Phytophthora species associated with roots of native and non-native trees in natural and managed forests

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ABSTRACT

Roots act as a biological filter that exclusively allows only a portion of the soil-associated microbial diversity to infect the plant. This microbial diversity includes organisms both beneficial and detrimental to plants. *Phytophthora* species are amongst the most important groups of detrimental microbes that cause various soil-borne plant diseases. We used a metabarcoding approach with *Phytophthora*-specific primers to compare the diversity and richness of *Phytophthora* species associated with roots of native and non-native trees, using different types of soil inocula collected from native and managed forests. Specifically, we analysed (i) roots of two non-native tree species (*Eucalyptus grandis* and *Acacia mearnsii*) and native trees, (ii) roots of two non-native tree species from an *in vivo* plant baiting trial, (iii) roots collected from the field versus those from the baiting trial, and (iv) roots and soil samples collected from the field. The origin of the soil and the interaction between root and soil significantly influenced *Phytophthora* species richness. Moreover, species richness and community composition were significantly different between the field root samples and field soil samples with a higher number of *Phytophthora* species in the soil than in the roots. The results also revealed a substantial and previously undetected diversity of *Phytophthora* species from South Africa.

KEYWORDS: Community composition, forestry, metabarcoding, species diversity, South Africa

INTRODUCTION

Plants live in a close association with the soil microbial community comprised of organisms including fungi, actinomycetes, bacteria, algae, nematodes, protozoa, viruses, and oomycetes. The rhizosphere represents a greater reservoir of microbial diversity in contrast to the remainder of the soil [1,2]. The interface between the roots and the soil accommodates a dynamic interplay between beneficial and detrimental organisms [3,4]. Rhizobacteria and mycorrhizae are examples of beneficial microbes while species of *Fusarium* and *Rhizoctonia* are detrimental to the plant health. For a plant to grow and survive in a non-native environment, it requires an association with beneficial microbes, such as is found in the *Pinus* – mycorrhizal fungus symbiosis [5].

Phytophthora species represent a distinct lineage of mycelial fungus-like microorganisms residing in the class Oomycota (water moulds). Since their first discovery, almost all *Phytophthora* species have been designated as plant pathogens [6]. However, various lines of evidence suggest that some *Phytophthora* species are successful saprotrophs [7-9], while others survive asymptomatically within host plants [10,11]. This is perhaps not surprising because oomycetes have been associated with plants for as long as they have been on earth [12,13] and their associations would logically include a diversity of lifestyles.

In South Africa, *Phytophthora* was first reported in 1913, as a pathogen causing crown rot of rhubarb. The pathogen was identified as *Phytophthora nicotianae* (published as *Phytophthora parasitica* var. *rhei*) [14]. Subsequently, several *Phytophthora* diseases have been recorded on agricultural and horticultural plants [15], as well as on commercially propagated non-native trees such as *Eucalyptus*, *Acacia* and *Pinus* [16-18]. *Phytophthora cinnamomi* causes a serious disease of native plants in the Cape Floristic Region of South Africa [19-21]. Yet, despite the growing importance of *Phytophthora* diseases on the African

continent, knowledge regarding the species diversity of these pathogens in both natural ecosystems and cultivated lands remains relatively limited [22-24].

Metabarcoding methods have significantly improved our ability to study community composition, diversity, and interaction between soil-inhabiting microbes and plants [25-29]. In a recent *Phytophthora* barcoding study [30], the authors recommended: (i) environmental sequencing as a preferable tool to catalogue *Phytophthora* diversity than soil-baiting, and (ii) field-collected roots and those from *in vivo* live plant baiting were excellent resources for *Phytophthora* diversity studies. However, none of the previous studies [22,31-35,29] has compared the diversity of *Phytophthora* species between: (i) plant roots from native and managed forests, and (ii) between soil and roots collected from these environments. Therefore, in this study, we used *Phytophthora*-specific primers coupled with pyrosequencing to bridge these gaps. We hypothesized that the diversity of *Phytophthora* species associated with the root systems of trees depends on both the tree species and the soil in which their roots develop. Specifically, we compared the community composition and richness of *Phytophthora* species associated with (i) roots of two non-native plantation species (*Eucalyptus grandis* and *Acacia mearnsii*) and native trees in the field, (ii) roots of two non-native plantation species (*E. grandis*, *A. mearnsii*) in an *in vivo* plant baiting trial, (iii) roots collected in the field and roots in an *in vivo* plant baiting trial and (iv) roots and soil collected in the field.

MATERIALS AND METHODS

Collection of root samples and inoculum soil

Root samples and soil to be used as inoculum were collected from three vegetation types (1) plantations of *E. grandis*, (2) plantations of *A. mearnsii* and (3) from adjacent

natural forests (data on native trees in Bose et al. [22]) at four sites in South Africa in March 2016. These sites were near Howick, Melmoth, Vryheid, and Commondale (near Paulpietersburg) in the Mpumalanga and KwaZulu-Natal Provinces (3 vegetation types × 4 sites; Figure. S1).

For each vegetation type per sampling site, two plots of 20 m × 20 m and 10 trees per plot were selected (3 vegetation types × 4 sites × 2 plots × 10 trees). Each plot within a vegetation type was located at least 20 m distant from the others. To collect the samples, about 4-5 cm of the topsoil was removed around the bases of the trees. Each sample per tree consisted of fine roots and rhizosphere soil, respectively. The root samples from the 10 trees per plot were mixed as one composite root sample per plot. The soil samples from 10 trees per plot within a vegetation type were mixed and 5 kg of this collection served as one composite soil sample per plot. Finally, a total of 24 composite root and 24 composite soil samples were collected (4 sites × 3 vegetation types × 2 plots; Figure. S2). The root samples were processed for metabarcoding and the soil samples were used as inoculum for an *in vivo* plant baiting trial.

In vivo plant baiting trial

Seeds of *E. grandis* and *A. mearnsii* were sourced from forestry companies in South Africa and these were germinated on sterile vermiculite. Three-week-old seedlings were then transferred to plastic potting bags containing one kilogram of the soil collected from the sampling sites. The soil collected from the field was used as natural inoculum for the three-week-old seedlings. Each of the 24 soil samples was divided into four parts, two each for *E. grandis* and *A. mearnsii* seedlings (Figure. S2). The seedlings were allowed to grow in these soils for five months in a phytotron. The temperature and relative humidity of the phytotron

was maintained at 19-21°C and 70-75% respectively. All of the plants were irrigated daily to water capacity for five months, after which roots were harvested from each seedling.

Processing of root samples

Root samples collected from the field and from the *in vivo* plant baiting trial were rinsed with sterilized deionized water to remove the soil and dried using paper towels. The roots were finely chopped and stored at -80°C until DNA extraction. These roots were then subjected to metabarcoding to detect the presence of *Phytophthora* species.

Metabarcoding analysis

DNA was extracted from 0.5 g of field-collected roots as well as those from the *in vivo* plant baiting trial using MOBIO PowerPlant® Pro DNA Isolation Kit (Carlsbad, CA) following the manufacturer's protocols. During extraction of DNA, for every batch of samples, two tubes of 0.5 g autoclaved fine sand served as controls (4 batches × 2 control samples/batch). The amplicon libraries for pyrosequencing were generated using a nested PCR approach [33]. In the first round of PCR, the DNA samples were amplified using the *Phytophthora*-specific primers 18Ph2F/5.8S-1R [33,36]. In the second round, the products from the first set of PCRs were re-amplified with fusion primer following the unidirectional sequencing protocol for library preparation [22,31]. In these reactions, ITS6 was used as the forward primer. The reverse primer 5.8S-1R was a fusion primer that allowed post-sequencing sample identification. Each fusion primer was attached to a pyrosequencing adaptor and a multiplex identifier (MID) [33]. Each sample along with controls was assigned a MID.

Agarose gel electrophoresis was used to visualize the PCR products. To standardize the DNA contribution for each sample, amplicons were pooled in groups based on band intensity. Each of the amplicon groups was cleaned twice with Agencourt AMPure XP PCR purification beads (Beckman Coulter Genomics, USA), after which, the amplicon groups were re-visualized on an agarose gel. The amplicon groups were further pooled into a single sample based on the band intensity. This final pooled sample was diluted to 1/5000 of the original concentration. 50 µl of this diluted sample was again cleaned with AMPure XP beads.

The amplicon libraries 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 raw pyrosequencing data were deposited in the NCBI Sequence Read Archive (https://submit.ncbi.nlm.nih.gov/subs/sra/) under the accession number PRJNA412577.

Identification of the MOTUs

Preliminary identification of molecular operational taxonomic units (MOTUs) was done using sequence similarity searches against a reference database that was comprised of complete ITS1 sequences from 192 *Phytophthora* taxa. This database included both formally described and undescribed (but designated) *Phytophthora* taxa (available through Centre for Phytophthora Science & Management, Murdoch University).

For the purpose of phylogenetic analyses, all MOTUs were separated into clades. Thereafter, the MOTUs for every taxon were aligned separately and the consensus sequences were extracted. These consensus sequences were used for downstream phylogenetic analyses. The ITS1 database was divided into four groups (i) clades 1 and 2, (ii) clades 3 and 4, (iii) clades 5 and 6, (iv) clades 7, 8 and 9 to achieve the best possible resolution within each clade.

Clade 10 was excluded because none of the MOTUs corresponded to this ITS group when preliminary identifications were made. All four datasets were aligned using MAFFT [37]. RAxML v8 [38] was used for tree construction using a maximum likelihood (ML) approach. The general time-reversible model along with gamma distribution (GTR GAMMA) was selected using jModelTest 2.1 [39,40]. Fifty replicated likelihood searches were executed for each dataset followed by 1000 bootstrap replicates. Resultant trees were rooted and modified using FigTree v1.4 and Adobe Illustrator CS6.

Additional data considered in the analysis

To compare the community composition and richness of *Phytophthora* species associated with roots and soil collected from the field (objective iv) we used the soil data from a previous study [22]. In that study, we used the same metabarcoding approach explained above, to investigate the *Phytophthora* species associated with soil from the same plantations of *E. grandis* and *A. mearnsii* and adjacent natural forests resampled in the present study. The data from the study by Bose et al. [22] is available at NCBI Sequence Read Archive under the accession number PRJNA412472.

Statistical analyses

The numbers of *Phytophthora* taxa found in the (i) roots of *E. grandis*, *A. mearnsii*, and native trees from the field, (ii) roots of *E. grandis* and *A. mearnsii* from the *in vivo* plant baiting trial, (iii) roots from the field and roots from the *in vivo* plant baiting trial and (iv) roots and soil from the field were visualized in Venn diagrams. The "VennDiagram" package in the R software [41] was used to construct the Venn diagrams and edited using Adobe Illustrator CS6.

To analyze *Phytophthora* species richness, the number of taxa per sample was calculated. The effect of root and soil types on species richness was analyzed with linear mixed models. The explanatory variables were (i) root type from the field (*E. grandis***,** *A. mearnsii* or native tree), (ii) root type from the *in vivo* trial (*E. grandis***,** *A. mearnsii*), soil used as inoculum (*E. grandis***,** *A. mearnsii* and native tree) and their interaction, (iii) root type (field and *in vivo* trial) and (iv) sample type (roots from the field and soil from the field). To include the among-block variation in the study, the effect of site was considered as a random effect in all models. The "lme4" package of the R software (R Core Team, 2018) was used to analyze species richness.

The *Phytophthora* taxa community composition for the different root and soil types (objectives i-iv) was analyzed visually with Principal Coordinate Analysis (PCoA) using Jaccard distance. Statistical assessment of differences in *Phytophthora* community composition between types of root and/or soil samples was carried out using permutational multivariate analysis of variance (PERMANOVA). The explanatory variables used for PERMANOVA were the same as those used to analyze species richness. A permutational multivariate analysis of dispersion [PERMDISP, 42] was used to compare among-group differences in the distance from observations to their group centroid (dispersion). PERMDISP shows whether the differences are differences in dispersion and not differences in community structure when the PCoA fails to provide evidence of a pattern but the PERMANOVA is statistically significant and the value of *R* not very large [43]. The "vegan" package of the R software (R Core Team, 2018) was used for PCoA, PERMANOVA and PERMDISP.

RESULTS

Phytophthora taxa identified

The pyrosequencing run generated 88,871 reads (about 74% of the wells provided good quality reads) corresponding to 174 Molecular Taxonomic Units (MOTUs). The average length of the reads was 282.5 bp and 95.3% of the reads could be allocated to *Phytophthora* taxa. The remaining 4.7% of the reads were chimaeras and incomplete sequences. Chimaeras were identified and discarded after generating consensus alignments for MOTUs for each barcode.

Phytophthora taxa were detected from both field-collected root samples as well as those from the *in vivo* plant baiting trial. Clustering of the MOTUs and phylogenetic identification revealed 27 distinct *Phytophthora* taxa residing in 9 of the 11 clades found within the *Phytophthora* phylogeny [44]. Most of the MOTUs represented well-defined taxa but seven represented informally described taxa (Table. S1; Figure. S3). Among the 27 *Phytophthora* taxa detected from roots using metabarcoding, the greatest number of reads were for *P. frigida* followed by *P. cinnamomi, P. multivora*, *P. thermophila* and *P. alticola*. There were seven other *Phytophthora* species including *P. amnicola, P. crassamura, P. constricta, P. kwongonina*, *P. rosacearum, P. rubi* and *P. thermophila* that have not previously been recorded in South Africa (Table. S1).

Phytophthora taxa associated with roots of non-native and native trees in the field

Roots samples collected from *E. grandis, A. mearnsii* and native trees in the field revealed a total of 14 *Phytophthora* taxa, six of which were shared between the three types of roots (Figure. 1a). Only three, two and one taxa were exclusively found in root samples of *E. grandis*, *A. mearnsii* and native trees, respectively (Figure. 1a). Species richness and

composition were not significantly different between the field roots of the three vegetation types (*E. grandis, A. mearnsii* non-native plantations, and native trees) (Table. 1 and 2; Figure. 1b).

Phytophthora taxa associated with roots of non-native trees in a plant baiting trial

Roots samples collected from *E. grandis* plants in the *in vivo* plant baiting trial revealed the presence of 16 *Phytophthora* taxa (Figure. 2a). Of these taxa, five were present in *E. grandis* roots independent of the source of the soil substrate used as inoculum (Figure. 2a). Moreover, six were present only in *E. grandis* roots planted in soil from *E. grandis* plantations and one taxon was exclusively found in *E. grandis* roots planted in soil from the natural forest (Figure. 2a).

A total of 20 *Phytophthora* taxa were found in *A. mearnsii* roots in the *in vivo* plant baiting trial and only four taxa were shared by *A. mearnsii* roots independently of the soil substrate used (Figure. 2b). The number of *Phytophthora* taxa on *A. mearnsii* roots was different depending on the soil in which the plants were grown; with ten, four and two taxa found in roots planted in *E. grandis*, *A. mearnsii* and native trees soil, respectively (Figure. 2b).

Phytophthora species richness in roots was significantly influenced only by the soil type in which the plants were grown (Table. 1). Specifically, roots (*E. grandis* and *A. mearnsii*) planted in soil from *E. grandis* plantations showed higher species richness than those planted in *A. mearnsii* soils or natural forest soils (mean ± S.E.: 5.9 ± 1.1, 3.1 ± 0.4, 2.6 ± 0.3, respectively). The PCoA plot did not provide a pattern in community composition (Figure. 2c). However, PERMANOVA showed that the soil in which plants were grown and the interaction between the roots and the soil (root × soil), influenced *Phytophthora* community composition (Table. 2). This influence on *Phytophthora* community composition

accounted for a low percentage of the statistical variance (low r^2; Table. 2). In this situation, PERMDISP demonstrated that the differences shown by the PERMANOVA (Table. 2) were due to significant differences in the dispersion of the samples (F = 3.628; P =0.035 and F = 4.101, P > 0.004, source of soil and interaction root × soil, respectively) and not by the *Phytophthora* community composition structure.

Phytophthora **taxa associated with roots in the field and roots in the baiting trial**

The roots collected in this study (roots in the field and roots in the baiting trial, independently of the tree species) revealed the presence of 27 *Phytophthora* taxa. Of these, 13 taxa were present both in roots collected in the field and roots from the *in vivo* plant baiting trial, 13 taxa were found only in roots from the baiting trial and one taxon was found in roots from the field (Figure. 3a).

Phytophthora species richness was not influenced by the origin of the roots (field or *in vivo* trial; Table. 1) Neither PCoA plot indicated a pattern in community composition, i.e. there were no significant differences in the *Phytophthora* community composition between roots from the field or the *in vivo* trial (Figure. 3b). PERMANOVA suggested a significant effect of root type (field and *in vivo* trial) on *Phytophthora* community composition (low r^2, Table. 2). This influence suggested by PERMANOVA can also be caused by differences in the dispersion of the groups (PERMDISP; F = 20.279; P < 0.001). However, due to the unequal sample size (n=24 field roots and n=48 *in vivo* trial roots), it is not possible to conclude that root type had a significant effect on *Phytophthora* community composition. This significant effect might arise from the dispersion of samples alone (PERMDISP) or both dispersion and centroid differences (PERMANOVA, differences between the two groups of roots).

Phytophthora taxa associated with roots and soil in the field

Root and soil samples collected from the three vegetation types in the field revealed the presence of 36 *Phytophthora* taxa (Table. S1). Four of these were found only in root samples and 22 were identified only in soil samples (Table. S1; Figure. 4a). *Phytophthora* species richness was significantly lower in root samples (4.9 ± 1.4, mean ± S.E.) than in soil samples (11.3 ± 0.4, mean ± S.E.) (Table. 1). Moreover, the PCoA plot supported a difference in *Phytophthora* community composition between the root and soil samples from the field (Figure. 4b), and this difference was significantly confirmed by the PERMANOVA (Table. 2).

DISCUSSION

We compared the community composition and richness of *Phytophthora* taxa associated with roots of two non-native tree (*E. grandis* and *A. mearnsii*) and native trees, using a pyrosequencing platform. Roots act as biological filters that exclusively allow a portion of the soil-associated microbial diversity to infect a plant. Therefore, we compared the diversity data from this study (roots) with data for soils samples collected from the same areas sampled [22] a year earlier. Statistical analyses showed that: (i) the community composition of *Phytophthora* taxa associated with the field roots did not differ significantly between three vegetation types; (ii) in the *in vivo* plant baiting trail, the origin of soil and the interaction between root and soil significantly influenced the *Phytophthora* species richness; and (iii) species richness and composition of *Phytophthora* taxa were significantly different between the field root and soil samples. We also reconfirmed the fact that: (i) *Phytophthora* species are associated with the root systems in asymptomatic hosts; and (ii) a significant proportion of the *Phytophthora* species present in the soil are not likely to infect plants, most probably due to host preference.

***Phytophthora* taxa associated with roots of two non-native plantation tree species and native trees in the field**

The *Phytophthora* community composition of the roots did not differ significantly between three vegetation types considered in this study. These results concur with those of our previous study [22], where the vegetation type was also not a significant factor influencing the diversity of *Phytophthora* taxa in the soil. A substantial diversity of *Phytophthora* taxa was shared between the roots collected from three vegetation types. This suggests that many of the *Phytophthora* species detected have a broad-host-range and this is well documented for many *Phytophthora* species [45,46,10,47].

Amongst the *Phytophthora* species detected from the roots of trees representing the three vegetation types, there were at least four taxa that were not present in the soil [22]. This indicates possible sources of *Phytophthora* inoculum other than the plantation soil, one of which could be commercial nurseries that supplied the non-native tree species. In this regard, previous studies have shown that commercial nurseries are an important source for the dispersal of *Phytophthora* inoculum [11,48-50].

A high level of *Phytophthora* diversity was found associated with trees in natural forests (22) that were resampled in the present study. The results of both studies show that natural forests are a likely source of *Phytophthora* species for adjacent plantations of non-native tree species. This was apparent because a significantly higher number of reads for *P. thermophila* were detected from the field-collected roots of native trees as well as the roots of non-native trees used for baiting the natural forest soils.

The overall *Phytophthora* species richness was relatively low in the field-collected roots compared to soil samples. This result is in contrast to those of Khaliq et al. [30], where the highest number of *Phytophthora* phylotypes were detected in fine roots collected from urban parks in Western Australia. This could be explained by the argument of Barber et al.

[51] that urban environments in Western Australia are known to harbour a higher diversity of *Phytophthora* species. This recorded diversity could be attributed to rigorous sampling in these areas, conducive environmental conditions and the fact that landscaping with nursery plants and associated soil is used.

Phytophthora **taxa associated with roots of two non-native plantations in an *in vivo* plant baiting trial**

A greater number of *Phytophthora* taxa were recovered from the roots of *A. mearnsii* than *E. grandis* in the *in vivo* baiting trial. This is consistent with the fact that roots can stimulate the growth and germination of *Phytophthora* propagules (oospores and chlamydospores), leading to successful infection [30]. Such stimulation of *Phytophthora* propagules is due to the organic compounds secreted by living plant roots into the surrounding environment [52,2]. Because *A. mearnsii* and *E. grandis* considered in this study are distantly related plants [53], the biochemical composition of their root exudates would be different. Consequently, the root exudates of *A. mearnsii* could have attracted a higher diversity of *Phytophthora* taxa than those of *E. grandis*. This would be in contrast to the fact that some *Acacia* species have the potential for the biological control of *P. cinnamomi* [54-57] and where a lower diversity of *Phytophthora* species would have been associated with *A. mearnsii* roots. The biological basis for our *A. mearnsii* results requires further study.

Phytophthora **taxa associated with roots in the field and roots in an *in vivo* plant baiting trial**

A greater number of exclusive *Phytophthora* taxa were detected in the root samples originating from the *in vivo* plant baiting trial than those from the field. The *in vivo* plant baiting trial simulated situations where: (i) the natural forests and grasslands are cleared to establish a new plantation (non-native trees grown in the natural forest soil), and (ii)

plantation of one non-native tree species is replaced by another (*E. grandis* growing in soil collected from *A. mearnsii* plantation and *vice versa*). The results of this trial suggested a relatively large diversity of *Phytophthora* taxa reside dormant in the soil and that they are activated when conditions become favourable for them. In the *in vivo* plant baiting trial, favourable temperature, humidity and regular irrigation could have interrupted the dormancy of those *Phytophthora* species triggering the infection of the seedlings [58].

The *in vivo* baiting trial showed that the roots of *E. grandis* and *A. mearnsii* growing in the natural forest soil had a lower number of *Phytophthora* reads than those growing in soil collected from established plantations. This was possibly due to *Phytophthora* inoculum in virgin natural forest soil being lower than that in the plantations. Such a situation could emerge from successive rotations of trees being planted on the same land. This would be consistent with examples from agriculture environments such as the 'apple replant disease' [59], and 'take-all' disease of wheat [60] where regular replanting with same or closely related species has a deleterious effect on plant health due to the microbial build-up [4,1].

Phytophthora **taxa associated with roots and soil collected from the field**

Plant roots release a wide range of signaling agents that allow for the selective infection of soil-inhabiting microbes [61]. This interaction between roots and microbes has been studied extensively in mycorrhizae [62-64] and *Rhizobium* [65]. Results of the present study revealed a significant difference in the *Phytophthora* community composition and richness between the field-collected soil and root samples collected at the same location. For all vegetation types, the diversity of *Phytophthora* taxa was higher in the soil than in the roots. This result is consistent with those of the *in vivo* baiting trial where a greater number of *Phytophthora* taxa was recovered from baited soil than from field roots. The difference in diversity of *Phytophthora* species between the roots and soil samples was remarkable. There

are two possible explanations for this result: (i) the roots acted as a biological filter allowing only a portion of the total *Phytophthora* diversity in the soil to infect them, and (ii) those *Phytophthora* species that were simultaneously recovered from soil and root samples were viable while the remaining species were either dead or dormant.

The high-throughput sequencing platform used in this study negated the possibility to verify the biological status (living/dead/dormant) of soil-associated *Phytophthora* taxa. It is possible that a substantial diversity of the *Phytophthora* species detected were either dead or dormant. As demonstrated by Khaliq et al. [30], a future approach would be to consider barcoding of the 'filtered bait water' rather than using soil. This strategy would allow barcoding of DNA originating from zoospores, which would substantially lower the chances of detecting biologically inactive *Phytophthora* species.

CONCLUSIONS

The present study compared the community composition and species richness for *Phytophthora* taxa associated with roots of native and non-native trees in plantation areas in South Africa. Results revealed a substantial number of *Phytophthora* taxa previously unknown from South Africa. Further studies considering different ecosystems in the country will most likely reveal an even greater diversity of these potentially important organisms. This and other diversity studies [31,66,15,67,68] across a broad range of environments highlights the need to reconsider long-held views regarding the biology of *Phytophthora*. This group of Oomycota is currently considered to be predominantly plant pathogenic, but their detection in the absence of symptoms suggests they may also have other ecological roles.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table legends

TABLE 1. Results of the linear mixed model for the analysis of the effects of root and soil types on *Phytophthora* species richness. The explanatory variables and results for each group of interactions are shown: (i) root type from the field (*Eucalyptus grandis***,** *Acacia mearnsii* or native tree), (ii) root type from the *in vivo* plant baiting trial (*E. grandis***,** *A. mearnsii*), soil used as inoculum (*E. grandis***,** *A. mearnsii* and native tree) and their interaction, (iii) root type (field and *in vivo* trial) and (iv) sample type (roots from the field and soil from the field). Each group of interactions match with the objectives of the study. Degrees of freedom (df) and *F*-ratios are shown. Significant *P*-value is indicated in bold ($P < 0.05$).

TABLE 2. Results of the PERMANOVA for the analysis of the effects of root and soil types on *Phytophthora* species composition. The explanatory variables and results for each group of interactions are shown: (i) root type from the field (*Eucalyptus grandis***,** *Acacia mearnsii* or native tree), (ii) root type from the *in vivo* plant baiting trial (*E. grandis***,** *A. mearnsii*), soil used as inoculum (*E. grandis***,** *A. mearnsii* and native tree) and their interaction, (iii) root type (field and *in vivo* trial) and (iv) sample type (roots from the field and soil from the field). Each group of interactions match with the objectives of the study. Degrees of freedom (df) and *F*-ratios are shown. Significant *P*-value is indicated in bold ($P < 0.05$).

Supplementary table legend

TABLE S1. A list of *Phytophthora* species detected through environmental sequencing of soil and root samples from South Africa (present and a previous study (Bose et al. 2018)). Data for all first reports for formally described taxa were sourced from IDphy (https://idtools.org/id/phytophthora/).

Figure legends

FIGURE 1. The richness and community composition of *Phytophthora* taxa associated with roots of two non-native plantations (*Eucalyptus grandis* and *Acacia mearnsii*) and native trees in the field. (a) Venn diagram, (b) Principal Coordinate Analysis (PCoA).

FIGURE 2. The richness and community composition of *Phytophthora* taxa associated with roots of two non-native plantations (*Eucalyptus grandis*, *Acacia mearnsii*) in an *in vivo* plant baiting trial. (a) Venn diagram of *E. grandis* roots, (b) Venn diagram of *A. mearnsii* roots and (c) Principal Coordinate Analysis (PCoA).

FIGURE 3. The richness and community composition of *Phytophthora* taxa associated with roots in the field and roots in an *in vivo* plant baiting trial. (a) Venn diagram, (b) Principal Coordinate Analysis (PCoA).

FIGURE 4. The richness and community composition of *Phytophthora* taxa associated with roots in the field and soil in the field. (a) Venn diagram, (b) Principal Coordinate Analysis (PCoA).

Supplementary figure legends

FIGURE S1. Soil and root collection sites across Mpumalanga and KwaZulu-Natal provinces of South Africa. (A, B) Soil and root samples were collected from four sites spanning across KwaZulu-Natal and Mpumalanga Provinces of South Africa; Close-up maps of the sampling sites at (C) Howick, (D) Vryheid, (E) Melmoth, and (F) Commondale. For

each close-up map of the collection sites, pointers in blue = *Eucalyptus grandis*, red = *Acacia mearnsii*, and purple = natural forest. Bar equals to 100 m.

FIGURE S2. Flowchart for soil and root sampling at each collection sites. At each site, a total of 12 soil and root samples (six each) were collected. For the field-collected root samples, amplicon library was directly prepared from the total genomic DNA. Each soil samples was divided into two parts and baited with sterile grown seedlings of *Eucalyptus grandis* and *Acacia mearnsii*. After five months of incubation, the roots were harvested from each seedling followed by preparation of amplicon library. In total, we sequenced 72 samples (18 samples per collection site × 4 sites) from four collection sites.

FIGURE S3. Maximum likelihood phylogenies using complete ITS1 gene region for *Phytophthora* species recovered from metabarcoding of root samples. The consensus sequence of MOTUs for each taxon was used for these five analyses. Suffix HTRSA indicates MOTUs recovered from the present study. Taxa names in pink font indicate new reports from South Africa. Numerical on the branches show bootstrap value >70%.

TABLE 1.

Interactions tested	Explanatory variable	df	*F*-ratio	*P* value
(i)	Root type	2	1.031	0.375
(ii)	Root type	1	0.103	0.751
	Source of soil	2	6.746	**0.003**
	Root × soil	2	0.551	0.580
(iii)	Root type	1	2.095	0.151
(iv)	Sample type	1	36.892	**< 0.001**

TABLE 2.

Interactions tested	Explanatory variable	df	*F*-ratio	r^2	*P* value
(i)	Root type	2	1.434	0.120	0.184
(ii)	Root type	1	1.701	0.033	0.109
	Source of soil	2	2.551	0.099	**0.004**
	Root × soil	2	2.321	0.090	**0.004**
(iii)	Root type	1	4.956	0.068	**< 0.001**
(iv)	Sample type	1	8.365	0.197	**< 0.001**

FIGURE 1.

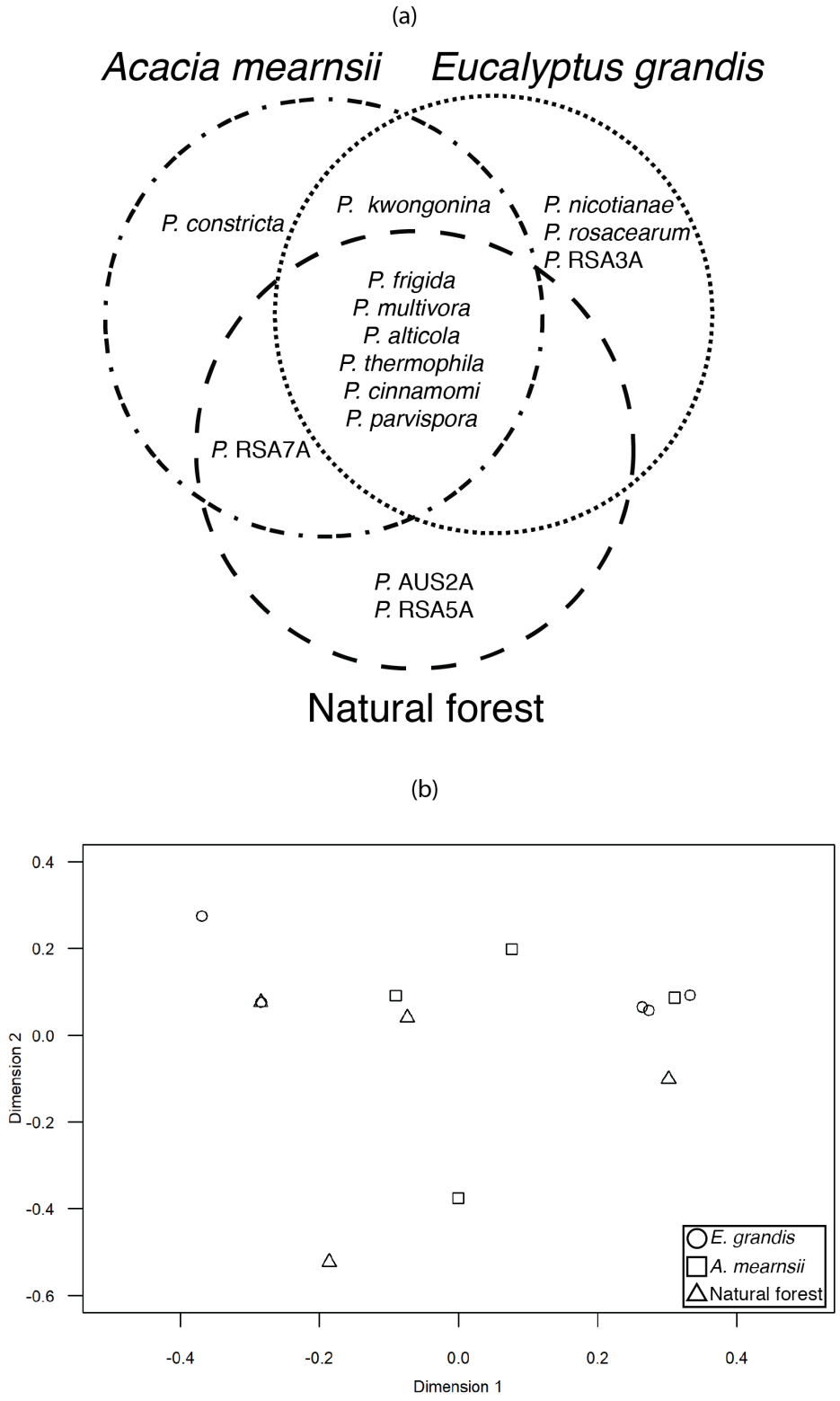

(a)
Acacia mearnsii
Eucalyptus grandis
P. constricta
P. kwongonina
P. nicotianae
P. rosacearum
P. RSA3A
P. frigida
P. multivora
P. alticola
P. thermophila
P. cinnamomi
P. parvispora
P. RSA7A
P. AUS2A
P. RSA5A
Natural forest
(b)
Dimension 2
Dimension 1
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
E. grandis
A. mearnsii
Natural forest

FIGURE 2.

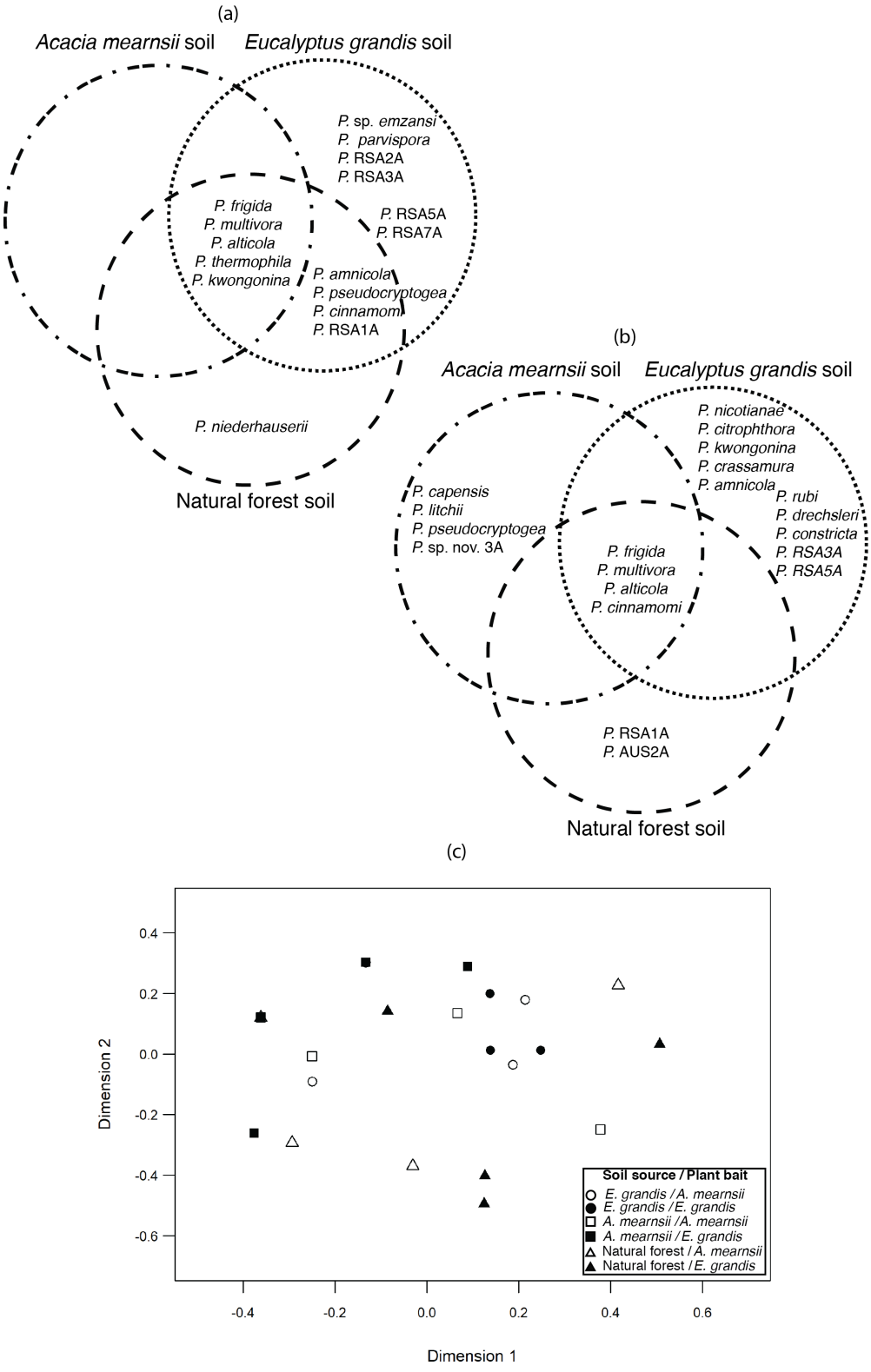

(a)
Acacia mearnsii soil
Eucalyptus grandis soil
P. sp. emzansi
P. parvispora
P. RSA2A
P. RSA3A
P. frigida
P. multivora
P. alticola
P. thermophila
P. kwongonina
P. RSA5A
P. RSA7A
P. amnicola
P. pseudocryptogea
P. cinnamomi
P. RSA1A
P. niederhauserii
Natural forest soil
(b)
Acacia mearnsii soil
Eucalyptus grandis soil
P. nicotianae
P. citrophthora
P. kwongonina
P. crassamura
P. amnicola
P. capensis
P. litchii
P. pseudocryptogea
P. sp. nov. 3A
P. rubi
P. drechsleri
P. constricta
P. RSA3A
P. RSA5A
P. frigida
P. multivora
P. alticola
P. cinnamomi
P. RSA1A
P. AUS2A
Natural forest soil
(c)
Dimension 2
Dimension 1
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
Soil source / Plant bait
E. grandis / A. mearnsii
E. grandis / E. grandis
A. mearnsii / A. mearnsii
A. mearnsii / E. grandis
Natural forest / A. mearnsii
Natural forest / E. grandis

FIGURE 3.

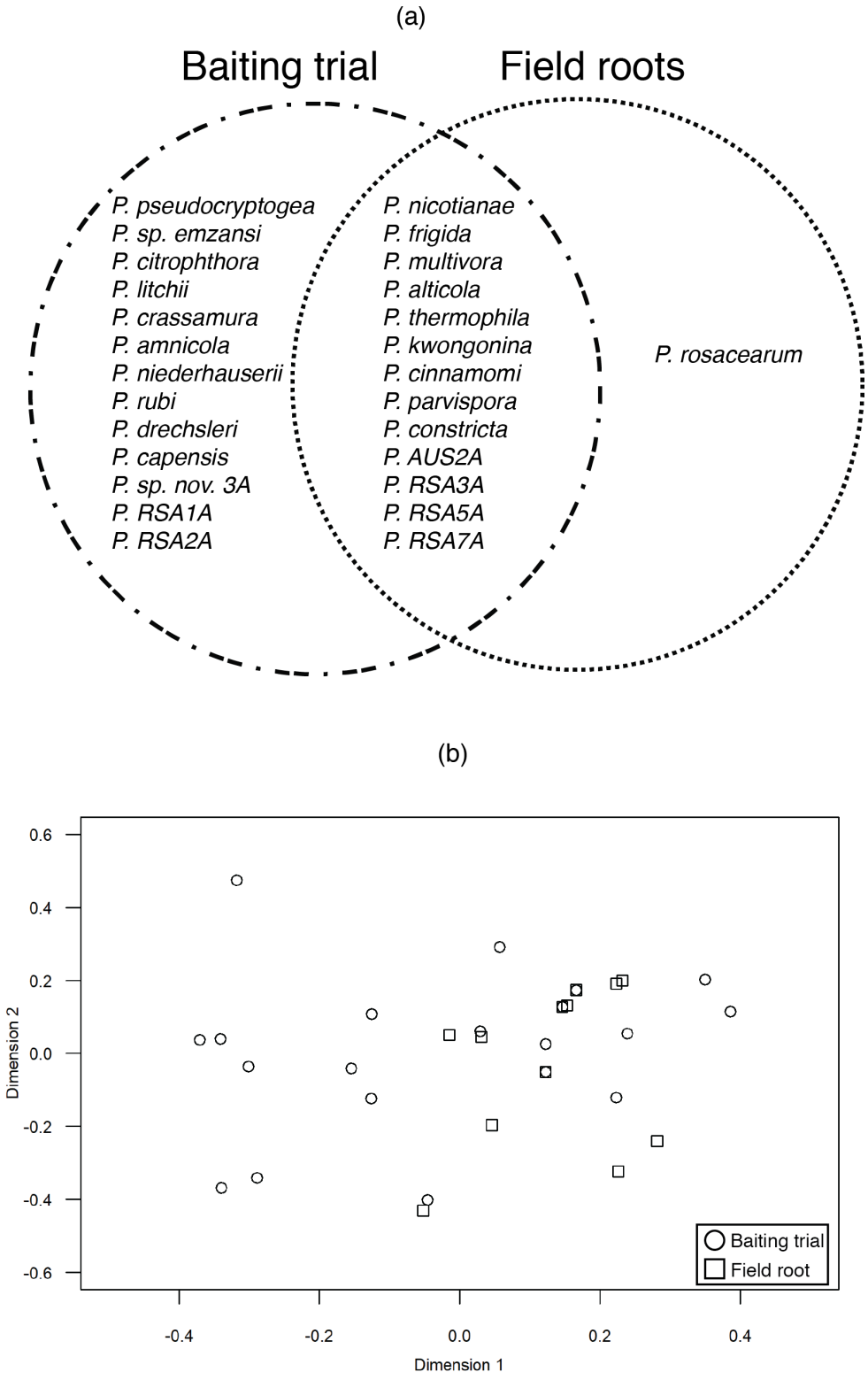
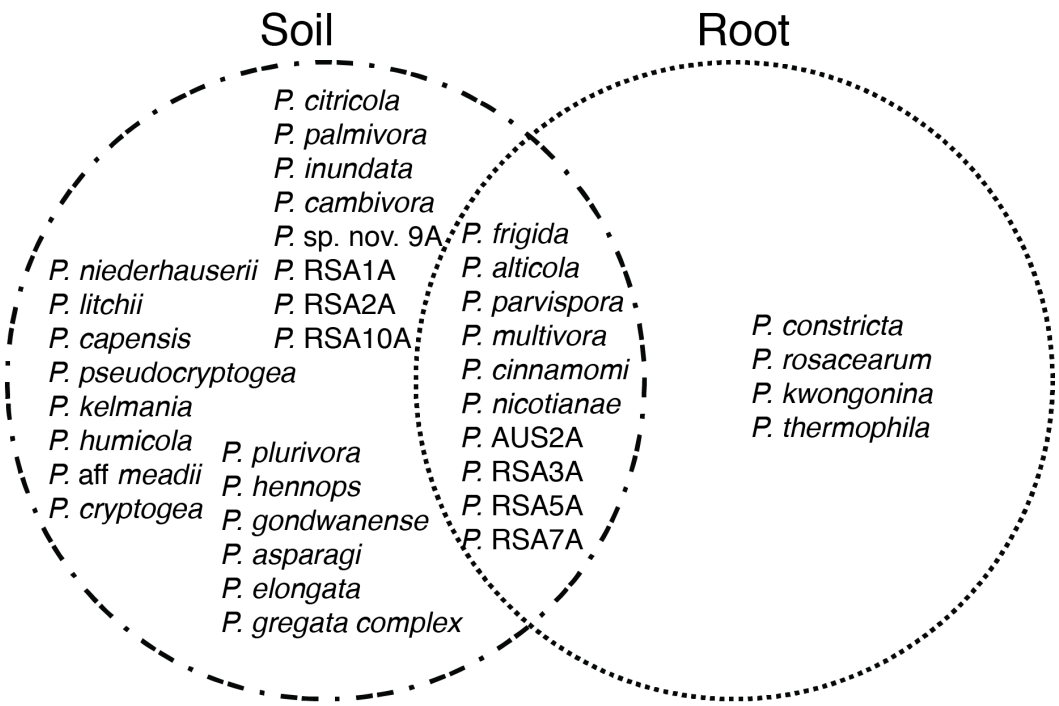

(a)
Baiting trial
Field roots
P. pseudocryptogea
P. sp. emzansi
P. citrophthora
P. litchii
P. crassamura
P. amnicola
P. niederhauserii
P. rubi
P. drechsleri
P. capensis
P. sp. nov. 3A
P. RSA1A
P. RSA2A
P. nicotianae
P. frigida
P. multivora
P. alticola
P. thermophila
P. kwongonina
P. cinnamomi
P. parvispora
P. constricta
P. AUS2A
P. RSA3A
P. RSA5A
P. RSA7A
P. rosacearum
(b)
Dimension 2
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
Dimension 1
Baiting trial
Field root

FIGURE 4.

(a)


Soil
Root
P. citricola
P. palmivora
P. inundata
P. cambivora
P. sp. nov. 9A
P. RSA1A
P. RSA2A
P. RSA10A
P. niederhauserii
P. litchii
P. capensis
P. pseudocryptogea
P. kelmania
P. humicola
P. aff meadii
P. cryptogea
P. plurivora
P. hennops
P. gondwanense
P. asparagi
P. elongata
P. gregata complex
P. frigida
P. alticola
P. parvispora
P. multivora
P. cinnamomi
P. nicotianae
P. AUS2A
P. RSA3A
P. RSA5A
P. RSA7A
P. constricta
P. rosacearum
P. kwongonina
P. thermophila

(b)

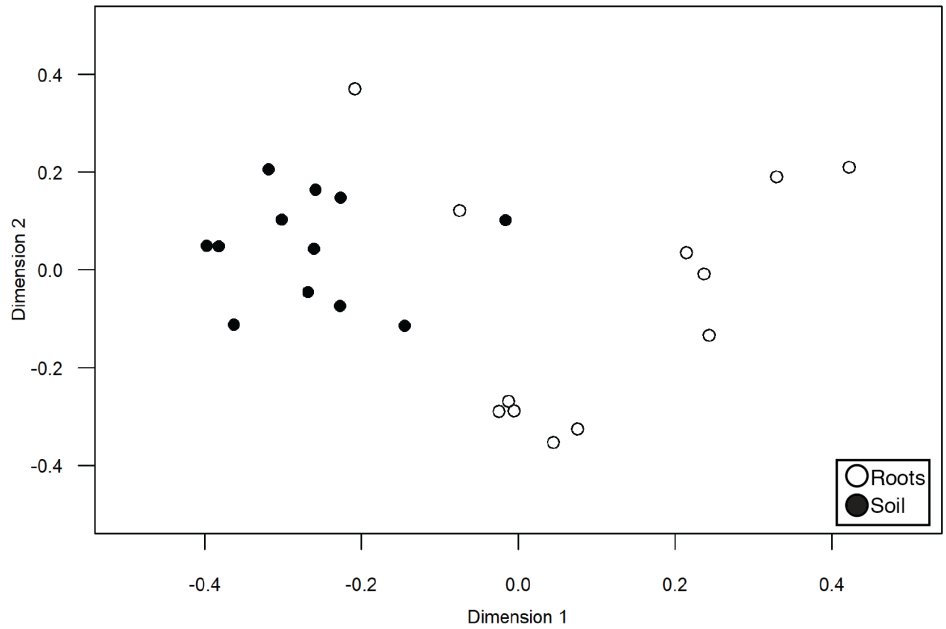

0.4
0.2
0.0
-0.2
-0.4
Dimension 2
-0.4
-0.2
0.0
0.2
0.4
Dimension 1
Roots
Soil

Table S1.

Taxon	Soil	Roots	First report - Global		In South Africa		References
			Year	Locations	Year	Locations	
P. alticola	+	+	2007	Kwa-Zulu Natal, South Africa	-	-	[1]
P. amnicola		+	2012	Western Australia, Australia	2020	Kwa-Zulu Natal and Mpumalanga	This study
P. asparagi	+		2012	Michigan, USA	2018	Kwa-Zulu Natal	[2]
P. AUS2A	+	+	2017	Multiple provinces, Australia	2018	Kwa-Zulu Natal	[2]
P. cambivora	+		1917	Torino, Italy	2018	Kwa-Zulu Natal	[2]
P. capensis	+	+	2010	Western Cape, South Africa	-	-	[3]
P. cinnamomi	+	+	1922	West Sumatra, Indonesia	1931	Kwa-Zulu Natal	[4]
P. citricola	+		1927	Taiwan	1989	Western Cape	[5]
P. citrophthora		+	1925	California, USA	1925	Limpopo and West Cape	[4]
P. constricta		+	2011	Western Australia, Australia	2020	Kwa-Zulu Natal and Mpumalanga	This study
P. crassamura		+	2012	Sardinia, Italy	2020	Kwa-Zulu Natal	This study
P. cryptogea	+		1919	Dublin, Ireland	1931	South Africa	[4]
P. drechsleri		+	1931	Idaho, USA	1988	Western Cape	[6]
P. elongata	+		2010	Western Australia, Australia	2018	Kwa-Zulu Natal	[2]
P. frigida	+	+	2007	Kwa-Zulu Natal, South Africa	-	-	[7]
P. gondwanensis	+		2016	New South Wales, Australia	2018	Mpumalanga	[2]
P. 'hennops'	+		2013	Gauteng and Kwa-Zulu Natal, South Africa	-	-	[8]
P. humicola	+		1985	Taiwan	2018	Kwa-Zulu Natal	[2]
P. inundata	+		2003	Kent, UK	2018	Kwa-Zulu Natal	[2]
P. 'kelmania'	+		2002	USA	2018	Kwa-Zulu Natal	[3]
P. kwongonina		+	2018	Western Australia, Australia	2020	Kwa-Zulu Natal	This study
P. litchii	+	+	2007	Taiwan	2018	Kwa-Zulu Natal	[1]
P. multivora	+	+	2009	Western Australia, Australia	1941	Western Cape	[4]

Continued…

Taxon	Soil	Roots	First report - Global		In South Africa		References
			Year	Locations	Year	Locations	
P. nicotianae	+	+	1896	Sumatra, Indonesia	1913	Limpopo	[4]
P. niederhauserii	+	+	2001	North Carolina, USA	2011	Western Cape	[9]
P. palmivora	+		1919	India	1981	Limpopo	[10]
P. parvispora	+	+	2014	Sardinia, Italy	2018	Kwa-Zulu Natal	[2]
P. pseudocryptogea	+	+	2006	Western Australia, Australia	2018	Kwa-Zulu Natal	[2]
P. rosacearum		+	2009	California, USA	2020	Kwa-Zulu Natal	This study
P. RSA1A	+	+	2018	Kwa-Zulu Natal, South Africa	x	x	[2]
P. RSA2A	+	+	2018	Kwa-Zulu Natal, South Africa	x	x	[2]
P. RSA3A	+	+	2018	Kwa-Zulu Natal and Mpumalanga, South Africa	x	x	[2]
P. RSA5A	+	+	2018	Kwa-Zulu Natal and Mpumalanga, South Africa	x	x	[2]
P. RSA7A	+	+	2018	Kwa-Zulu Natal and Mpumalanga, South Africa	x	x	[2]
P. RSA10A	+		2018	Kwa-Zulu Natal and Mpumalanga, South Africa	x	x	[2]
P. rubi		+	2007	Scotland, UK	2020	Mpumalanga	This study
P. sp. emzansi		+	2010	Western Cape, South Africa	x	x	[3]
P. sp. nov. 9A	+		2017	New South Wales, Victoria and Tasmania, Australia	2018	Kwa-Zulu Natal	[2]
P. sp. nov. 3A		+	2017	Victoria and Western Australia, Australia	2018	Kwa-Zulu Natal	[2]
P. thermophila		+	2011	Western Australia, Australia	2020	Kwa-Zulu Natal and Mpumalanga	This study

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FIGURE S1.

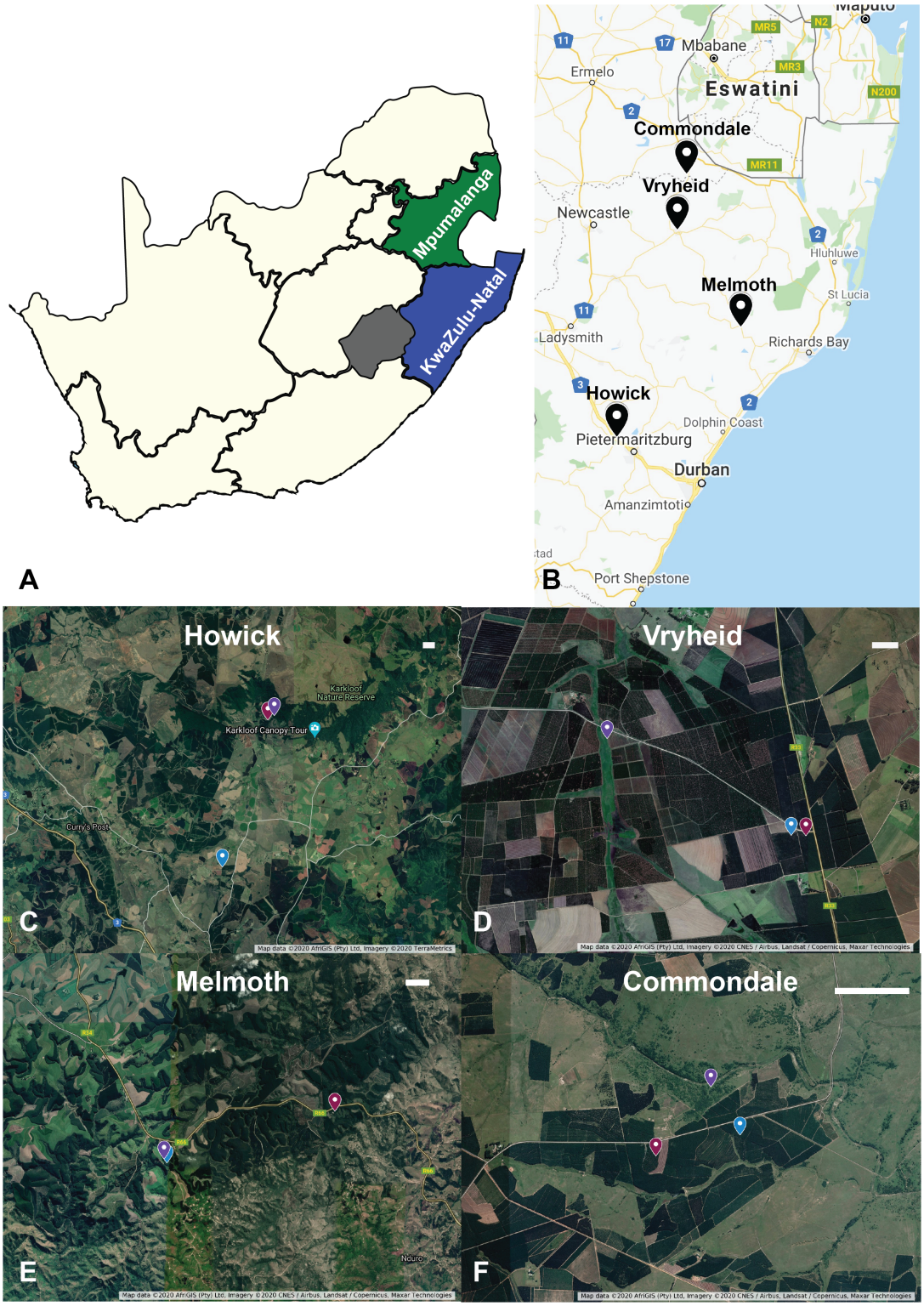

Mpumalanga
KwaZulu-Natal
A
Mbabane
Ermelo
Eswatini
Commondale
Vryheid
Newcastle
Hluhluwe
Melmoth
St Lucia
Ladysmith
Richards Bay
Howick
Dolphin Coast
Pietermaritzburg
Durban
Amanzimtoti
Port Shepstone
B
Howick
Karkloof Nature Reserve
Karkloof Canopy Tour
Curry's Post
C
Vryheid
D
Melmoth
E
Commondale
F

FIGURE S2.

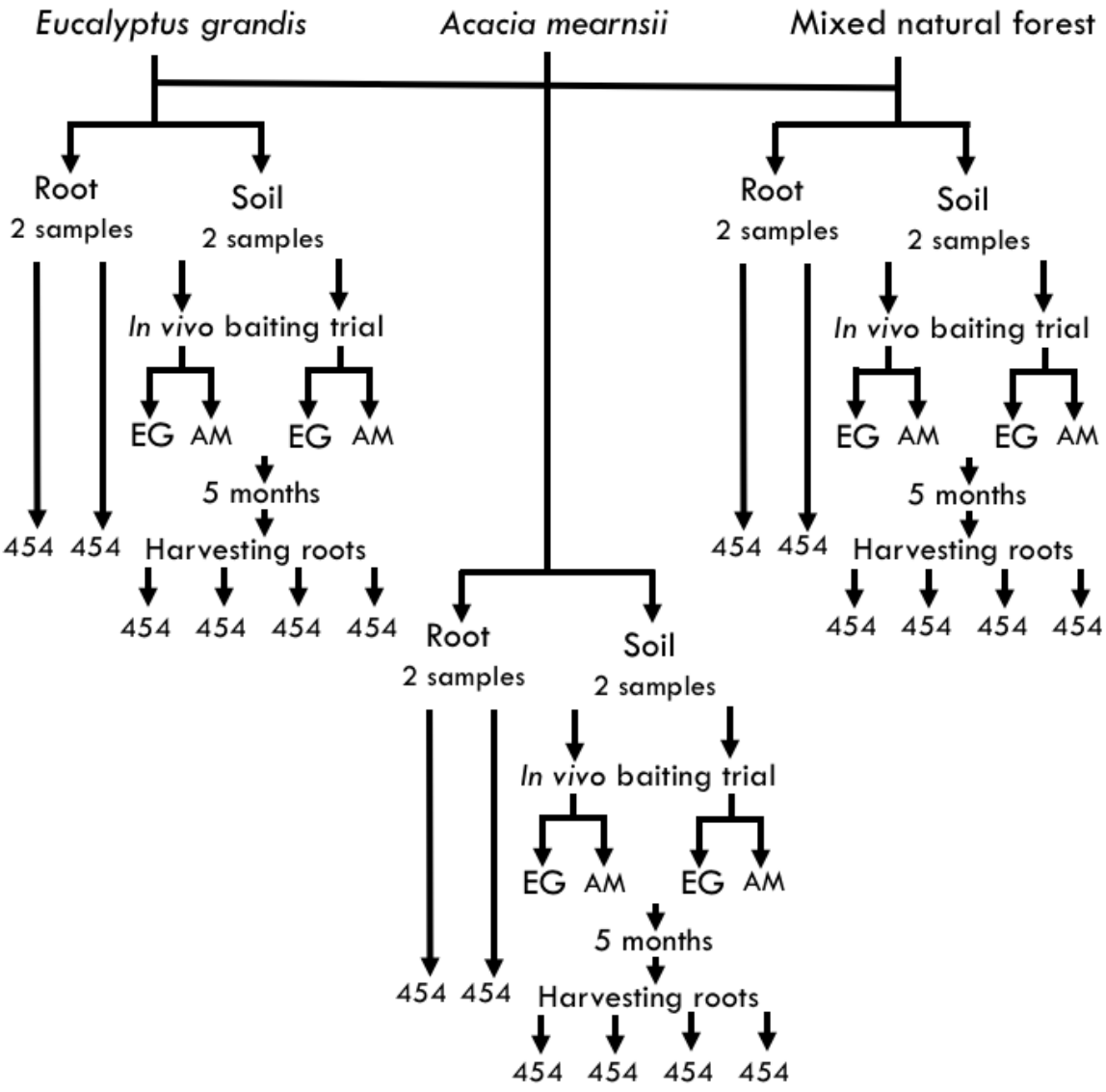
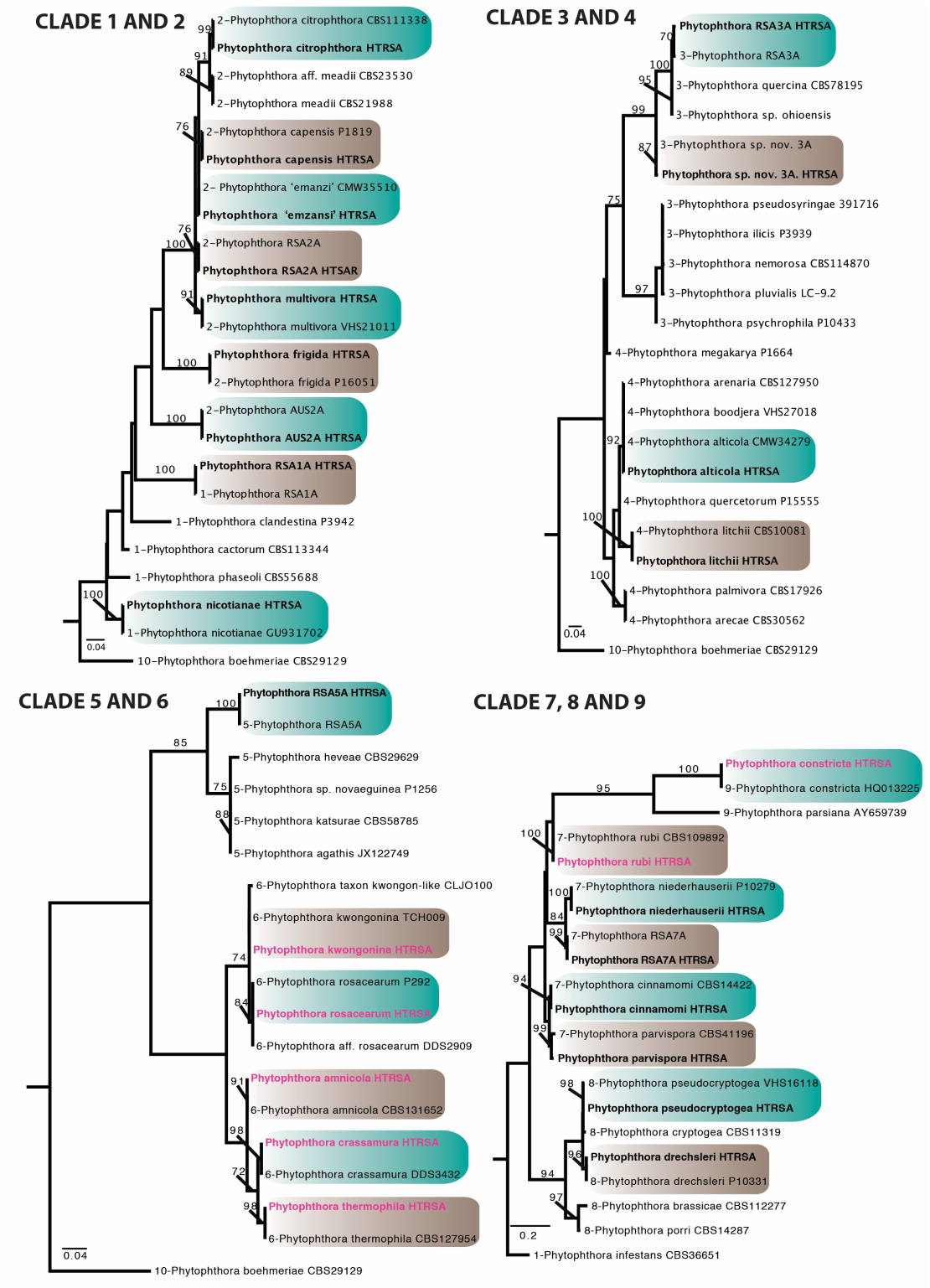

Eucalyptus grandis
Acacia mearnsii
Mixed natural forest
Root
2 samples
Soil
2 samples
In vivo baiting trial
EG AM EG AM
5 months
Harvesting roots
454 454 454 454
454 454

FIGURE S3.


CLADE 1 AND 2
2-Phytophthora citrophthora CBS111338
Phytophthora citrophthora HTRSA
2-Phytophthora aff. meadii CBS23530
2-Phytophthora meadii CBS21988
2-Phytophthora capensis P1819
Phytophthora capensis HTRSA
2- Phytophthora 'emanzi' CMW35510
Phytophthora 'emzansi' HTRSA
2-Phytophthora RSA2A
Phytophthora RSA2A HTSAR
Phytophthora multivora HTRSA
2-Phytophthora multivora VHS21011
Phytophthora frigida HTRSA
2-Phytophthora frigida P16051
2-Phytophthora AUS2A
Phytophthora AUS2A HTRSA
Phytophthora RSA1A HTRSA
1-Phytophthora RSA1A
1-Phytophthora clandestina P3942
1-Phytophthora cactorum CBS113344
1-Phytophthora phaseoli CBS55688
Phytophthora nicotianae HTRSA
1-Phytophthora nicotianae GU931702
0.04
10-Phytophthora boehmeriae CBS29129
CLADE 3 AND 4
Phytophthora RSA3A HTRSA
3-Phytophthora RSA3A
3-Phytophthora quercina CBS78195
3-Phytophthora sp. ohioensis
3-Phytophthora sp. nov. 3A
Phytophthora sp. nov. 3A. HTRSA
3-Phytophthora pseudosyringae 391716
3-Phytophthora ilicis P3939
3-Phytophthora nemorosa CBS114870
3-Phytophthora pluvialis LC-9.2
3-Phytophthora psychrophila P10433
4-Phytophthora megakarya P1664
4-Phytophthora arenaria CBS127950
4-Phytophthora boodjera VHS27018
4-Phytophthora alticola CMW34279
Phytophthora alticola HTRSA
4-Phytophthora quercetorum P15555
4-Phytophthora litchii CBS10081
Phytophthora litchii HTRSA
4-Phytophthora palmivora CBS17926
4-Phytophthora arecae CBS30562
0.04
10-Phytophthora boehmeriae CBS29129
CLADE 5 AND 6
Phytophthora RSA5A HTRSA
5-Phytophthora RSA5A
5-Phytophthora heveae CBS29629
5-Phytophthora sp. novaeguinea P1256
5-Phytophthora katsurae CBS58785
5-Phytophthora agathis JX122749
6-Phytophthora taxon kwongon-like CLJO100
6-Phytophthora kwongonina TCH009
Phytophthora kwongonina HTRSA
6-Phytophthora rosacearum P292
Phytophthora rosacearum HTRSA
6-Phytophthora aff. rosacearum DDS2909
Phytophthora amnicola HTRSA
6-Phytophthora amnicola CBS131652
Phytophthora crassamura HTRSA
6-Phytophthora crassamura DDS3432
Phytophthora thermophila HTRSA
6-Phytophthora thermophila CBS127954
0.04
10-Phytophthora boehmeriae CBS29129
CLADE 7, 8 AND 9
Phytophthora constricta HTRSA
9-Phytophthora constricta HQ013225
9-Phytophthora parsiana AY659739
7-Phytophthora rubi CBS109892
Phytophthora rubi HTRSA
7-Phytophthora niederhauserii P10279
Phytophthora niederhauserii HTRSA
7-Phytophthora RSA7A
Phytophthora RSA7A HTRSA
7-Phytophthora cinnamomi CBS14422
Phytophthora cinnamomi HTRSA
7-Phytophthora parvispora CBS41196
Phytophthora parvispora HTRSA
8-Phytophthora pseudocryptogea VHS16118
Phytophthora pseudocryptogea HTRSA
8-Phytophthora cryptogea CBS11319
Phytophthora drechsleri HTRSA
8-Phytophthora drechsleri P10331
8-Phytophthora brassicae CBS112277
8-Phytophthora porri CBS14287
0.2
1-Phytophthora infestans CBS36651