

Molecular testing uncovers new *Phytophthora* taxa from natural ecosystems in Western Australia

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Introduction

Verification of mapping of the extent of *Phytophthora* dieback disease, based on shadowless colour aerial photography, involves the routine testing of soil and root samples -- collected from beneath dying, *Phytophthora*-sensitive native plant "indicator species" -- for the presence of the pathogen. In addition to *P. cinnamomi*, six other *Phytophthora* species have been reported from native vegetation in Western Australia (WA) since the 1980s during these operations. They were identified, using morphological characters, as *P. citricola*, *P. megasperma*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae* and *P. boehmeriae*¹.

The recent advent and availability of DNA sequencing techniques for the identification of *Phytophthora* species² has enabled the testing of new isolates that were difficult to identify from their morphology, as well as a range of historical isolates dating back to the 1980s from the Department of Environment and Conservation culture collection.

Methods and Materials

DNA was extracted from pure cultures of *Phytophthora* grown on cornmeal agar, and the Internal Transcribed Spacer (ITS) regions of the rRNA gene were amplified using primers ITS6 and ITS4. BLASTn searches of sequence data were conducted in GenBank to determine the most closely related *Phytophthora* spp. Sequences were then aligned and parsimony and distance analyses conducted in PAUP.

Results and Discussion

Based on phylogenetic analysis, ten potentially new and undescribed taxa of *Phytophthora* can be distinguished (Fig. 1). Several of these are morphologically indistinguishable from known species (eg *P. citricola*, *P. megasperma*, *P. cryptogea*, *P. drechsleri*). In some cases the new taxa are indeed most closely related to the known species (eg P.sp. 4 and *P. citricola*, P.sp. 5 and *P. cryptogea*). However, the DNA sequences of other new taxa show that they are not closely related to the morphologically similar species (eg P.sp. 3 and *P. drechsleri*, P.sp. 9 and *P. megasperma*). Multiple isolates have been sequenced for most of the new taxa, except for P.sp. 6 (two isolates) and P.sp. 10 (one).

One of the new species (P.sp. 2), with morphology similar to *P. citricola* but most closely related phylogenetically to *P. bisheria* and *P. multivesiculata*, has been isolated from the lower stem and roots of dying 1- to 2-year-old jarrah (*Eucalyptus marginata*) seedlings in rehabilitated open-cut bauxite mine pits¹. *Phytophthora inundata*, described in Europe in 2003, has been identified based on phylogenetic analysis from several locations in the south-west of WA where it has been associated with dying native plants (Table 1). Some of these isolates were recovered in the 1980s.

Further work is planned to describe the new taxa and their relationships, and to test their pathogenicity, so that an estimate of the level of threat they pose to native vegetation can be made.

Fig. 1. Phylogram showing ten putative new *Phytophthora* taxa (P.sp. 1-10) from Western Australia based on ITS DNA sequences, and representative *Phytophthora* species from seven clades after Cooke (2000)². Species known to exist in WA are shown in green text.

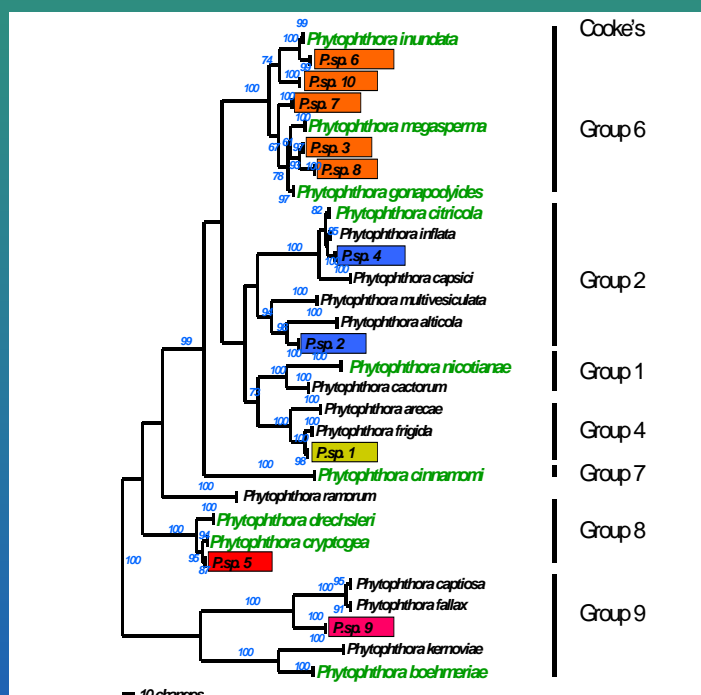


Table 1. Undescribed *Phytophthora* taxa and new records from Western Australia, with associated dying native plant species.

P.sp. 1	<i>Banksia menziesii</i> , <i>B. littoralis</i> , <i>B. attenuata</i>
P.sp. 2	<i>Eucalyptus marginata</i> , <i>B. grandis</i> , <i>Patersonia xanthina</i>
P.sp. 3/8	<i>E. marginata</i> , <i>B. attenuata</i> , <i>B. grandis</i>
P.sp. 4	<i>E. marginata</i> , <i>B. attenuata</i> , <i>B. grandis</i> , <i>B. littoralis</i> , <i>B. menziesii</i> , <i>B. prionotes</i> , <i>Xanthorrhoea gracilis</i> , <i>Podocarpus drouyniana</i> , <i>Patersonia</i> sp.
P.sp. 5	<i>B. grandis</i> , <i>X. gracilis</i> , <i>Patersonia</i> sp., <i>Crowea angustifolia</i> , <i>Isopogon buxifolius</i>
P.sp. 6	<i>Grevillea mccutcheonii</i> , <i>X. preissii</i>
P.sp. 7	<i>X. preissii</i>
P.sp. 9	<i>B. attenuata</i> , <i>Dryandra cirsioides</i> , <i>Isopogon</i> sp.
P.sp. 10	<i>B. prionotes</i>
<i>P. inundata</i>	<i>B. littoralis</i> , <i>X. preissii</i> , <i>Adenanthos cuneata</i>

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