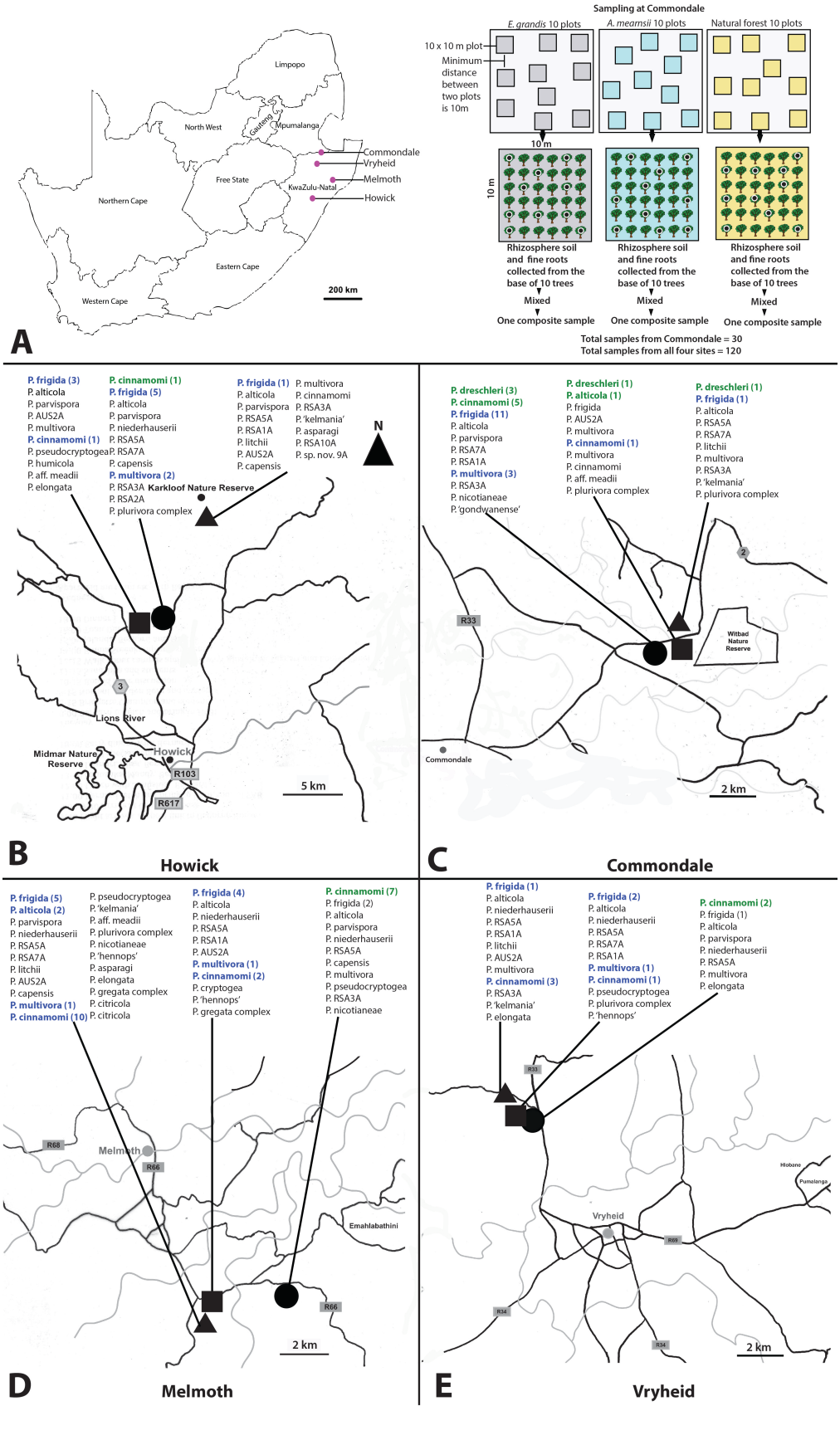

A
Limpopo
North West
Gauteng
Mpumalanga
Free State
KwaZulu-Natal
Northern Cape
Eastern Cape
Western Cape
Commondale
Vryheid
Melmoth
Howick
200 km
Sampling at Commondale
E. grandis 10 plots
A. mearnsii 10 plots
Natural forest 10 plots
10 x 10 m plot
Minimum distance between two plots is 10m
10 m
Rhizosphere soil and fine roots collected from the base of 10 trees
Mixed
One composite sample
Total samples from Commondale = 30
Total samples from all four sites = 120
B
Howick
P. frigida (3)
P. alticola
P. parvispora
P. AUS2A
P. multivora
P. cinnamomi (1)
P. pseudocryptogea
P. humicola
P. aff. meadii
P. elongata
P. cinnamomi (1)
P. frigida (5)
P. alticola
P. parvispora
P. niederhauserii
P. RSA5A
P. RSA7A
P. capensis
P. multivora (2)
P. RSA3A
P. RSA2A
P. plurivora complex
P. frigida (1)
P. alticola
P. parvispora
P. RSA5A
P. RSA1A
P. litchii
P. AUS2A
P. capensis
P. multivora
P. cinnamomi
P. RSA3A
P. 'kelmania'
P. asparagi
P. RSA10A
P. sp. nov. 9A
Karkloof Nature Reserve
N
Lions River
Midmar Nature Reserve
Howick
R103
R617
5 km
C
Commondale
P. dreschleri (3)
P. cinnamomi (5)
P. frigida (11)
P. alticola
P. parvispora
P. RSA7A
P. RSA1A
P. multivora (3)
P. RSA3A
P. nicotianeae
P. 'gondwanense'
P. dreschleri (1)
P. alticola (1)
P. frigida
P. AUS2A
P. multivora
P. cinnamomi (1)
P. multivora
P. cinnamomi
P. aff. meadii
P. plurivora complex
P. dreschleri (1)
P. frigida (1)
P. alticola
P. RSA5A
P. RSA7A
P. litchii
P. multivora
P. RSA3A
P. 'kelmania'
P. plurivora complex
R33
2
Witbad Nature Reserve
2 km
D
Melmoth
P. frigida (5)
P. alticola (2)
P. parvispora
P. niederhauserii
P. RSA5A
P. RSA7A
P. litchii
P. AUS2A
P. capensis
P. multivora (1)
P. cinnamomi (10)
P. pseudocryptogea
P. 'kelmania'
P. aff. meadii
P. plurivora complex
P. nicotianeae
P. 'hennops'
P. asparagi
P. elongata
P. gregata complex
P. citricola
P. citricola
P. frigida (4)
P. alticola
P. niederhauserii
P. RSA5A
P. RSA1A
P. AUS2A
P. multivora (1)
P. cinnamomi (2)
P. cryptogea
P. 'hennops'
P. gregata complex
P. cinnamomi (7)
P. frigida (2)
P. alticola
P. parvispora
P. niederhauserii
P. RSA5A
P. capensis
P. multivora
P. pseudocryptogea
P. RSA3A
P. nicotianeae
R68
Melmoth
R66
Emahlabathini
R66
2 km
E
Vryheid
P. frigida (1)
P. alticola
P. niederhauserii
P. RSA5A
P. RSA1A
P. litchii
P. AUS2A
P. multivora
P. cinnamomi (3)
P. RSA3A
P. 'kelmania'
P. elongata
P. frigida (2)
P. alticola
P. niederhauserii
P. RSA5A
P. RSA7A
P. RSA1A
P. multivora (1)
P. cinnamomi (1)
P. pseudocryptogea
P. plurivora complex
P. 'hennops'
P. cinnamomi (2)
P. frigida (1)
P. alticola
P. parvispora
P. niederhauserii
P. RSA5A
P. multivora
P. elongata
R33
Hlobane
Pumalanga
Vryheid
R69
R34
R34
2 km

FIGURE 2

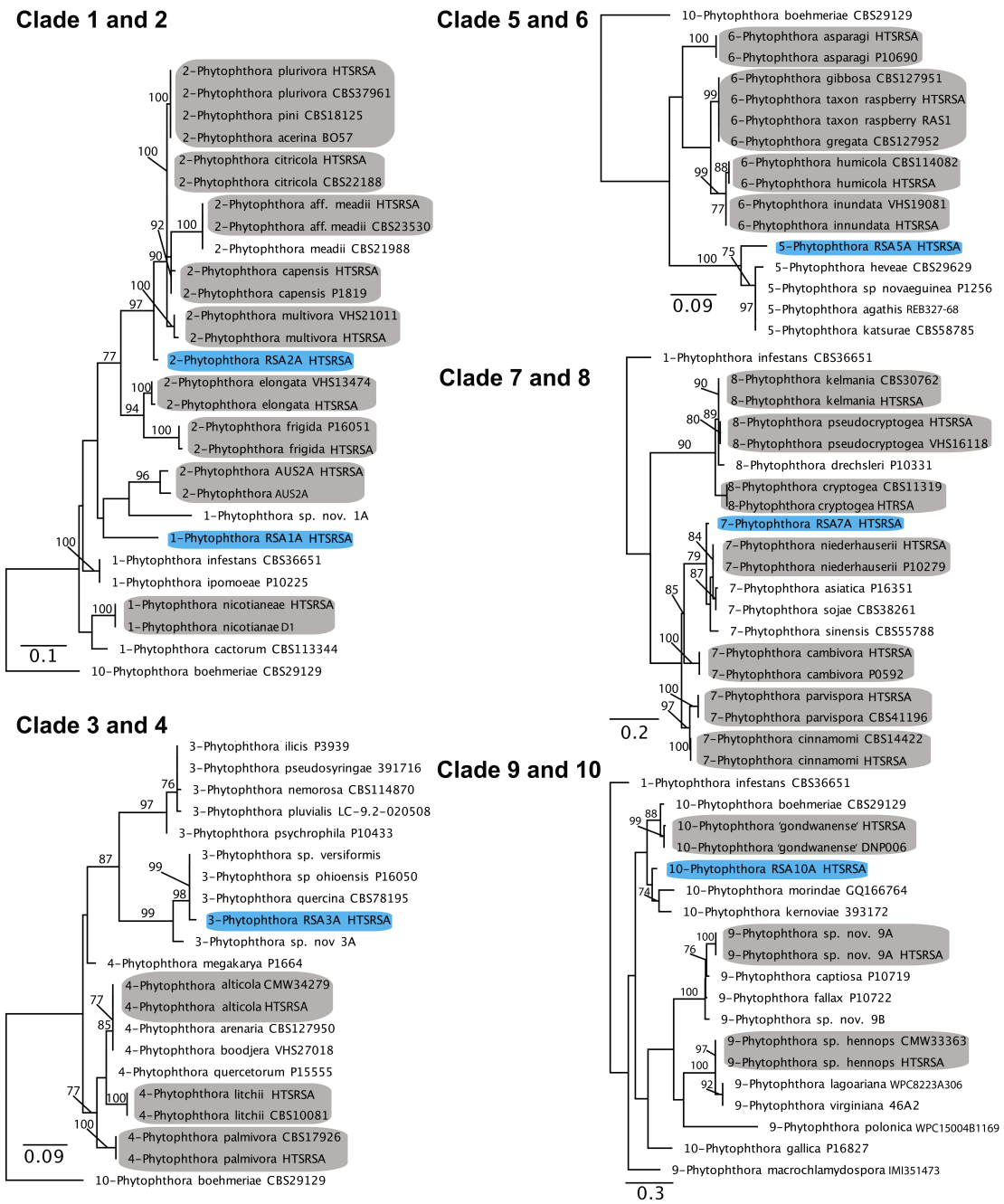

Clade 1 and 2
2-Phytophthora plurivora HTSRSA
2-Phytophthora plurivora CBS37961
2-Phytophthora pini CBS18125
2-Phytophthora acerina BO57
2-Phytophthora citricola HTSRSA
2-Phytophthora citricola CBS22188
2-Phytophthora aff. meadii HTSRSA
2-Phytophthora aff. meadii CBS23530
2-Phytophthora meadii CBS21988
2-Phytophthora capensis HTSRSA
2-Phytophthora capensis P1819
2-Phytophthora multivora VHS21011
2-Phytophthora multivora HTSRSA
2-Phytophthora RSA2A HTSRSA
2-Phytophthora elongata VHS13474
2-Phytophthora elongata HTSRSA
2-Phytophthora frigida P16051
2-Phytophthora frigida HTSRSA
2-Phytophthora AUS2A HTSRSA
2-Phytophthora AUS2A
1-Phytophthora sp. nov. 1A
1-Phytophthora RSA1A HTSRSA
1-Phytophthora infestans CBS36651
1-Phytophthora ipomoeae P10225
1-Phytophthora nicotianeae HTSRSA
1-Phytophthora nicotianae D1
1-Phytophthora cactorum CBS113344
0.1
10-Phytophthora boehmeriae CBS29129
Clade 3 and 4
3-Phytophthora ilicis P3939
3-Phytophthora pseudosyringae 391716
3-Phytophthora nemorosa CBS114870
3-Phytophthora pluvialis LC-9.2-020508
3-Phytophthora psychrophila P10433
3-Phytophthora sp. versiformis
3-Phytophthora sp ohioensis P16050
3-Phytophthora quercina CBS78195
3-Phytophthora RSA3A HTSRSA
3-Phytophthora sp. nov 3A
4-Phytophthora megakarya P1664
4-Phytophthora alticola CMW34279
4-Phytophthora alticola HTSRSA
4-Phytophthora arenaria CBS127950
4-Phytophthora boodjera VHS27018
4-Phytophthora quercetorum P15555
4-Phytophthora litchii HTSRSA
4-Phytophthora litchii CBS10081
4-Phytophthora palmivora CBS17926
4-Phytophthora palmivora HTSRSA
0.09
10-Phytophthora boehmeriae CBS29129
Clade 5 and 6
10-Phytophthora boehmeriae CBS29129
6-Phytophthora asparagi HTSRSA
6-Phytophthora asparagi P10690
6-Phytophthora gibbosa CBS127951
6-Phytophthora taxon raspberry HTSRSA
6-Phytophthora taxon raspberry RAS1
6-Phytophthora gregata CBS127952
6-Phytophthora humicola CBS114082
6-Phytophthora humicola HTSRSA
6-Phytophthora inundata VHS19081
6-Phytophthora innundata HTSRSA
5-Phytophthora RSA5A HTSRSA
5-Phytophthora heveae CBS29629
5-Phytophthora sp novaeguinea P1256
5-Phytophthora agathis REB327-68
5-Phytophthora katsurae CBS58785
0.09
Clade 7 and 8
1-Phytophthora infestans CBS36651
8-Phytophthora kelmania CBS30762
8-Phytophthora kelmania HTSRSA
8-Phytophthora pseudocryptogea HTSRSA
8-Phytophthora pseudocryptogea VHS16118
8-Phytophthora drechsleri P10331
8-Phytophthora cryptogea CBS11319
8-Phytophthora cryptogea HTRSA
7-Phytophthora RSA7A HTSRSA
7-Phytophthora niederhauserii HTSRSA
7-Phytophthora niederhauserii P10279
7-Phytophthora asiatica P16351
7-Phytophthora sojae CBS38261
7-Phytophthora sinensis CBS55788
7-Phytophthora cambivora HTSRSA
7-Phytophthora cambivora P0592
7-Phytophthora parvispora HTSRSA
7-Phytophthora parvispora CBS41196
7-Phytophthora cinnamomi CBS14422
7-Phytophthora cinnamomi HTSRSA
0.2
Clade 9 and 10
1-Phytophthora infestans CBS36651
10-Phytophthora boehmeriae CBS29129
10-Phytophthora 'gondwanense' HTSRSA
10-Phytophthora 'gondwanense' DNP006
10-Phytophthora RSA10A HTSRSA
10-Phytophthora morindae GQ166764
10-Phytophthora kernoviae 393172
9-Phytophthora sp. nov. 9A
9-Phytophthora sp. nov. 9A HTSRSA
9-Phytophthora captiosa P10719
9-Phytophthora fallax P10722
9-Phytophthora sp. nov. 9B
9-Phytophthora sp. hennops CMW33363
9-Phytophthora sp. hennops HTSRSA
9-Phytophthora lagoariana WPC8223A306
9-Phytophthora virginiana 46A2
9-Phytophthora polonica WPC15004B1169
10-Phytophthora gallica P16827
9-Phytophthora macrochlamydospora IMI351473
0.3

FIGURE 3

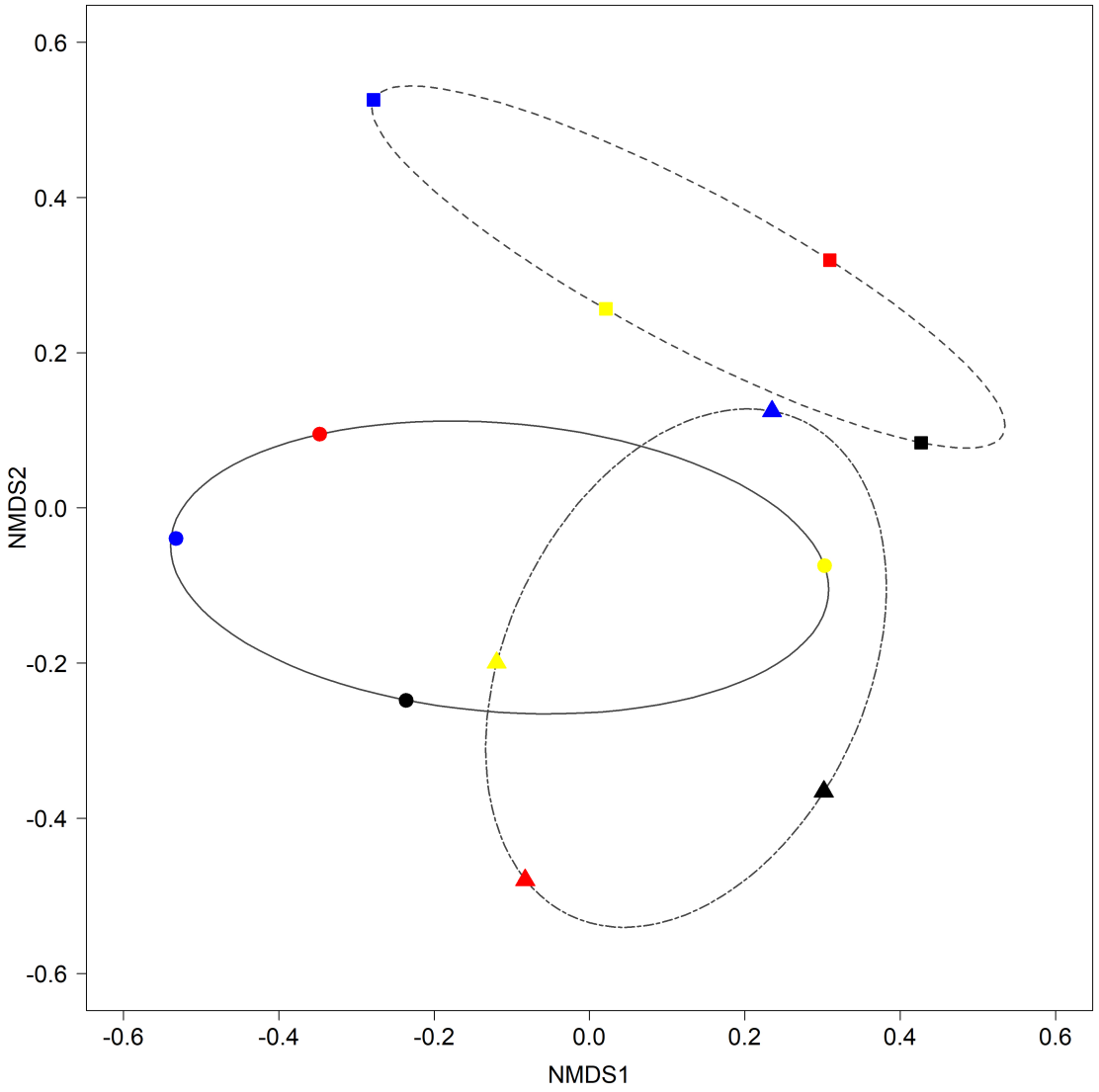
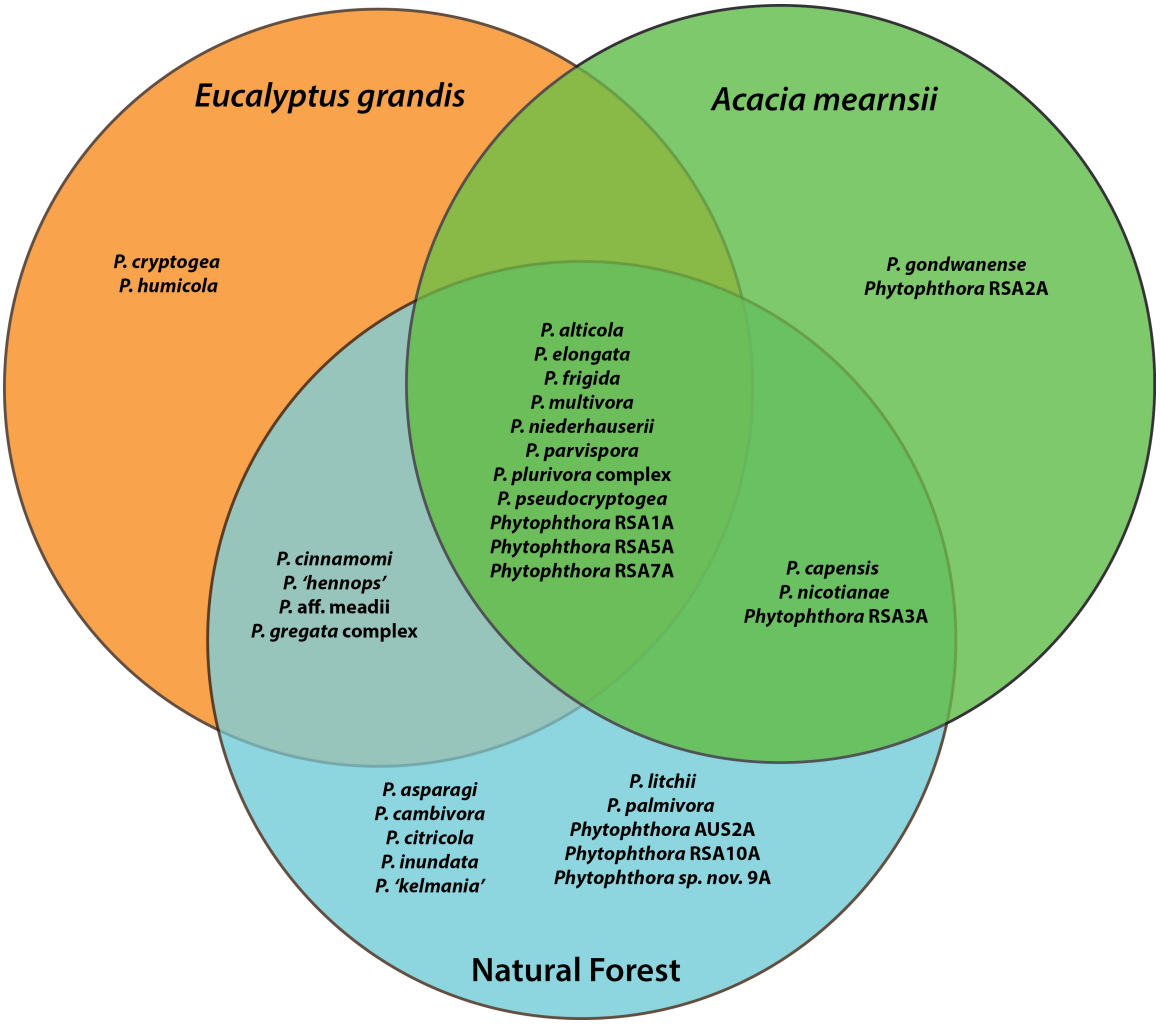


FIGURE 4


Eucalyptus grandis
Acacia mearnsii
Natural Forest
P. cryptogea
P. humicola
P. gondwanense
Phytophthora RSA2A
P. alticola
P. elongata
P. frigida
P. multivora
P. niederhauserii
P. parvispora
P. plurivora complex
P. pseudocryptogea
Phytophthora RSA1A
Phytophthora RSA5A
Phytophthora RSA7A
P. cinnamomi
P. 'hennops'
P. aff. meadii
P. gregata complex
P. capensis
P. nicotianae
Phytophthora RSA3A
P. asparagi
P. cambivora
P. citricola
P. inundata
P. 'kelmania'
P. litchii
P. palmivora
Phytophthora AUS2A
Phytophthora RSA10A
Phytophthora sp. nov. 9A

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
P. alticola	48711	**KX247599**	Commondale	S27 14.384 E31 00.904	EG	*E. sieberi*
	48712	**KX247599**	Melmoth	S28 39.919 E31 25.597	NF	Rose petal
	48713	**KX247600**	Melmoth	S28 40.505 E31 25.135	NF	*E. sieberi*
P. cinnamomi	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	*R. indicum*
	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	*H. helix*
	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	*R. indicum*
	48778	**KU726781**	Melmoth	S28 39.745 E31 25.601	NF	Rose petal
	48780	**KU726783**	Melmoth	S28 39.936 E31 25.612	NF	*H. helix*
	48781	**KU726787**	Melmoth	S28 40.505 E31 25.135	NF	Rose petal
	48782	**KU726789**	Melmoth	S28 39.032 E31 28.511	AM	*E. sieberi*
	48783	**KU726793**	Melmoth	S28 39.745 E31 25.601	EG	*H. helix*
	48784	**KU726794**	Melmoth	S28 40.498 E31 25.084	NF	Rose petal
	48785	**KU726795**	Melmoth	S28 39.910 E31 25.586	NF	*R. indicum*
	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	*H. rosa-sinensis*
	48791	**KU726803**	Melmoth	S28 39.745 E31 25.601	NF	*D. repens*
	48792	**KU726804**	Melmoth	S28 39.991 E31 28.507	AM	Rose petal
	48793	**KU726807**	Melmoth	S28 39.750 E31 25.636	EG	*E. sieberi*
	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	*D. repens*
	48797	**KU726812**	Melmoth	S28 40.498 E31 25.084	NF	*D. repens*
	48799	**KU726816**	Melmoth	S28 39.991 E31 28.507	AM	Rose petal
	48779	**KU726782**	Vryheid	S27 39.061 E30 43.049	AM	*E. sieberi*
	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	*E. sieberi*
P. frigida	48726	**KU726738**	Commondale	S27 14.471 E31 00.140	AM	*D. repens*
	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	*R. indicum*
	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	*R. indicum*
	48744	**KU726770**	Commondale	S27 14.558 E31 00.136	AM	Rose petal
	48745	**KU726772**	Commondale	S27 14.068 E31 00.585	NF	*H. rosa-sinensis*
	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	*D. repens*
	48737	**KU726757**	Howick	S29 18.852 E30 14.138	AM	*D. repens*
	48738	**KU726758**	Howick	S29 18.829 E30 14.116	AM	*H. rosa-sinensis*
	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	*R. indicum*
	48747	**KU726776**	Howick	S29 18.690 E30 14.364	NF	Rose petal

Taxa	CMW #	GenBank #	Site	Coordinates	Vegetation type	Baits
P. frigida	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	*E. sieberi*
	48753	**KU726797**	Melmoth	S28 39.745 E31 25.601	NF	*H. helix*
	48754	**KU726800**	Melmoth	S28 39.804 E31 25.650	EG	*H. rosa-sinensis*
	48755	**KU726806**	Melmoth	S28 39.750 E31 25.636	EG	*D. repens*
	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	*H. helix*
	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
P. multivora	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	*R. indicum*
	48805	**KU726761**	Howick	S29 18.658 E30 14.260	NF	Rose petal
	48808	**KU726773**	Howick	S29 18.868 E30 14.120	AM	*D. repens*
	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	*H. rosa-sinensis*
P. pseudocryptogea	48814	**KU726754**	Commondale	S27 14.574 E31 00.133	AM	Rose petal
	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	*E. sieberi*
	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
Phytophthora frigida	24759	+	+	+	+	+	+	+	+	+	+	+	+
Phytophthora alticola	15083		+	+	+	+	+	+	+	+	+	+	+
Phytophthora parvispora †	11320		+		+	+	+		+			+	+
Phytophthora niederhauserii	9860					+		+	+	+	+	+	+
Phytophthora RSA5A**	8204			+		+	+	+	+	+	+	+	+
Phytophthora RSA7A**	6830		+	+		+		+					+
Phytophthora RSA1A**	6630		+				+	+		+	+		
Phytophthora litchii †	6120			+			+			+			+
Phytophthora AUS2A †	5890	+			+		+			+	+		+
Phytophthora capensis	4538					+	+					+	+
Phytophthora multivora	4530	+	+	+	+	+	+	+	+	+	+	+	+
Phytophthora cinnamomi	4229	+			+		+	+		+	+		+
Phytophthora pseudocryptogea †	1978				+			+				+	+
Phytophthora RSA3A**	1674		+	+		+	+			+		+	
Phytophthora 'kelmania' †	1002			+			+			+			+
Phytophthora RSA2A**	288					+							
Phytophthora humicola †	190				+								
Phytophthora aff. meadii †	154	+			+								+
Phytophthora cryptogea	133										+		
Phytophthora plurivora complex	123	+		+		+		+					+
Phytophthora nicotianeae	120		+									+	+
Phytophthora 'hennops'	88							+			+		+
Phytophthora gondwanense †	66		+										
Phytophthora asparagi †	55						+						+
Phytophthora elongata †	37				+				+	+			+
Phytophthora gregata complex †	22										+		+
Phytophthora RSA10A**	16						+						
Phytophthora citricola	12												+
Phytophthora AUS9A †	12						+						
Phytophthora palmivora	11												+
Phytophthora inundata †	6												+
Phytophthora cambivora †	2												+