*PHYLOGENETIC AND POPULATION ANALYSES OF THE INVASIVE BROWN ROOT-ROT PATHOGEN (*PHELLINUS NOXIUS*) HIGHLIGHT THE EXISTENCE OF AT LEAST TWO DISTINCT POPULATIONS

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INTRODUCTION

Phellinus noxius (Corner) G. H. Cunn is a vastly destructive, fast-growing pathogen that affects a wide range of woody hosts in pan-tropical areas, including Asia, Australia, Africa, and Oceania (Ann *et al*. 2002). This invasive pathogen causes brown root-rot disease on cacao, coffee, and rubber, as well as diverse fruit, nut, ornamental, and other native/exotic trees, and little host specificity is known to occur (Sahashi *et al*. 2010). Symptoms of *P. noxius* infection can include reduced tree growth, defoliation, and branch dieback; however, *P. noxius* can also survive as a saprophyte by colonizing the heart wood and other organic matter. Brown root-rot disease can develop over several years, or in some cases, *P. noxius* infection can cause tree mortality within a year. Understanding the genetic diversity and evolutional history of *P. noxius* populations worldwide well help assess the evolutionary origins, worldwide movement, and potential ecological differences within *P. noxius*, which will contribute to the development of management strategies. Our objective was to understand the genetic diversity and evolutional history of *P. noxius* populations worldwide to assess the evolutionary origins, worldwide movement, and potential ecological differences within populations of *P. noxius*.

METHODS

Isolates and DNA Sequencing

A total of 147 isolates were included from Japan, Taiwan, Australia, China (Hong Kong), Malaysia, and the Pacific islands of American Samoa, Saipan, Guam, Palau, Yap, Pohnpei, and Kosrae were sequenced at four loci including internal transcribed spacer (ITS), large subunit (LSU-rDNA), translation elongation factor-1 alpha (*tef1*), and RNA polymerase subunit II (*RPB2*) (Table 1).

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Statistical Analyses

Genotype sequences were phased into haplotypes for each locus and DnaSP v.5 (Rozas *et al*. 2003) was used to estimate the number of unique haplotypes, pairwise nucleotide diversity (π), and the number of segregating sites for each locus. Haplotype networks were constructed for each locus by statistical parsimony with the program TCS v1.21 using a 92% similarity cutoff (Clement *et al*. 2000). Phylogenies were inferred for collapsed haplotypes for each locus with Bayesian inference using MrBayes v3.0 (Huelsenbeck *et al*. 2001). DT-ModSel (Minin *et al*. 2003) was used to estimate the best-fit, nucleotide-substitution models for each dataset. Bayesian inference was performed with parameter settings suggested by the best-fit, nucleotide-substitution models. The Markov chain Monte Carlo (MCMC) search was run with four chains for 3,000,000 generations generating 30,001 trees; the first 6,000 trees were discarded as "burnin" of the chains.

Table 1. Number and location (Country/ Island) of isolates used in this study.

Country / Island	Number of isolates
American Samoa	30
Australia	25
China	21
Guam	15
Japan	10
Kosrae	2
Malaysia	15
Palau	8
Pohnpei	7
Saipan	12
Taiwan	2
Yap	2

Table 2. Total number of sites, haplotypes, sequence diversity, segregating sites and nucleotide diversity for each lous.

Locus	N [a]	# of hap [b]	Seg. sites [c]	π [d]	H*d* [e]
LSU	880	20	11	0.002	0.837
ITS	639	78	83	0.013	0.924
RBP2	861	86	103	0.014	0.983
tef1	1077	60	83	0.008	0.923

[a] Total number of bps; [b] Number of unique haplotypes; [c] Number of segregating sites; [d] Nucleotide diversity; [e] Haplotype diversity

RESULTS

On the basis of these preliminary analyses, the most diverse locus was the *RBP2* with a nucleotide diversity (π) of 0.014 and a haplotype diversity (H*d*) of 0. 983 (Table 2). The least diversity locus was the LSU region with $\pi = 0.002$ and a haplotype diversity of 0.837 (Table 2). The LSU Bayesian phylogeny highlighted a general grouping of *P. noxius* sequences, while showing that the genus *Phellinus* is polyphyletic, indicating that further research is needed to clarify *Phellinus* taxonomy (Figure 1).

The *tef1* locus showed the most phylogenetic signal of the four loci (Figure 2). The Bayesian phylogeny showed two distinct clades of *P. noxius* that separated sequences by general geographic regions. All sequenced isolates from Malaysia (15) and Taiwan (2) grouped with high posterior probably into an eastern Asia/American Samoa clade, which also contained some isolates from Japan, China (Hong Kong), and American Samoa. Separate from the eastern Asia/American Samoa clade, isolates from Australia, Guam, Saipan, Palau, Yap, Pohnpei, and Kosrae grouped in a distinct eastern Asia/Oceania/Pacific islands clade that also contained some isolates from Japan and China (Hong Kong). Isolates from Australia formed a distinct monophyletic subclade (PP = 0.92) within but distinct in the eastern Asia/Oceania/Pacific island clade. Interestingly, isolates from American Samoa formed a distinct, monophyletic subclade (PP = 0.86) within the eastern Asia/American Samoa clade.

Haplotype networks of the *RBP2* and ITS also highlighted the existence of these two major phylogenetic groups based on each *P. noxius* sequences (data not shown). However, signals of admixture or migration were observed where isolates from the eastern Asia/American Samoa and eastern Asia/Oceania/Pacific islands regions share haplotypes. However, in both networks, isolates from American Samoa formed a distinct group. These American Samoa isolates do not share *RPB2* haplotypes; however, one ITS haplotype is shared with an isolate from Australia.

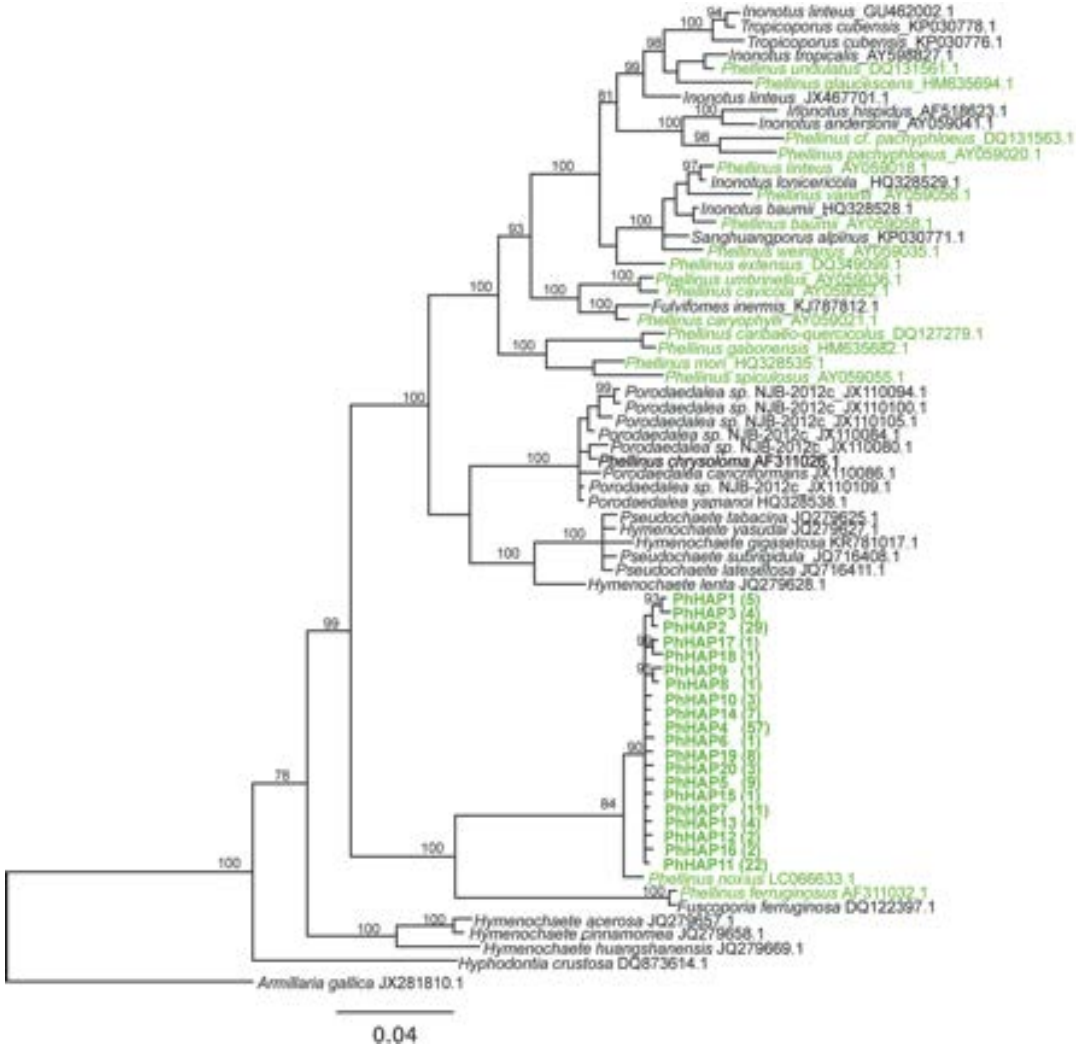

Figure 1. Bayesian phylogeny of the large subunit (LSU; 25S) of rDNA. Node support as posterior probability is labeled above each node. Isolates from this study are labeled as PhHAP. note: these isolates are grouped together in these analyses.

DISCUSSION

From these preliminary sequencing and phylogenetic analyses, we have evidence to suggest the existence of at least two genetic distinct populations of *P. noxius* from eastern Asia/American Samoa (Japan, Taiwan, China, Malaysia, and American Samoa) and eastern Asia/Oceania/Pacific islands (Australia, Guam, Saipan, Palau, Yap, Pohnpei, Kosrae, China, and Japan). However, our data suggests that admixture or migration

of genotypes across these two populations is likely to have occurred, which can be compared to regional population structure that has been previously studied in Japan (Akiba *et al*. 2015) and Taiwan (Chung *et al*. 2015). In addition, the results suggest that isolates from American Samoa are genetically distinct, and at the *tef1* locus, group with isolates from the eastern Asia/American Samoa clade. Future analyses will include sequencing data generated by Illumina sequencing of double-digest reduced representation libraries, which will allow high-resolution analyses. Continued analyses will assess regional population structure, gene flow among populations, diversity in recently observed populations, and potential suitable climate space for specific genotypes.

Continued studies of the genetic diversity and population structure of *P. noxius* is essential to understand the pathways of dispersal for this destructive, invasive pathogen. Furthermore, understanding of *P. noxius* populations will contribute to management strategies by better characterizing host range, suitable climate space, and potential/existing hybridization among *P. noxius* populations, which will allow informed predictions of geographic regions that are at risk of invasion.

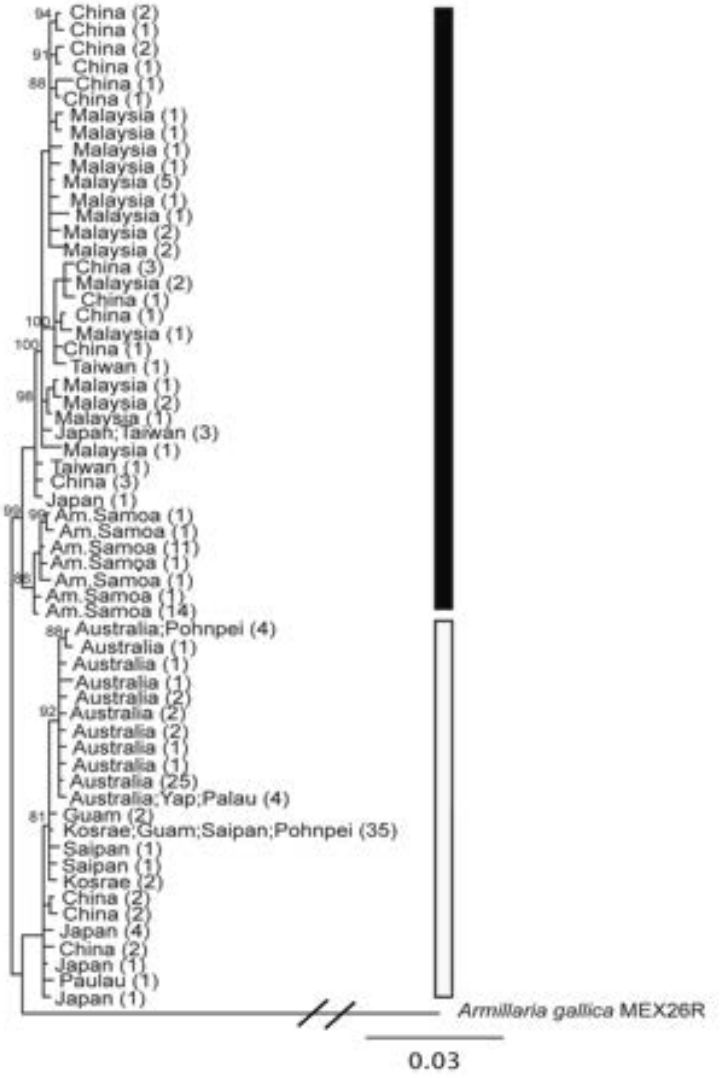

Figure 2. Bayesian phylogeny of the translation elongation factor 1-alpha (tef1) with Armillaria gallica used as the outgroup. Note: support as posterior probability is labeled above each node. Isolates are labeled by their country of origin. There are two major clades, the eastern Asia/American Samoa clade (black) and the eastern Asia/Oceania/Pacific islands clade (white). Isolates from American Samoa form a distinct subclade within the eastern Asia/American Samoa clade.

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