

Abstract

When this study commenced in April 2007, a recent molecular re-evaluation of a number of historical isolates of *Phytophthora* kept by the Vegetation Health Service (VHS) at the Department of Environment and Conservation (DEC) of Western Australia (WA) had indicated the presence of at least nine undescribed taxa. This study has described three of these as novel pathogenic species of *Phytophthora* and expands our knowledge of the number and diversity of species occurring within this vast landscape and the role they play in the ecology of the ecosystems in which they occur. Together with the description of *P. multivora* and five ITS clade 6 species a total of nine species have now been described following their isolation from natural (and disturbed) ecosystems in WA

Phytophthora elongata, a pathogen predominantly isolated in association with diseased *Eucalyptus marginata*, was the first species to be described as part of this study. *Phytophthora elongata* shares many morphological similarities with *P. citricola* to which it had traditionally been mistakenly classified. It serves as an excellent example of the utility of molecular taxonomic approaches to delineate morphologically cryptic species. In agreement with previous studies the results of sequencing both the ITS rDNA and *cox1* gene of this species in this study revealed no genetic variation at these loci suggesting that this population is clonal and likely to have been recently introduced to the jarrah forest. The discovery of *P. bisheria*, the closest described phylogenetic relative of *P. elongata*, in undisturbed native forest in Taiwan suggests a possible Asian origin for *P. elongata*. However, the occurrence of the closest known phylogenetic taxon of *P. elongata*, *P. taxon elongata*-like, in the southern jarrah forest is noteworthy.

The other species described in this study, *P. constricta* and *P. arenaria*, occur in the sandy heath-land vegetation referred to in WA as kwongan and both appear to be well-adapted physiologically to the ecosystems in which they occur. However, these two species differ considerably in morphology and are distant phylogenetically. Like *P. elongata*, the use of a molecular phylogenetic approach clearly elucidates these taxa as species distinct from morphologically similar species. Indeed in the case of *P. constricta* (ITS clade 9) and *P. arenaria* (ITS clade 4), the most morphologically similar species, *P. megasperma* (clade 6) and *P. cactorum* (clade 1), respectively, belong to distant ITS clades. The study of both *P. constricta* and *P. arenaria* also illustrates the importance of fully characterising species to gain insights into their biology and physiology, that explain how these species survive in the ecosystems in which they occur. For example, the high oospore-wall index and the relationship between temperature and growth for *P. arenaria* are indicative of adaptation to survival in the kwongan vegetation of the northern sandplains. This, coupled with the ITS rDNA and *cox1* sequence diversity observed for the isolates examined in this study suggests that this species may be endemic to WA. Similarly, the sequence diversity observed for the *cox1* gene for *P. constricta* suggest that it too may be endemic, whilst its slightly lower optimal temperature for growth and oospore wall index make it well adapted to the cooler southern kwongan from which it has predominantly been isolated. Thus, although species identification using traditional morphological methods is being increasingly overlooked in favour of molecular diagnostic tools, these studies illustrate the importance of insights gained by characterising all aspects of the biology of an organism. This may be particularly relevant in the context of plant biosecurity, when one is interested in the invasive potential of a pathogen or its suitability to persist in a given situation. In addition to confirming the pathogenic ability of the three described

species on hosts they have been isolated from natural ecosystems, the results of this study have also demonstrated that *P. elongata* is a pathogen of *Banksia attenuata*, a potential host that is widespread throughout the south-west botanical province.

The diversity observed within the *cox1* locus lead to the hypothesis that *P. multivora* may be endemic to WA. In the present study DNA sequence variation at four nuclear and three mitochondrial loci was assessed for a putative worldwide collection of *P. multivora* isolates and subjected to coalescent-based genealogical and population genetic analysis. Overall the results clearly show that the isolates from the Republic of South Africa (RSA), taken as a population, have greater values for all measures of genetic diversity, including a greater number of multi-locus nuclear and mitochondrial genotypes than those from WA. It therefore appears likely that *P. multivora* has been introduced to WA, possibly from RSA.

This study also analysed the genetic diversity within *P. plurivora* and closely related taxa from *Phytophthora* ITS clade 2a. The loci assessed in this study were the same as those used in the study of *P. multivora*. Compared to *P. multivora*, markedly lower levels of genetic diversity were observed amongst the assessed isolates of *P. plurivora*. Interestingly, the genealogical analysis of the predominantly European *P. plurivora* isolates revealed two clonal lineages, consistent with *P. plurivora* being introduced to Europe on at least two occasions. Furthermore, this study revealed two undescribed taxa closely related to *P. citricola* sensu stricto amongst isolates obtained from the Royal Horticultural Society gardens in Surrey, England, and the first recorded isolation of *P. plurivora* in Australia.

In addition to describing three novel pathogenic species of *Phytophthora* and examining the genetic diversity and evolutionary history of *P. multivora*, *P. plurivora* and closely related taxa, the present study emphasises the role played by the movement of plants across international borders in spreading plant pathogens around the globe, highlighting the potential risk that this trade poses to biosecurity.