New and Undescribed *Phytophthora* Species in Natural Ecosystems in WA: Position Statement and Recommendations

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1. Background

Traditionally, *Phytophthora* isolates have been identified in pure culture by microscopic examination of their morphological characters, and checking these morphological data against published taxonomic keys. Based upon these methods, the following seven species were previously known to be present in WA natural ecosystems: ***P. cinnamomi, P. citricola, P. megasperma, P. cryptogea, P. drechsleri, P. nicotianae*** (Stukely *et al.*, 1997), and ***P. boehmeriae*** (D'Souza *et al.*, 1997). Often, new isolates obtained by the Vegetation Health Service (VHS) cannot be satisfactorily identified to species – either because they are sterile in culture (and formation of the necessary structures cannot be induced), or because there is not a unique or unequivocal match to any of the published species on the keys.

The adoption and use of DNA sequencing techniques for the identification of *Phytophthora* species is causing something of a revolution in *Phytophthora* taxonomy, worldwide. These methods are providing a far more precise definition of the species, and of their phylogenetic relationships. As a result, several new *Phytophthora* species have been described recently, in various countries, including four species that are causing declines in eucalypts (see notes, **Section 6**). The availability of these DNA techniques in WA, through the **Centre for *Phytophthora* Science and Management** (CPSM) at Murdoch University, has enabled the testing of some WA isolates that could not be identified satisfactorily by traditional morphological examination. New isolates as well as historical isolates from the VHS Culture Collection (some dating back to the 1980s) have been tested, with some surprising results.

> DNA sequencing and data analysis: DNA was extracted from pure cultures of *Phytophthora* grown on cornmeal agar, and the Internal Transcribed Spacer (ITS) regions of the rDNA 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. **Phylogenetic trees** were constructed (see **Attachment**).

2. New and Undescribed Phytophthoras in WA

We have now received ITS rDNA sequence data for a total of over 160 recent and historical VHS isolates of *Phytophthora* tested by CPSM. Most were isolated from samples collected in natural ecosystems in WA (see also: VHS Annual Report, Stukely *et al.* 2007). In brief:

- There are now known to be as many as **ten undescribed new species of *Phytophthora*** present in natural ecosystems in WA (designated "**P. spp. 1 - 10**"). See the **phylogenetic tree** (**Attachment**); explanatory notes are given in **Section 6** (p. 4).
- All ten undescribed *Phytophthora* species have been found associated with dying plants in native forest or heath-land in WA (**Table 1**).
- For most of the new taxa, multiple isolates have been identified (more than five), and many are distributed across several DEC Regions.
- Two new records (for WA) of other known *Phytophthora* species (***P. inundata*** and ***P. gonapodyides***) have been confirmed. *P. inundata* was associated with dying native plants in several locations.
- Publications produced (see **Section 5**): 'P.sp.2' in WA – Stukely et al. 2007; New *Phytophthora* taxa in WA (poster) – Stukely et al. 2007; *P. inundata* in WA – Stukely et al. 2007.

Table 1 shows the associations of the 'new' *Phytophthora* taxa with dying native plant species that have been recorded in WA so far. Distribution maps are now being produced.

Table 1. Undescribed *Phytophthora* taxa (P. spp. 1 – 10) and new species records from Western Australia to 30th June 2007, with associated dying native plant species.

***Phytophthora* sp.**	**Dying native plant species**
P. sp. 1	*Banksia menziesii, B. littoralis, B. attenuata*
P. sp. 2	*Eucalyptus marginata, B. grandis, Patersonia xanthina*
P. sp. 3 / 8	*E. marginata, B. attenuata, B. grandis*
P. sp. 4 *'P. citricola'*	*E. marginata, B. attenuata, B. grandis, B. littoralis, B. menziesii, B. prionotes, Xanthorrhoea gracilis, Podocarpus drouyniana, Patersonia* sp.
P. sp. 5	*B. grandis, X. gracilis, Patersonia* sp., *Crowea angustifolia, Isopogon buxifolius*
P. sp. 6	*Grevillea mccutcheonii, X. preissii*
P. sp. 7	*X. preissii*
P. sp. 9	*B. attenuata, Dryandra cirsioides, Isopogon* sp.
P. sp. 10	*B. prionotes*
***P. inundata*[1]**	*B. littoralis, X. preissii, Adenanthos cuneata*

[1]***P. inundata*** – This species was formally named only recently (Brasier *et al.* 2003). It was first reported in Australia in 2006, from Victoria (Cunnington *et al.* 2006), where it was not associated with plant disease although isolated from soil in horticultural sites. However, in Europe and South America, *P. inundata* is a recognised pathogen of woody trees and shrubs, including *Olea, Prunus, Salix* and *Vitis*. Some of these known host species are grown commercially in WA.

3. Management Implications and the Need for Further Investigation

The implementation of **Best Practice methods and standards** for managing *Phytophthora* dieback is based upon the key steps of detection, diagnosis, demarcation and mapping of infested areas, and hence the identification of non-infested areas (CALM, 2002). This enables appropriate measures to be applied to protect non-infested areas, on a priority basis, where this is feasible.

Current management and interpretation procedures rightly focus primarily on *P. cinnamomi* (Interpreter Guidelines, CALM 2001). Guidelines on demarcation of "Other *Phytophthora* species" state that the infested area is "not usually taped unless it has significant impact" (ie, such areas are often treated as **non-infested**). There is now a compelling case for this to be revised so as to minimise the spread of **all** Phytophthoras from infested sites, irrespective of the current level of impact observed on those sites. Recommendations are given in **Section 4.**

It is not known whether the newly discovered *Phytophthora* taxa are **indigenous or were introduced** to WA ecosystems, or if **hybrids** may be involved. It appears that they are not particularly new introductions, with at least some isolates of most taxa having been obtained from samples collected in WA in the 1980s and 1990s, as well as more recently.

It is likely that the 'new' Phytophthoras will all have at least some level of pathogenic ability, and so it can be expected that they will damage native vegetation under some circumstances. It is known, for example, that the *'P. megasperma'* group causes most damage following summer rainfall in coastal heath-land. Clearly, this high impact occurs at irregular intervals. It is also likely that increased levels of human activity in and around infested areas in the short term, together with the consequences of climate change and other stressors in the longer term, will exacerbate the effects and impact of at least some of these Phytophthoras on native flora.

Some of the 'new' taxa, but not all of them, are associated mostly with low-impact dieback sites (eg 'P.sp.4', which had previously always been identified as *P. citricola*). However, **it is very important at this stage that they should not all be treated as 'indigenous' (and hence not threatening), until they are investigated further.** In one recent case, an undescribed *Phytophthora* ('P.sp.6') has been associated with deaths of the rare and endangered *Grevillea mccutcheonii* in a site where *P. cinnamomi* is not present (Table 1).

The key point is that the introduction of any *Phytophthora* to a previously non-infested site could lead to unexpectedly high impacts, given the right conditions and host plants.

Some of the ten undescribed new *Phytophthora* taxa are indeed closely related to the named species that they resemble morphologically (eg 'P.sp.4' and *P. citricola*; 'P.sp.5' and *P. cryptogea*) (see **Attachment**). However, several are genetically very distinct from all of the named Phytophthoras found to date in natural ecosystems in WA (eg 'P.sp.1', 'P.sp.2', 'P.sp.9'), despite having strong physical similarities in culture to the known Phytophthoras. The '*P. megasperma*' group or morphospecies, as previously suggested, is very diverse genetically and includes at least three undescribed taxa that are distinct from *P. megasperma*.

These unexpected phylogenetic differences among the new taxa are a particular cause for concern. The differences suggest that some of the 'new' Phytophthoras may have a distinct set of capabilities, strengths and vulnerabilities, and may pose different immediate and potential levels of threat to biodiversity. These questions must now be investigated.

It is imperative that these undescribed Phytophthoras are all properly described and documented as separate, individual components of our biodiversity.

The undescribed Phytophthoras are all potential pathogens, as has already been indicated by their associations with dying native flora (**Table 1**). It must be remembered that no *Phytophthora* has yet been documented as a "benign" organism – all are pathogens, as their name implies. Detailed investigations must therefore be conducted on each new species to determine its host range, pathogenicity, distribution, ease of spread, preferred environmental conditions, and other characteristics such as its response to the inhibitor, phosphite.

New research projects that are now starting (CPSM, Murdoch University, with input by VHS) will provide answers to some of the key questions above. **An estimate of the level of threat that each *Phytophthora* poses to our biodiversity is required,** so decisions can then be made, based on sound science, as to whether specific management strategies involving intervention are appropriate or necessary. Controls that are applied currently for *Phytophthora cinnamomi* (such as phosphite application) may not always be appropriate, nor directly applicable without modification, for managing the 'new' *Phytophthora* species.

Information on the distribution of <u>all</u> *Phytophthora* species must be up-to-date, for land management to be most effective. Intervention (if deemed appropriate) at the earliest possible opportunity, to confine and possibly eradicate small *Phytophthora* infestations in priority areas, can be expected to pay very large dividends in future nature conservation efforts, and could also significantly benefit the commercial sector.

The analogy must be drawn here to *Phytophthora cinnamomi*. Localised dieback symptoms were first recorded in the Jarrah forest in the 1920s, but their cause was not discovered until over 40 years later – and by then, irreparable environmental damage had been set in motion, following the uncontrolled spread of the pathogen. Today, we have the benefit of rapidly increasing knowledge of the suite of widely different Phytophthoras that are also present as potentially serious pathogens in native vegetation, and we should now heed the warning and treat them with caution. We should minimise their opportunities to reach non-infested areas.

4. Recommendations

- The undescribed *Phytophthora* species (and also 'other' named species) should now all be regarded as a threat, and managed accordingly, until it is proven otherwise.
- Known infested areas should be identified and demarcated for **<u>all</u>** *Phytophthora* spp.
- Consistent efforts should be made to prevent the spread of **<u>all</u>** Phytophthoras to protectable non-infested areas, irrespective of their present levels of expression and impact in known infested areas.
- Efforts should continue through the VHS and CPSM to identify and document the new Phytophthoras, and research projects investigating them should receive continued support (via CPSM).

This is clearly an issue that now needs to be addressed by land managers with a well-considered, adaptive management approach. **Guidelines** should be amended accordingly.

5. References

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6. Attachment; Notes and Acknowledgment

1. **Phylogenetic tree (based on ITS rDNA sequences) showing a limited selection of the known species of *Phytophthora*, and ten undescribed WA taxa (indicative only).**

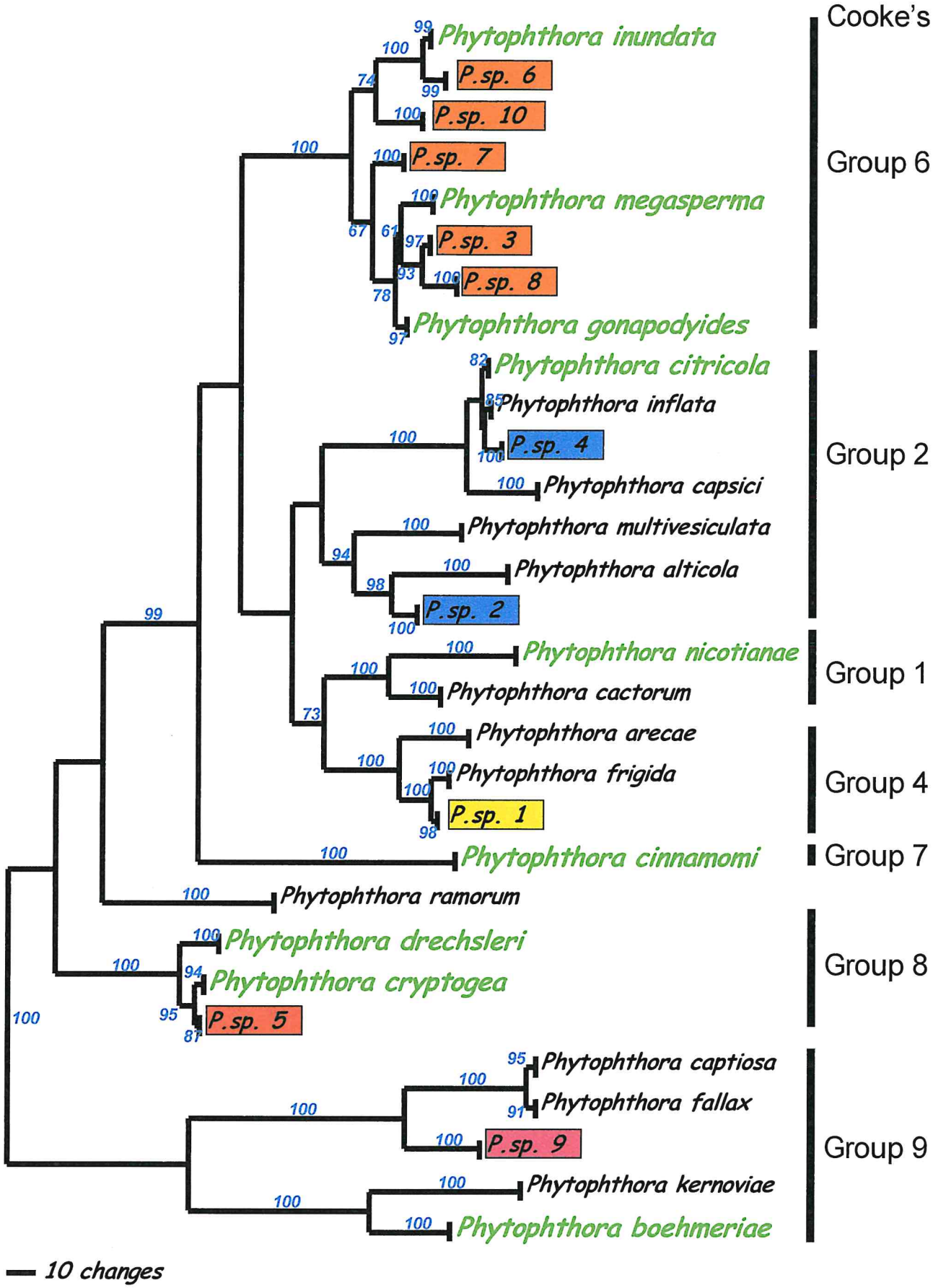
   The ten undescribed taxa isolated from WA samples ("**P. spp. 1-10**") are colour-coded according to the groupings proposed by Cooke *et al.* (2000), and Cooke's Groups 1, 2, 4, and 6-9 are shown at the right.

   Phytophthora species shown in green text are those known previously to be present in WA natural ecosystems, based on the traditional morphological identification of pure cultures (Stukely *et al.* 1997; D'Souza *et al.* 1997).

   Other species shown include those most closely related to each of the 'new' WA taxa, based on analysis of their ITS rDNA sequences, from the GenBank database.

   Four newly described *Phytophthora* species causing declines of eucalypts, in South Africa (*P. alticola, P. frigida* – Maseko *et al.* 2007) and New Zealand (*P. captiosa, P. fallax* – Dick *et al.* 2006), are also shown.

   Acknowledgment: Analysis of rDNA sequence data and the construction of the phylogenetic tree were carried out by Dr Treena Burgess (Centre for *Phytophthora* Science and Management, Murdoch University).


Phytophthora inundata
P.sp. 6
P.sp. 10
P.sp. 7
Phytophthora megasperma
P.sp. 3
P.sp. 8
Phytophthora gonapodyides
Phytophthora citricola
Phytophthora inflata
P.sp. 4
Phytophthora capsici
Phytophthora multivesiculata
Phytophthora alticola
P.sp. 2
Phytophthora nicotianae
Phytophthora cactorum
Phytophthora arecae
Phytophthora frigida
P.sp. 1
Phytophthora cinnamomi
Phytophthora ramorum
Phytophthora drechsleri
Phytophthora cryptogea
P.sp. 5
Phytophthora captiosa
Phytophthora fallax
P.sp. 9
Phytophthora kernoviae
Phytophthora boehmeriae
Cooke's
Group 6
Group 2
Group 1
Group 4
Group 7
Group 8
Group 9
10 changes