

# Molecular Testing Uncovers New *Phytophthora* Taxa From Natural Ecosystems in Western Australia<sup>1</sup>

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## Abstract

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*, other isolates have been recovered on selective agar following the baiting of soil, or the direct plating of plant tissue, during these operations. These have been identified, using morphological characters, as *P. citricola*, *P. megasperma*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae*, and *P. boehmeriae*.

The recent advent and availability of DNA sequencing techniques for the identification of *Phytophthora* species 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.

DNA was extracted from pure cultures grown on cornmeal agar and the Internal Transcribed Spacer (ITS) regions of the rRNA 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. Based on phylogenetic analysis, seven potentially new and undescribed taxa of *Phytophthora* can be distinguished. Several of these are morphologically indistinguishable from known species (eg *P. citricola*, *P. megasperma*, and *P. cryptogea*). In some cases the new taxa are indeed most closely related to the known species (eg *P. citricola*), but in others their DNA sequences show that they are not closely related to the morphologically similar species (eg *P. megasperma*).

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

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.

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