A new homothallic *Phytophthora* from the jarrah forest in Western Australia

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Abstract. A homothallic *Phytophthora*, isolated at irregular intervals since the 1980s from Western Australian jarrah (*Eucalyptus marginata*) forest sites, has been previously misidentified as *P. citricola* based on morphological characters. Based on rDNA sequencing, it has been shown to be an undescribed species that is provisionally designated '*Phytophthora* sp. WA2' pending a full description. It has been associated with lower stem lesions and root necrosis on dying 1- to 2-year-old jarrah seedlings growing in rehabilitated open-cut bauxite mine pits.

Additional keywords: DNA sequencing, *Phytophthora cinnamomi*.

Mapping of the extent of *Phytophthora* dieback disease based on shadowless colour aerial photography (Shearer and Tippet 1989) is a continuing process in the management of the Western Australian jarrah (*Eucalyptus marginata*) forest and other ecosystems. The disease is caused by the water mould *Phytophthora cinnamomi* (Podger 1972) and verification of mapping involves the routine testing of soil samples collected from beneath dying, *Phytophthora* sensitive 'indicator species' for the presence of the pathogen. Testing for *Phytophthora* is also regularly carried out in rehabilitated open-cut bauxite mine pits in the jarrah forest 1–2 years after completion of rehabilitation operations (Colquhoun and Hardy 2000; Gardner and Stoneman 2003), based on sampling of dead and dying vegetation and associated soil (including direct plating from diseased tissue).

Other *Phytophthora* species including *P. citricola*, *P. megasperma*, *P. cryptogea*, *P. drechsleri* and *P. nicotianae* (Stukely *et al.* 1997), and at one location *P. boehmeriae* (D'Souza *et al.* 1997), have also been recovered, either alone or in the same samples as *P. cinnamomi*. *Phytophthora inundata* has recently been identified by DNA sequencing in samples collected from the Southern Jarrah Forest in 2005 and from *Banksia* heathlands on the South Coast in 1986 (Stukely *et al.* 2007).

The identity of homothallic isolates that had been identified as *P. citricola* (Waterhouse Group 3) based on the morphology of their sporangia, oogonia, oospores and related structures (Erwin and Ribiero 1996), but having distinct colony morphology on cornmeal agar plates, was tested by DNA sequencing. The isolates were found to constitute a new taxon, provisionally

designated '*Phytophthora* sp. WA2' pending a full description. This paper describes its isolation and essential features.

Samples of soil and root material were baited with *Eucalyptus sieberi* cotyledons (Marks and Kassaby 1974), which were plated after 5 or 10 days onto P₁₀ VPH (Tsao and Guy 1977) or NARPH (Hüberli *et al.* 2000) selective agar, from which pure cultures were then isolated. Roots of dying *E. marginata* plants from rehabilitated mine pits were surface-sterilised in 70% ethanol and rinsed four times in distilled water before being cut into short lengths (1.0–1.5 cm) and plated onto selective media as above.

Cultures grown on cornmeal agar (BBL, Beckton, Dickinson and Co.) consistently had sparse, rough-textured but uniform growth patterns at the margins and within the colonies. They clearly differed from the denser, radiate colonies with well-defined circular margins and narrow lanceolate central sectors that are typical of *P. citricola* (Waterhouse and Waterston 1966). Growth rates on cornmeal agar at 25°C were similar to those of *P. citricola*.

Production of sporangia was induced by suspending freshly colonised squares of 10% V8 juice agar in non-sterile soil extract at 25°C in daylight. In total, 7–10 sporangia were measured for each of 10 isolates (VHSC 13482, 13502, 13559, 13523, 13607, 13615, 13663, 13694, 13708, 13713). The mean dimensions across the 10 isolates were: length 38.0 µm (s.e. 1.45 µm) and breadth 21.5 µm (s.e. 0.87 µm), with a length/breadth ratio of 1.5–2.2. Sporangia were consistently ovoid-obpyriform, and semi-papillate (exit pore average 6.95 µm diam.). Lateral attachment of sporangia, variable sporangia shape, and basal swellings of sporangiophores were

all observed occasionally. No chlamydospores were observed. In pure cultures of single isolates grown on 10% V8 juice agar ($n > 100$ examined), abundant oospores were formed, with only paragynous antheridia being observed. The species is thus homothallic. In total, 7–10 organs were measured in each of the above 10 isolates. The mean dimensions across the 10 isolates were: oogonium diameter $28.2\ \mu\text{m}$ (s.e. $0.40\ \mu\text{m}$) and oospore diameter $25.8\ \mu\text{m}$ (s.e. $0.41\ \mu\text{m}$). Oospores were slightly apleurotic, with a mean wall thickness of $2.5\ \mu\text{m}$ (range 2.2 – $2.7\ \mu\text{m}$). These dimensions are generally within the published range for *P. citricola* (Erwin and Ribiero 1996), except that the oospore walls of these isolates are thicker (*P. citricola* mean $1.5\ \mu\text{m}$), and antheridia are considerably broader at 12 – $14\ \mu\text{m}$ (*P. citricola* 7 – $8\ \mu\text{m}$).

Subcultures (10 isolates) grown on cornmeal agar at 25°C for 8 days were used for DNA extractions. DNA was extracted using the method described by Graham *et al.* (1994) and the internal transcribed spacer (ITS) regions of the rDNA were amplified using primers ITS6 and ITS4 (Cooke *et al.* 2000). ITS regions were sequenced using the same primers and BigDye 3.1 technology (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

Sequence comparison with GenBank accessions (Altschul *et al.* 1997) and further sequence analysis showed that *Phytophthora* sp. WA2 is well resolved within the major ITS clade 2 of *Phytophthora* (*sensu* Cooke *et al.* 2000). *Phytophthora* sp. WA2 is most closely related to *P. bisheria* sp. nov. (Abad *et al.* 2007) and *P. multivesiculata*. Differences within the complete ITS and 5.8 S rDNA regions are up to one nucleotide between isolates of *Phytophthora* sp. WA2, and are 18–21 nucleotides and 27–29 nucleotides between *Phytophthora* sp. WA2 and *P. bisheria* and *P. multivesiculata*, respectively. Bunny (1996) used isozyme analysis to separate Australian collections that had initially been identified as *P. citricola* into three discrete subgroups, one of which (SG1) we identify as *Phytophthora* sp. WA2. A single isolate from the same subgroup and study was sequenced independently of this work and was also putatively identified as an undescribed species (D. E. L. Cooke, pers. comm.). In a study of isozyme subgroups within *P. citricola* by Oudemans *et al.* (1994), one isozyme subgroup included the type culture of *P. citricola* (IMI21173) and a Western Australian isolate recovered from *Citrus aurantium* (IMI129904). In Bunny (1996), isolate IMI129904 falls into a different subgroup than the SG1 (= *Phytophthora* sp. WA2) isolates.

VHSC isolates with these sequences have been designated '*Phytophthora* sp. WA2' pending their full description. GenBank accessions for the complete ITS sequences from two isolates of *Phytophthora* sp. WA2 are EF121961 (isolate VHSC13615, WAC13038) and EF121962 (isolate VHSC13713, WAC13039).

Phytophthora sp. WA2 has been isolated frequently from samples collected in rehabilitated open-cut bauxite mine pits in the Northern Jarrah Forest since 2003. Unusually large numbers of isolations were made from the Dwellingup area, on the Darling Scarp ~100 km south-east of Perth, in the summer of 2004–05. DNA sequencing has been carried out on isolates with similar colony forms from the WA Department of Environment and Conservation culture collection (VHSC), dating back to the mid-1980s. This has shown that *Phytophthora* sp. WA2 has also

been isolated from forest locations up to 180 km apart, which were not associated with mining disturbance, in the Dwellingup and Bridgetown-Nannup districts (in the Northern and Southern Jarrah Forests, respectively).

The pathogenicity of *Phytophthora* sp. WA2 to native flora in the jarrah forest and elsewhere in Western Australia is unknown and requires investigation. It appears to have significant pathogenic ability with *E. marginata* as it has frequently been associated with lower stem lesions and root necrosis on dying 1- to 2-year-old seedlings growing in rehabilitated bauxite mine pits near Dwellingup, where intensive sampling has been carried out. Its distribution appears to be widespread in the south-west of the state, and further testing of old and new collections will provide additional information on its distribution and host range.

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