

IDENTIFICATION OF GENETIC GROUPS WITHIN THE INVASIVE BROWN ROOT ROT PATHOGEN, *PYRRHODERMA NOXIUM* (FORMERLY *Phellinus noxius*)

Andrea R. Garfinkel¹, Phil G. Cannon², Ned B. Klopfenstein³, Jane E. Stewart¹, and Mee-Sook Kim⁴

Abstract

Recent taxonomic investigation into genus *Pyrrhoderma* has determined that the genus currently contains six species, including *Pyrrhoderma noxium* (formerly *Phellinus noxius*), and one unnamed, but likely novel species (Zhou et al. 2018). *Pyrrhoderma noxium* is considered one of the most important pathogens in this genus due to its worldwide distribution in pan-tropical regions, including Asia, Australia, Africa, and Oceania. *Pyrrhoderma noxium* has a wide host range of over 200 plant species and causes mortality of >100 tropical tree species (e.g., breadfruit, mango) that are integral to economies, native cultures, and forest ecosystems of the Pacific Islands (e.g., Ann et al. 2002). In a previous study, over 100 isolates of *P. noxium* (as *P. noxius*) were collected from the Pacific Islands (e.g., Guam, Rota, Saipan, Pohnpei, Yap, Palau, Kosrae, and American Samoa) and other locations (e.g., Japan, Taiwan, Hong Kong, Malaysia, and Australia) and phylogenetic analyses were conducted based on ITS (internal transcribed spacer) and LSU (28S nuclear large sub unit) rDNA gene sequences. Results from this study found that both the ITS and LSU regions provide strong evidence that *P. noxium* isolates differ from the ex-type culture of *P. noxium*, suggesting that *P. noxium* isolates potentially represent one or more undescribed species or distinct genetic groups. Our current work aims to identify the appropriate taxonomic status for the previously characterized distinct genetic groups of *P. noxium*. Accurate identification of species and/or genetic groups is essential to understanding the true host range and geographic distribution of brown root rot pathogens that are currently comprised within *P. noxium*. Further, correct identification of the pathogen species or sub-species is critical to develop subsequent measures for detecting and managing the pathogen and its spread, such as creating and enforcing quarantine measures, especially for the widespread and devastating pathogens that are currently considered as *P. noxium*.

Introduction

- The genus *Pyrrhoderma* has recently undergone taxonomic restructuring, including the addition of two species formerly within genus *Phellinus*, and now is made-up of six named and one unnamed species (Zhou et al. 2018).
- *Pyrrhoderma noxium* (formerly *Phellinus noxius*) is an aggressive pathogen (Figure 1) on over 100 tropical tree species, including many of which are ecologically, culturally, and economically valuable (e.g., Ann et al. 2002, Brooks 2002, Sahashi et al. 2015).
- Over 100 isolates of a *Pyrrhoderma* species were collected across the Pacific Islands (including Guam, Rota, Saipan, Pohnpei, Yap, Palau, Kosrae, and American Samoa) and other locations where this pathogen is present (such as Japan, Taiwan, Hong Kong, Malaysia, and Australia).
- The taxonomic placement of these isolates is unknown in the context of the newly-revised phylogenies.

¹Department of Bioagricultural Sciences & Pest Management, Colorado State University, Fort Collins, CO. ²USDA Forest Service, Vallejo, CA. ³USDA Forest Service, Rocky Mountain Research Station, Moscow, ID. ⁴USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR



Figure 1: Dried mycelial mat of *Pyrrhoderma noxium* on a moso'oi (*Cananga odorata*) tree in American Samoa (Photo: Fred Brooks).

Goal

To correctly identify and characterize the collected isolates of *Pyrrhoderma* by phylogenetic analyses that include the sequences of type specimens and other related species described in the literature.

Materials and Methods

- DNA extraction of 95 isolates of *Pyrrhoderma* species from around the world.
- PCR and sequencing of the ITS (internal transcribed spacer), LSU (28S nuclear large subunit), *RPB2* (RNA polymerase II), and *tef1* (translation elongation factor – 1 alpha) genes of selected isolates.
- Alignment and trimming of sequences using Clustal W implemented in MEGA 6.
- Maximum likelihood (implemented in MEGA 6) and Bayesian analysis (implemented in Mr. Bayes).

Results

- Analysis of the ITS and LSU loci suggests the *Pyrrhoderma* isolates (Figure 2; white circles) are not members of *P. noxium* (Figure 2; blue circles), as previously described (Figure 2) and additional isolates in GenBank characterized as *P. noxium* do not align with the type specimen, but with our isolates (data not shown).
- Our collection of *Pyrrhoderma* isolates (Figure 2; white circles) are more closely related to the unnamed *Pyrrhoderma* species (Figure 2; green circles) partially described by Zhou et al. (2018).
- *RPB2* and *tef1* (data not shown) suggest notable genetic diversity among our isolates and up to three phylogenetic species and/or groups may be present (Figure 3). A group from American Samoa (indicated in green branches) appears to be genetically distinct from other geographic populations (Figure 3).

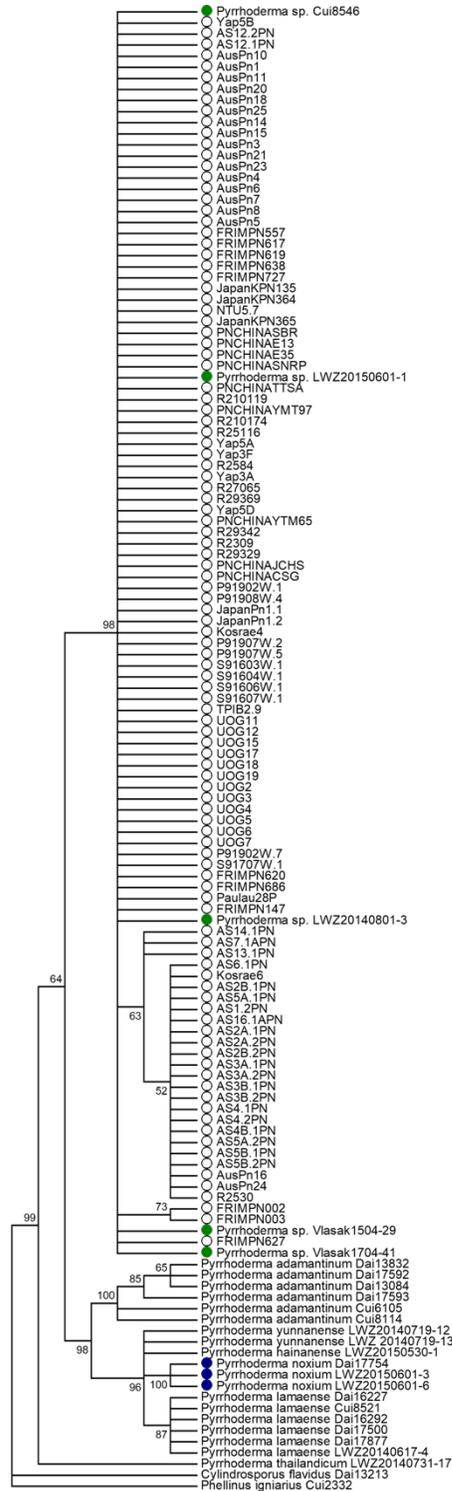


Figure 2: Combined ITS+LSU ML tree describing the relationship of *Pyrrhoderma* sp. isolates collected globally (white circles) to voucher specimens (blue circles) described by Zhou et al. (2018). The unnamed *Pyrrhoderma* species (green circles) was partially described by Zhou et al. (2018). *Cylindrosporium flavidus* and *Phellinus igniarius* are used as outgroups. The Hasegawa-Kishino-Yano model with gamma distribution was used to model evolutionary rates. Branches with less than 50% bootstrap support are not shown. Bootstrap replicates = 1000.

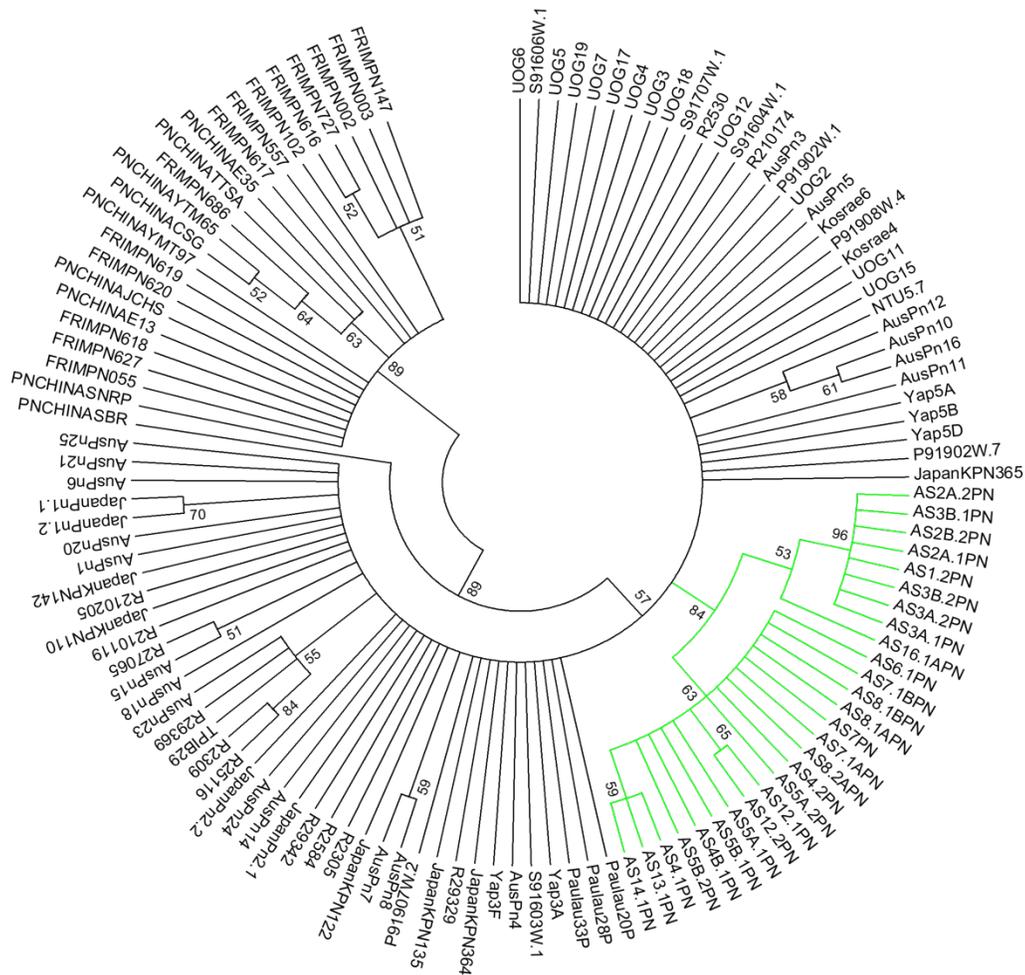


Figure 3: RPB2 maximum likelihood tree describing genetic diversity among *Pyrrhoderma* sp. isolates previously collected around the world. A group from American Samoa (indicated in green branches) appears to be genetically distinct from other geographic populations. The Kimura 2-parameter model was used to model evolutionary rates. Branches with less than 50% bootstrap support are not shown. Bootstrap replicates = 1000.

Implications

- Multiple *Pyrrhoderma* species besides *P. noxium* may be important pathogens of tropical and subtropical forests.
- These multiple species may differ in host range, virulence, ecological niches, environmental requirements, or other factors that may influence the epidemiology of the diseases they cause and their management.
- The putatively novel *Pyrrhoderma* species suggested by this study has not been reported in some U.S. states/territories (e.g., Hawaii, Florida, Puerto Rico), where it represents a major invasive threat based on bioclimatic models (Stewart et al., in revision).
- Taxonomy and correct species identification are key to understanding hosts and geographical range, which are important for establishing regulatory processes, such as quarantines, that can help prevent pathogen spread.

Future Goals

- Additional genetic analyses to confirm any new species present in our collection.
- Morphological characterization of the isolates to corroborate genetic data.
- Development of molecular detection methods to identify the pathogen (or genetic groups) and evaluate management treatments in tropical forests.

Acknowledgements

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