New species from *Phytophthora* Clade 6a: evidence for recent radiation

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**Key words**
- biodiversity hotspot
- heathland
- native vegetation

**Abstract** During routine vegetation health surveys in the southwest of Western Australia (SWWA), several *Phytophthora* isolates with affinity to Clade 6a have been recovered. In this study, all known taxa from Clade 6a, *P. inundata*, *P. humicola*, *P. gemini*, *P. ‘walnut’* and *P. ‘personi’*, and the new isolates were compared based on morphology and DNA sequence data from three nuclear genes and two mitochondrial genes resulting in the description of five new species, *P. balyanboodja*, *P. kondillina*, *P. cooljarloo*, *P. kwongonina* and *P. pseudorosacearum*. With the exception of *P. gemini* and *P. humicola*, all species from Clade 6a have been recovered from natural ecosystems in SWWA. These species are morphologically similar, with predominantly ovoid sporangia and nested and extended internal proliferation. If oospores are present, they tend to be aplerotic with paragynous antheridia mostly attached adjacent to the oggonial stalk. They can all grow at 35 °C and have a fast growth rate on most agar media. These species have all been recovered from the rhizosphere soil and dead and dying plants within dry kwongon heathlands, often from water gaining sites and frequently from very isolated areas. The radiation, origin and potential ecological role of these species are discussed.

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**INTRODUCTION**

Before molecular systematics became commonplace, there were approximately 60 described species of *Phytophthora* (Cooke et al. 2000, Erwin & Ribeiro 1996). Clade 6 was represented by three species: *P. gonapodyides*, *P. megasperma* and *P. humicola*, described in 1927, 1931 and 1985, respectively (Buismann 1927, Drechsler 1931, Ko & Ann 1985). Post 2000, 108 new species have been described of which 20 reside in Clade 6, which is now divided into three sub-clades. Clade 6b is the largest clade with 18 described species and numerous designated but undescribed taxa. Clade 6c is represented by a single species *P. asparagi* (Ganke et al. 2012), *Phytophthora inundata* (Brasier et al. 2003b), *P. gemini* (Man in’t Veld et al. 2011) and *P. rosacearum* (Hansen et al. 2009) now cluster with *P. humicola* in Clade 6a. Two designated but undescribed taxa also reside in Clade 6a, *P. ‘personi’* and *P. ‘walnut’*.

Most Clade 6b species are considered aquatic specialists (Jung et al. 2011), and although many have been reported as pathogens, there are generally contributing factors such as extensive flooding associated with the disease reports. The exception within this sub-clade is *P. pinifolia*, a serious foliar pathogen of *Pinus radiata* in Chile (Durán et al. 2010). All species from Clade 6a have been reported as associated with woody plants, and while species such as *P. inundata* and *P. gemini* are commonly found in brackish water, other species do not appear to have the same dominant aquatic lifestyle.

Routine surveys of dying natural vegetation in the southwest of Western Australia (SWWA), have recovered numerous new *Phytophthora* species (Burgess et al. 2009), 15 of which have now been described including eight species from Clade 6b. However, several isolates with affinity to Clade 6a have also been recovered. In this study, all known taxa from Clade 6a and the new isolates were compared based on morphology and DNA sequence data from three nuclear genes and two mitochondrial genes resulting in the description of five new species, *P. balyanboodja*, *P. kondillina*, *P. cooljarloo*, *P. kwongonina* and *P. pseudorosacearum*.

**MATERIAL AND METHODS**

*Phytophthora* isolates

Isolates obtained from soil and root samples collected beneath dying *Phytophthora*-susceptible species in native ecosystems, parks and reserves were provided by the Vegetation Health Service at the Western Australian Department of Biodiversity, Conservation and Attractions or the Centre of Phytophthora Science and Management, Murdoch University. Additional isolates were obtained from CBS (Westerdijk Fungal Biodiversity Institute, Utrecht) and the World Phytophthora Collection (WPC). Isolates were maintained in 90 mm Petri dishes on V8 agar (V8A, 0.1 L filtered V8 juice, 17 g agar, 0.1 g CaCO₃, 0.9 L distilled water) and on 5 mm V8A discs stored in 20 mL sterile water in McCartney bottles at room temperature. All isolates used in this study are detailed in Table 1.

**DNA isolation, amplification and sequencing**

The *Phytophthora* isolates were grown on half-strength potato dextrose agar PDA (19 g PDA Becton, Dickinson and Company, Sparks, MD 21152, USA, 7.5 g of agar and 1 L of distilled water) at 20 °C for 2 wk in the dark, and the mycelium was harvested by scraping from the agar surface with a sterile blade and placed in a 1.5 mL sterile Eppendorf® tube. The mycelia were frozen...
Identity, host information, collection location, date, and GenBank accession numbers for Phytophthora spp. considered in this study.

| Isolate | Identity | Substrate | Host | Location | Date | GenBank Accession no. |
|---------|----------|-----------|------|----------|------|-----------------------|
| CBS 143058 | P. balyanboodja | Soil | Native vegetation | Australia, WA, Alfred Cove | 2011 | KJ372258 MF326806 MF326892 MF326882 MF326927 |
| VHS25567 R3 | P. balyanboodja | Soil | Native vegetation | Australia, WA, Alfred Cove | 2011 | KJ372259 MF326807 MF326893 MF326883 MF326926 |
| MUC768 | P. condilina | Water | Native vegetation | Australia, WA, Esperance | 2008 | HQ012959 MF326808 HQ012927 HQ012883 MF326923 |
| MUC769 | P. condilina | Water | Native vegetation | Australia, WA, Esperance | 2008 | HQ012960 MF326809 HQ012928 HQ012884 MF326924 |
| MUC806 | P. condilina | Soil | Casuarina obesa | Australia, WA, Alfred Cove | 2011 | KC748465 MF326810 MF326867 MF326839 MF326917 |
| MUC807 | P. condilina | Soil | Casuarina obesa | Australia, WA, Alfred Cove | 2011 | KJ372264 MF326811 MF326870 MF326840 MF326918 |
| VHS19278 | P. condilina | Soil | Native vegetation | Australia, WA, Ravensthorpe | 2008 | JN547640 MF326812 MF326872 MF326843 MF326920 |
| VHS25241 | P. condilina | Soil | Casuarina obesa | Australia, WA, Alfred Cove | 2011 | KJ372263 MF326813 MF326858 MF326842 MF326919 |
| VHS25242 | P. condilina | Soil | Eucalyptus wandoo | Australia, WA, Lake Toolibin | 2013 | KJ372266 MF326815 MF326871 MF326844 MF326916 |
| HAS2313 | P. cooljarloo | Swamp | Native vegetation | Australia, WA, Cooljarloo | 1996 | HQ012961 MF326817 HQ012929 HQ012885 MF326911 |
| CBS 143062 | P. cooljarloo | Soil | Hibbertia sp. | Australia, WA, Cooljarloo | 2008 | HQ012957 MF326816 MF326872 MF326841 MF326920 |
| CBS123381 | P. gemini | Seed | Zostera marina | Taiwan | 1981 | AF266792 MF326818 MF326891 MF326850 MF326932 |
| WPC P6702 | P. humicola | Citrus | Praseoclus sp. | Taiwan | | FJ801938 JN359945 JN359946 JN359947 JN359948 |
| DDS3481 | P. inundata | Soil | Native vegetation | Australia, WA, Northern Sandplains | 1991 | KJ372261 MF326819 MF326844 MF326845 MF326912 |
| IMI 390121 | P. inundata | Roots | Osea sp. | Spain, Seville, Ecija | 1996 | EF210201 EF210203 JN935947 EF210207 JN936043 |
| VHS1638 | P. inundata | Soil | Xanthorrhoea preissii | Australia, WA, Boyup Brook | 2007 | HQ012944 MF326820 MF326860 HQ012885 MF326926 |
| VHS190812 | P. inundata | Soil | Banksia attenuata | Australia, WA, Bold Park | 2008 | HQ012945 MF326821 MF326866 HQ012886 MF326922 |
| DSD3999 | P. kwongonina | Soil | Xanthorrhoea platypylta | Australia, WA, Fitzgerald River NP | 1993 | EU593258 MF326822 MF326875 MF326846 MF326913 |
| IMI 326669 | P. kwongonina | Roots | Banksia priorutes | Australia, WA, Cervantes | 1986 | EU593265 MF326823 HQ012932 HQ012889 MF326912 |
| CBS 1430601 | P. kwongonina | Soil | Banksia grandis | Australia, WA, Bunbury | 2010 | JN547636 MF326824 MF326876 MF326847 MF326914 |
| HAS1959 | P. lacustis | Soil | Native vegetation | Australia, WA, Welshpool | 1994 | HQ012956 JN547618 HQ012924 HQ012880 JN547706 |
| HAS2530 | P. pseudosoraceain | Swamp | Native vegetation | Australia, WA, Cooljarloo | 1998 | HQ012963 MF326825 HQ012931 HQ012887 MF326908 |
| VHS24266 | P. pseudosoraceain | Soil | Xanthorrhoea platypylta | Australia, WA, Albany | 2010 | JN547637 MF326826 MF326877 MF326857 MF326909 |
| CBS 143061 | P. pseudosoraceain | Soil | Persoonia longifolia | Australia, WA, Jarrahdale | 2013 | KJ372267 MF326827 MF326876 MF326858 MF326907 |
| CBS 124696 | P. rosaceain | Malus domestica | USA, California | | | |
| AHS1658 | P. rosaceain | Swamp | Native vegetation | Australia, WA, Cooljarloo | 1993 | KJ372274 MF326830 MF326884 MF326851 MF326906 |
| IMI 389749 | P. rosaceain | Malus domestica | USA, California, Sonoma County | 1979 | AF541911 JN35980 JN359852 JN35985 JN36032 JN36033 |
| OSU55 | P. rosaceain | Prunus armerica | USA, Maryland | | | |
| OSU62 | P. rosaceain | Prunus avium | USA, California | | | |
| OSU63 | P. rosaceain | Prunus domestica | USA, California | | | |
| OSU65 | P. rosaceain | Malus domestica | USA, California | | | |
| DDS2909 | P. rosaceain | Soil | Pinus radiata | Australia, WA, Albany | 1998 | HQ012958 HQ012930 HQ012888 MF326890 MF326905 |
| HAS1650 | P. rosaceain | Swamp | Native vegetation | Australia, WA, Cooljarloo | 1993 | KJ372268 MF326829 MF326886 MF326850 MF326906 |
| HAS2529 | P. rosaceain | Swamp | Native vegetation | Australia, WA, Cooljarloo | 1998 | HQ012961 MF326831 HQ012930 HQ012886 MF326899 |
| VHS25476 | P. rosaceain | Soil | Banksia repens | Australia, WA, Wellstead | 2011 | KJ372269 MF326838 MF326881 MF326850 MF326897 |
| VHS6186 | P. rosaceain | Soil | Native vegetation | Australia, WA, Manjimup | 1999 | JN547638 MF326837 MF326879 MF326849 MF326900 |
| CBS127954 | P. thermophila | Soil | Eucalyptus marginata | Australia, WA, Dwellingup | 2004 | EU301155 JN547613 HQ012916 HQ012872 JN547709 |
| MUC767 | P. 'personii' | Water | Native vegetation | Australia, VIC, Ti-Tree Creek | 2008 | HQ012958 MF326804 MF326889 MF326881 MF326900 |
| SA728 | P. 'personii' | Soil | Rubus anglocandicans | Australia, WA, Walpole | | |
| VHS14801 | P. 'personii' | Soil | Grevillea mucrotheoni | Australia, WA, Busselton | 2005 | EU301169 MF326805 MF326890 HQ012877 MF326928 |
| IMI 389735 | P. 'walnut' | Juglans hindii | USA, California, Merced County | 1988 | AF541910 JN35980 JN359856 JN359871 JN36042 |

1 Ex-type isolates.
2 Isolated not included in the morphological studies.
in liquid nitrogen and crushed to a fine powder, and genomic DNA was extracted using ZR Fungal/Bacterial DNA Miniprep™ (Zymo Research, Irvine, California, CA). For all isolates, five gene regions were amplified and sequenced:

i. the region spanning the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal DNA was amplified using the primers DC6 (Cooke et al. 2000) and ITS-4 (White et al. 1990);

ii. the mitochondrial gene cox1 (COX) was amplified with primers FM77 and FM 84 (Martin & Tooley 2003);

iii. heat shock protein 90 (HSP) was amplified with HSP90-F and HSP90-R1 primers (Blair et al. 2008);

iv. β-tubulin (TUB) was amplified with BTF1A and BTR1 primers; and

v. NADH dehydrogenase subunit 1 was amplified with NADH-F1 and NADH-R1 primer (Kroon et al. 2004).

The PCR reaction mixture contained 12.5 μL GoTaq® Green Master Mix 2X (Promega Corporation, Madison, Wisconsin, USA), 0.5 μL of each primer (10 μM), 10 μL water and 1.5 μL of DNA. PCR conditions were 3 min at 94 °C, 35 cycles of 30 s at 95 °C, 30 s at annealing temperature and 60 s at 72 °C with a final extension of 5 min at 72 °C. Annealing temperature was 55 °C for ITS, 60 °C TUB and HSP and 52 °C for COX and NADH. All gene regions were sequenced in both directions with primers used in amplification. PCR and sequencing products were cleaned using Sephadex® G-50 columns as described previously (Sakalidis et al. 2011). All sequences derived in this study were added to GenBank and accession numbers are provided in Table 1.

**Phylogenetic analysis**

Excluding outgroups, the aligned datasets for Clade 6a consisted of sequences from 41 isolates, representing new species from SWWA, four known species and two undescribed taxa (Table 1). Isolates of two species from Clade 6b, *Phytophthora laucistris* (HSA1959) and *P. thermophila* (CBS 127954) were included as outgroup taxa. Sequences were mostly obtained during this study, but some were obtained from GenBank (http://www.ncbi.nlm.nih.gov). Sequence data were compiled and manually edited in Geneious v. 10 (Biomatters; available from http://www.geneious.com/). Analysis was conducted for each gene region separately and on the concatenated nuclear (ITS, TUB and HSP) or mitochondrial (COX and NADH) gene regions. Phylogenetic analyses of sequence data were performed within Geneious software using plugins for Bayesian analysis using MrBayes (Ronquist et al. 2011). Alignment files and resultant phylogenetic trees are available from Dryad Digital Repository (http://datadryad.org/).

**Colony morphology, growth rates and cardinal temperatures**

Morphology and colony growth, and colony growth patterns of representative isolates (Table 1) were defined from 10-d-old cultures grown at 20 °C in the dark on V8A, malt extract agar (MEA) (20 g malt extract, 17 g agar and 1 L distilled water), carrot agar (CA) (0.1 L filtered carrot juice, 17 g agar and 0.9 L distilled water) and half-strength PDA (all from BBL, Becton, Dickinson & Co, Sparks MD 21152, USA). Circular inoculum plugs (5 mm diam) were taken from the margin of 10-d-old cultures on V8A and placed in the centre of 90 mm Petri dishes of the test media. Colony morphology was described according to Erwin & Ribeiro (1996).

For temperature-growth relationships, representative isolates (Table 1) were sub-cultured onto V8A plates and incubated for 24 h at 20 °C to stimulate onset of growth. Then three replicate plates per isolate were transferred to 5, 10, 15, 20, 25, 30, 32.5, 35 and 37.5 °C. Radial growth rate was measured 4–7 d after the onset of linear growth, along two lines crossing the middle of the inoculum plug at right angles, and the mean growth rates (mm per day) were assessed. Plates with no colony growth were returned to 20 °C for 7 d to check the isolate viability.

**Morphology of sporangia and gametangia**

Morphological features of representative isolates (Table 1) were examined. Sporangia were produced by flooding 15 × 15 mm square agar plugs, removed from the growing edge of 3–5-d-old colonies on V8A in 90 mm Petri dishes, with V8 broth (100 mL clarified V8 juice and 900 mL distilled water) at 18–25 °C with their surfaces submerged, in natural daylight for 4 h. This broth was then decanted and replaced with filtered tap water, which was decanted and replaced thrice (every 2–3 h). In the final change, 0.2 mL of non-sterile soil extract was also added and the Petri dishes were incubated overnight. The soil extract was made by suspending 10 g of rhizosphere soil from beneath a *Quercus* sp. in 100 mL distilled water and incubated for 12 h at 20 °C. The supernatant from the soil extract was added directly to the Petri dishes. After 18–24 h, dimensions and characteristic features of 50 mature sporangia of each isolate, selected at random, were ascertained at 400× magnification (BX51 Olympus). After 3–10 d, 25 hyphal swellings and 50 chlamydospores, if formed, were also measured.

Isolates grown in the dark on V8A plates supplemented with 10 mg/mL Beta-Sitosterol, a plant sterol shown to induce oospore formation in oomycetes (Ribeiro et al. 1975), at 25 °C for up to 30 d were examined for the presence of oogonia. Isolates which did not produce oogonia in single culture were paired on V8A with isolates of the same species and with A1 and A2 tester strains of *P. cinnamomii* (MP94-48, DCE25, respectively). Inoculum plugs (5 mm diam) of the isolate to be tested and the tester isolate were placed on opposite sides of a 9 cm Petri dish, 2 cm from the edge. The plates were incubated at 20 °C in darkness and scored for oogonial formation 30 d after the two colonies had met. For each isolate producing oogonia (either in single culture or when paired), dimensions and characteristic features of 50 mature oogonia, oospores and antheridia chosen at random were measured at ×400. The oospore wall index was calculated as the ratio between the volume of the oospore wall and the volume of the entire oospore (Dick 1990).

**RESULTS**

**Phylogenetic analysis**

The alignments for TUB, HSP, ITS, COX and NADH were consisted of 1187, 957, 826, 1196 and 864 characters, respectively. Trees for the individual datasets produced similar topology (doi: https://doi.org/10.5061/dryad.d22g0) and the nuclear and mitochondrial gene regions were combined separately for the analyses presented here.

Excluding outgroups, the percentage similarity between taxa in Clade 6a ranged from 87 to 99.3 % for concatenated nuclear gene regions and 90.5 to 99.1 % for concatenated mitochondrial gene regions (Table 2). *Phytophthora balyanbooda* and *P. gemini* were the most different to each other and to all other taxa in the clade (Table 2). There are two groups of closely related species (>98 % similarity): i) *P. condilina, P. humicola* and *P. inundata*; and ii) *P. cooljarloo, P. kwongonina, P. rosacearum* and *P. pseudorosacearum*.

Support for terminal clades and their clustering was equivalent in both analyses and the Bayesian analysis is presented here (Fig. 1–2). All species reside in highly supported terminal clusters, the two groups of species previously recognised in Clade 6a (Jung et al. 2011) are reinforced by the addition of
new isolates and species. *Phytophthora ‘walnut’* is basal to the first group which also contains *P. cooljarloo, P. kwongonina, P. rosacearum* and *P. pseudorosacearum*. *Phytophthora gemini* is basal to the second group which contains *P. condilina, P. humicola, P. inundata, P. balyanboodja* and *P. ‘personii’. *Phytophthora rosacearum* itself falls into two sub-groups, one containing the isolates from the USA and one isolate from Australia (*P. rosacearum I*), the other containing the remaining isolates from Australia (*P. rosacearum II*).

**Colony morphology, growth rates and cardinal temperatures**

For clarity, the data for the growth rates on V8A have been divided between two graphs (Fig. 3) corresponding to the two clusters observed in the phylogenetic trees (Fig. 1–2). All species from Clade 6a have fast growth rates and can tolerate high temperatures. The minimum temperature for growth was 4 °C, and the lethal temperature is higher than 37.5 °C for all species. *Phytophthora balyanboodja* had the highest optimum of 32.5 °C, *P. ‘walnut’, P. pseudorosacearum* and *P. gemini* had optimum of 30 °C and all other species had optimum between 25 and 30 °C.

![Bayesian inference tree based on concatenated sequence data from nuclear genes regions, ITS, TUB and HSP, generated in MrBayes using the GTR + G substitution model showing relationship between all Clade 6a. The posterior probability is shown at the nodes. *Phytophthora lacustris* and *P. thermophila* were used as outgroup taxa.](image-url)

**Table 2** Percent nucleotide identity between pairs of *Phytophthora* species from Clade 6a. The upper triangle is for the concatenated nuclear sequence data and the lower triangle is for the concatenated mitochondrial data.
Fig. 2 Bayesian inference tree based on concatenated sequence data from mitochondrial gene regions, COX and NADH, generated in MrBayes using the GTR + G substitution model showing relationship between all Clade 6a. The posterior probability is shown at the nodes. *Phytophthora lacustris* and *P. thermophila* were used as outgroup taxa.

Fig. 3 Average radial growth rate (mm/d ± SE) of all Clade 6a species on V8 agar across the temperature range from 5–37.5 °C.
Fig. 4 Colony morphology of Phytophthora kwongonina, P. cooljaroo, P. pseudorosacearum, P. rosacearum I, P. rosacearum II and P. ‘walnut’ (from top to bottom) after 5 d growth at 20 °C on carrot agar, V8 agar, malt extract agar and potato-dextrose agar (from left to right).
Fig. 5 Colony morphology of Phytophthora condilina, P. humicola, P. inundata, P. balyanboodja, P. ‘personii’ and P. gemini (from top to bottom) after 5 d growth at 20 °C on carrot agar, V8 agar, malt extract agar and potato-dextrose agar (from left to right).
Colony morphologies on different media are also similar (Fig. 4–5). PDA was the most useful media for comparison as the different species varied in both growth rate and growth pattern. *Phytophthora rosacearum* has a rosaceous growth pattern, *P. cooljarloo* is petaloid, *P. kwongonina* and *P. pseudorosacearum* have a faint petaloid pattern, *P. ‘walnut’* grows more slowly with an irregular pattern (Fig. 4). *P. balyanboodja*, *P. ‘personii’* and *P. gemini* have no growth pattern. *Phytophthora condilina*, *P. humicola* and *P. inundata* have identical petaloid patterns (Fig. 5).

**TAXONOMY**

*Phytophthora balyanboodja* T.I. Burgess, *sp. nov. — MycoBank MB822009; Fig 6*

Etymology. Name for wetlands in Noongar (local Aboriginal) language.

*Typus. Australia*, Western Australia, Alfred Cove, from rhizosphere soil of mixed native vegetation, isolated by the VHS, 2015 (holotype MURU 475, dried culture on V8A, Herbarium of Murdoch University, Western Australia, culture ex-type CBS 143058, ITS, TUB, HSP, COX and NADH sequences GenBank KJ372258, MF326806, MF326892, MF326862, MF326927, respectively).

Sporangia, chlamydospores and hyphal swellings (Fig. 6a–g) — Sporangia of *P. balyanboodja* were not observed on solid agar but were produced abundantly in non-sterile soil extract. Sporangia were typically borne terminally on unbranched sporangiophores. Sporangia were persistent and non-papillate, although on first observation 20% of sporangia had apical protrusions (c, e–f), which later led to direct germination (h). Sporangia were exclusively ovoid to elongated ovoid in shape (a–g). Internal nested and extended proliferation of sporangia occurred in chains (d). Exit pores were 12.5–22 µm wide (av. 15.5 ± 2.0 µm), zoospore cysts were spherical and 10–12.5 µm diam (av. = 10.9 ± 0.6 µm). Sporangial dimensions of two isolates of *P. balyanboodja* averaged 63.3 ± 8.3 × 39.7 ± 5.8 µm (overall range 40.9–75.7 × 21.2–51.1 µm). The length/breadth ratio ranged from 1.19–2.23 (av. = 1.56 ± 0.17). Chlamydospores and hyphal swellings were absent.

Oogonia, oospores and antheridia — Gametangia were not produced in single culture or when paired with tester strains and this species is considered to be sterile in culture.

Colony morphology, growth rates and cardinal temperatures — Colonies on all media are wolly with no pattern (Fig. 5). The minimum, maximum and lethal temperatures for growth were around 4, 37.5 and > 37.5 °C, respectively. The average radial growth rate on V8A at the optimum temperature of 32.5 °C was 6.8 ± 0.15 mm d⁻¹ (Fig. 3b).

*Additional material examined. Australia*, Western Australia, Alfred Cove, from rhizosphere soil of mixed native vegetation, isolated by the VHS, 2015, VHS23675-R3.

*Phytophthora condilina* T.I. Burgess, *sp. nov. — MycoBank MB822010; Fig. 7*

 Etymology. From the Noongar (local Aboriginal) name for *Casuarina*, a known host of this species.

*Typus. Australia*, Western Australia, Alfred Cove, from rhizosphere soil of dying *Casuarina obesa*, isolated by VHS, 2011 (holotype MURU 476, dried culture on V8A, Herbarium of Murdoch University, Western Australia, culture ex-types CBS 143059 and VHS25244. ITS, TUB, HSP, COX and NADH sequences GenBank KJ372262, MF326814, MF326869, MF326843 and MF326915, respectively).

Fig. 6 *Phytophthora balyanboodja*. a–g. Persistent sporangia formed on V8 agar flooded with soil extract. a–b. ovoid with flat apex; c, e–f. ovoid with a pointed apex giving the appearance of papilla; d. chains of empty ovoid sporangia with internal nested and extended proliferation; h. direct germination of ovoid sporangia — Scale bars d and h = 25 µm; bar in h. applies for all images except d.
Sporangia, chlamydospores and hyphal swellings (Fig. 7a–j) — Sporangia of *P. condilina* were not observed on solid agar, but were produced abundantly in non-sterile soil extract. Sporangia were typically borne terminally on unbranched sporangiophores. Sporangia were persistent and non-papillate. Sporangia were ovoid in shape (a–d, f–i), ranging from broad ovoid (c–d, h) to occasionally elongated ovoid. Both nested (f–h) and extended (i) internal proliferation of sporangia was observed. Exit pores were 6.5–21 µm wide (av. 13.6 ± 2.9 µm), zoospore cysts were spherical and 7.5–14.5 µm diam (av. = 11.6 ± 1.5 µm). Sporangial dimensions of six isolates of *P. condilina* averaged 48.0 ± 7.4 × 36.3 ± 6.2 µm (overall range 29.8–69.3 × 20.1–51.4 µm).

![Figure 7](phytophthoracondilina.jpg)

Fig. 7 *Phytophthora condilina*. a–d, f–i. Persistent, non-papillate, ovoid sporangia formed on V8 agar flooded with soil extract. f–h. empty sporangia with internal nested proliferation; i. empty sporangium with internal extended proliferation; c. spherical hyphal swellings with radiating hyphae; j. intercalary chlamydospore. — k–p. Mature oogonia formed in single culture in V8 agar. k–p. golden brown, oogonia with wavy walls containing aplerotic oospores with large ooplasts; m–o. paragynous unicellular antheridia; p. amphigynous antheridium; q. mature oogonium with slightly tapering base; r. aborted oospore with slightly tapering base. — Scale bar = 25 µm.
Fig. 8 Phytophthora cooljarloo. a–i. Persistent, non-papillate sporangia formed on V8 agar flooded with soil extract. a–d. ovoid; e. elongated ovoid; f. limoniform; g. empty ovoid sporangia; h. empty ovoid sporangium showing internal extended proliferation; i. empty ovoid sporangium showing internal nested proliferation. — j–o. Mature oogonia formed in single culture in V8 agar. j–m. o. oogonia with wavy walls containing aplerotic, pale brown oosporang with large ooplasts and paragynous unicellular antheridia situated adjacent to the oogonial stalk; n. aborted oospore with large paragynous antheridium. — Scale bar = 25 µm.
The length/breadth ratio ranged from 1.00–1.86 (av. = 1.33 ± 0.15), intercalary chlamydospores (j) were present and ranged from 19.8–59.2 µm diam (av. = 38.1 ± 10.6). Hyphal swellings were regular, and they were predominantly spherical and intercalary with radiating hyphae and from their morphology appear like small chlamydospores (e) except that the wall did not form between the swelling and the hyphae. They ranged in size from 11.5–44.5 µm diam (av. = 24.1 ± 7.2).

Oogonia, oospores and antheridia (Fig. 7k–r) — Gametangia were inconsistently produced in single culture by five of the six isolates of P. condilina within 30 d. Oogonia were generally borne terminally ranging from 27–57.5 µm diam (av. = 42.0 ± 4.7). Oogonia often had wavy walls (k–l, o–p) and a slightly tapering base (q–r). Oospores were apleurotic, globose to slightly eccentric with a large ooplast, turning golden-brown on maturity (k–r), ranging in size from 23.5–42.5 µm diam (av. = 35.6 ± 3.8). The oospores were relatively thick-walled (3.31 ± 0.72 µm), with a mean oospore wall index of 0.46 ± 0.07. On average 80 % of the oogonia aborted after oospore formation (r). The antheridia were predominantly paragynous (m–o), terminal, round- to club-shaped and situated at the side of the oogonia, averaging 15.6 ± 3.3 × 10.7 ± 2.0 µm. Amphilgonous antheridia were occasionally seen (p). This species is considered to be homothallic.

Colony morphology, growth rates and cardinal temperatures — Colonies on V8A and CA were cottony with a slight petaloid pattern, growth was appressed with striations on MEA and cottony and rosaceous on PDA (Fig. 4). The minimum, maximum and lethal temperatures for growth were around 4, 35 and > 37.5 °C, respectively. The average radial growth rate on V8A at the optimum temperature of 25 °C was 4.8 ± 0.39 mm/d (Fig. 3a).

Additional material examined: AUSTRALIA, Western Australia, Cooljarloo, from rhizosphere soil of mixed native vegetation, R. Hart, 1996, HSA2313.

**Phytophthora kwonganina** T.I. Burgess, sp. nov. — MycoBank MB822012; Fig. 9

**Etymology.** Refers to the kwongan vegetation in the southwest of Western Australia.

**Typos.** AUSTRALIA, Western Australia, Bunbury, from rhizosphere soil of dying Banksia grandis, isolated by the VHS, 2010 (holotype MURU 477, dried culture on V8A, Herbarium of Murdoch University, Western Australia, culture ex-types CBS 143060 and VHS23289. ITS, TUB, HSP, COX and NADH sequences GenBank JN547638, MF326824, MF326876, MF326847 and MF326914, respectively).

Sporangia, chlamydospores and hyphal swellings (Fig. 9a–i) — Sporangia of *P. kwonganina* were not observed on solid agar but were produced abundantly in non-sterile soil extract. Sporangia were typically borne terminally on unbranched sporangiophores. Sporangia were persistent and non-papillate. Sporangia were predominantly ovoid to elongated ovoid (a, c–d, f) in shape although limoniform (e), ellipsoid (b) and broad ovoid shapes were observed. Both nested (h–i) and extended (f–g, j) internal proliferation of sporangia was observed. Exit pores were 9.5–19.5 µm wide (av. 14.5 ± 2.5 µm), zoospore cysts were spherical and 11–18 µm diam (av. = 13.1 ± 1.5 µm). Sporangial dimensions of three isolates of *P. kwonganina* averaged 57.5 ± 11.2 × 36.0 ± 6.9 µm (overall range 34.5–87 × 23–56.5 µm). The length/breadth ratio ranged from 1.15–2.34 (av. = 1.61 ± 0.21). Chlamydospores were absent. Hyphal swellings were common; they were predominantly spherical (sometimes catenulate) and intercalary with radiating hyphae and from their morphology appear like small chlamydospores (j) except that the wall did not form between the swelling and the hyphae. They ranged in size from 12–46.5 µm diam (av. = 21.5 ± 6.1).

Oogonia, oospores and antheridia (Fig. 9k–q) — Game-tangia were produced in single culture within 14 d. Oogonia were generally borne terminally ranging from 24–49 µm diam (av. = 35.8 ± 4.9). Oogonia had wavy walls. Oospores were highly apleurotic, globose, and pale on maturity, ranging in size from 32–44 µm diam (av. = 37.1 ± 2.9). The oospores were very thick-walled (4.89 ± 0.81 µm), with a mean oospore wall index of 0.60 ± 0.05. The antheridia were exclusively paragynous, terminal, round- to club-shaped and situated adjacent to the oogonial stalk averaging 16.2 ± 3.5 × 11.8 ± 2.2 µm. This species is considered to be homothallic.

Colony morphology, growth rates and cardinal temperatures — Colonies on V8A, CA and PDA were cottony with a slight petaloid pattern, growth was appressed with striations on MEA and cottony and rosaceous on PDA (Fig. 4). The minimum, maximum and lethal temperatures for growth were around 4, 35 and > 37.5 °C, respectively. The average radial growth rate on V8A at the optimum temperature of 25 °C was 6.8 ± 0.32 mm/d (Fig. 3a).

Additional material examined: AUSTRALIA, Western Australia, Cervantes, from rhizosphere soil of dying *Banksia prionotes*, T.C. Hill, 1986, TCH009; Fitzgerald River National Park, from rhizosphere soil of dying Xanthorrhoea platyphylla, isolated by the VHS, 1993, DDS3599.
Phytophthora pseudorosacearum T.I. Burgess, sp. nov. — MycoBank MB822013; Fig. 10

Etymology. Refers to close relationship to Phytophthora rosacearum.

Type. Australia, Western Australia, Jarrahdale, from rhizosphere soil of dying Persoonia longifolia, isolated by the VHS, 2013 (holotype MURU 478, dried culture on V8A, Herbarium of Murdoch University, Western Australia, culture ex-types CBS 143061 and VH29692. ITS, TUB, HSP, COX and NADH sequences GenBank KJ372287, MF326827, MF326876, MF326858 and MF326907, respectively).

Sporangia, chlamydospores and hyphal swellings (Fig. 10a–h) — Sporangia of P. pseudorosacearum were not observed on solid agar but were produced abundantly in non-sterile soil extract. Sporangia were typically borne terminally on unbranched sporangiophores. Sporangia were persistent and non-papillate. Sporangia were predominantly ovoid to elongated ovoid in shape although limoniform (c), ellipsoid (d) and broad ovoid (b) shapes were observed. Both nested (f) and extended (g–h) internal proliferation of sporangia was observed. Exit pores were...
9–20 µm wide (av. 14.9 ± 2.7 µm), zoospore cysts were spherical and 8–20 µm diam (av. = 11.6 ± 1.8 µm). Sporangial dimensions of three isolates of *P. pseudorosacearum* averaged 52.7 ± 10.0 × 34.1 ± 5.6 µm (overall range 32.7–59.3 × 19.4–38.3 µm). The length/breadth ratio ranged from 1.02–2.48 (av. = 1.57 ± 0.31). Intercalary chlamydospores (i) were present and ranged from 20–42.5 µm diam (av. = 28.4 ± 5.3). Hyphal swellings were common; they were predominantly spherical (sometimes catenulate) and intercalary with radiating hyphae and from their morphology appear like small chlamydospores (j) except that the wall did not form between the swelling and the hyphae. They ranged in size from 6–31 µm diam (av. = 17.8 ± 6.0).

Oogonia, oospores and antheridia (Fig. 10k–s) — Gametangia were produced in single culture within 14 d. Oogonia were generally borne terminally ranging from 24–49 µm diam (av. = 35.8 ± 4.9). Oogonia had wavy walls and sometimes a slightly tapering base (n). Oosporles were aplerotic, globose to eccentric (n, p–q), turning slightly golden-brown on maturity, ranging in size from 22.5–38 µm diam (av. = 30.8 ± 3.3). The oospores were relatively thick-walled (2.46 ± 0.47 µm), with a mean oospore wall index of 0.41 ± 0.06. On average 20% of the oogonia aborted after oospore formation (r–s). The antheridia were exclusively paragynous, terminal, round- to club-shaped.
### Table 3

Comparison of morphological characters and dimensions, and temperature-growth relations of *Phytophthora* *rosacearum*, *P. pseudorosacearum*, *P. kwongonina*, *P. cooljarloo* and *P. 'walnut'*. The two clusters within *P. rosearum* were considered separately. All measurements are in µm.

| Species | *P. rosacearum I* | *P. rosacearum II* | *P. pseudorosacearum* | *P. kwongonina* | *P. cooljarloo* | *P. 'walnut'* |
|---------|------------------|-------------------|-----------------------|----------------|----------------|----------------|
| No of isolates | 7 | 5 | 3 | 3 | 2 | 1 |
| Sporangia | | | | | | |
| LxB mean ± SD | 44.8 ± 5.3 x 27.4 ± 5.0 | 47.6 ± 10.5 x 29.7 ± 4.5 | 52.7 ± 10.0 x 34.1 ± 5.6 | 57.5 ± 11.2 x 36.0 ± 6.9 | 55.0 ± 9.5 x 37.6 ± 5.5 | 59.5 ± 6.0 x 38.1 ± 4.8 |
| Total range | 32.0–59.3 x 16.9–38.3 | 22.5–73.4 x 16.7–40.1 | 32.7–59.5 x 19.4–38.3 | 34.8–87.0 x 23.2–36.5 | 30.6–79.1 x 25.1–40.8 | 43.2–89.4 x 30.8–57.3 |
| Range of isolates means | 43.7–47.9 x 23.7–31.9 | 36.2–57.0 x 24.7–31.4 | 49.4–60.3 x 30.7–37.8 | 53.9–60.3 x 31.9–38.3 | 51.7–73.8 x 37.2–37.9 | 43.2–90.8 x 30.8–57.3 |
| L/B ratio (range) | 1.67 ± 0.26 (1.17–2.27) | 1.60 ± 0.24 (1.05–2.36) | 1.57 ± 0.31 (1.02–2.48) | 1.61 ± 0.21 (1.15–2.34) | 1.47 ± 0.24 (1.10–2.18) | 1.57 ± 0.15 (1.05–1.99) |
| Features | terminal, persistent, non-papillate | terminal, persistent, non-papillate | terminal, persistent, non-papillate | terminal, persistent, non-papillate | terminal, persistent, non-papillate | terminal, persistent, non-papillate |
| Shapes | oval 60% | oval 50% | oval 55% | oval 48% | oval 68% | oval 90% |
| Sporangiospheres | elongated oval 20% | elongated oval 34% | limoniform 5% | elongated oval 20% | elongated oval 12% | elongated oval 10% |
| Morphology | elliptoid 12% | limoniform 4% | broad oval 5% | limoniform 25% | elliptoid 5% | limoniform 4% |
| Proportion of Sporangiospheres | internal, both nested and extended | internal, both nested and extended | internal, both nested and extended | internal, both nested and extended | internal, both nested and extended | internal, both nested and extended |

| Exit pores | | | | | | |
| Width (range) | 11.3 ± 2.5 (5.7–17.3) | 13.6 ± 2.6 (7.9–18.6) | 14.9 ± 2.7 (8.8–20.2) | 14.5 ± 2.5 (9.5–19.3) | 17.5 ± 2.9 (11.4–22.4) | 13.4 ± 2.0 (10.5–15.9) |
| Zoosporangia cysts | | | | | | |
| Chlamydospores | | | | | | |
| Diameter (range) | 28.4 ± 5.3 (20.1–42.7) | | | | | |
| Features | | | | | | |
| Hyphal swellings | | | | | | |
| Oogonia | | | | | | |
| Mean diam | 17.6 ± 5.7 (9.0–27.8) | | | | | |
| Breeding system | homothallic | homothallic | homothallic | homothallic | homothallic | sterile in culture |
| Oogonial wall thickness | 1.93 ± 0.43 | 2.21 ± 0.46 | 2.46 ± 0.47 | 4.89 ± 0.81 | 2.76 ± 0.59 | 0.40 ± 0.07 |
| Antheridia | | | | | | |
| Mean diam | 35.7 ± 3.7 (23.8–45.4) | 36.6 ± 4.0 (25.3–47.3) | 35.8 ± 4.9 (23.5–49.0) | 45.4 ± 3.4 (36.7–52.4) | 41.9 ± 4.0 (31.9–48.3) | 40.2–43.5 |
| Mean diam | 32.6–38.8 | 31.8–38.9 | 33.1–37.4 | 42.8–47.9 | 48.7–53.6 | 43.9–50.3 |
| Oospores | | | | | | |
| Mean diam | 35.7 ± 3.7 (23.8–45.4) | 36.6 ± 4.0 (25.3–47.3) | 35.8 ± 4.9 (23.5–49.0) | 45.4 ± 3.4 (36.7–52.4) | 41.9 ± 4.0 (31.9–48.3) | 40.2–43.5 |
| Mean diam | 32.6–38.8 | 31.8–38.9 | 33.1–37.4 | 42.8–47.9 | 48.7–53.6 | 43.9–50.3 |
| Mean diam | 35.7 ± 3.7 (23.8–45.4) | 36.6 ± 4.0 (25.3–47.3) | 35.8 ± 4.9 (23.5–49.0) | 45.4 ± 3.4 (36.7–52.4) | 41.9 ± 4.0 (31.9–48.3) | 40.2–43.5 |
| Mean diam | 32.6–38.8 | 31.8–38.9 | 33.1–37.4 | 42.8–47.9 | 48.7–53.6 | 43.9–50.3 |
| Growth characteristics | | | | | | |
| Max temp (°C) | 37.5 | 37.5 | 37.5 | 35 | 35 | 37.5 |
| Opt temp (°C) | 25–30 | 25–30 | 25–30 | 25–30 | 25–30 | 25–30 |
| Min temp (°C) | 4 | 4 | 4 | 4 | 4 | 4 |
| Lethal temp (°C) | > 37.5 | > 37.5 | > 37.5 | > 37.5 | > 37.5 | > 37.5 |
| Growth rate on V8A at optimum (mm/day) | > 5.8 | > 6.3 | > 5.2 | > 0.4 | > 6.8 | > 0.3 |
| Growth rate on V8A at optimum (mm/day) | > 5.8 | > 6.3 | > 5.2 | > 0.4 | > 6.8 | > 0.3 |
Table 4  Comparison of morphological characters and dimensions, and temperature-growth relations of Phytophthora inundata, P. humicola, P. condilina, P. balyanboodja, P. gemini and P. ‘personii’. All measurements are in µm.

| Species | P. inundata | P. humicola | P. condilina | P. balyanboodja | P. gemini | P. ‘personii’ |
|---------|-------------|-------------|--------------|-----------------|-----------|--------------|
| No of isolates | 3 | 1 | 6 | 2 | 1 | 3 |
| Sporangia L:B ratio (mean ± SD) | 59.7 ± 13.3 | 40.6 ± 6.8 | 60.6 ± 43.1 | 44.0 ± 7.4 | 61.8 ± 39.7 | 49.6 ± 10.3 |
| Total range | 31.4–84.5 | 23.7–29.0 | 18.9–29.0 | 29.8–91.9 | 43.7–89.7 | 40.9–75.7 |
| Range of isolates | 54.4–65.3 | 38.3–52.4 | 58.7–65.4 | 44.4–49.8 | 68.1–86.9 | 40.9–75.7 |
| Features | terminal, persistent, non-papillate | terminal, persistent, non-papillate | terminal, persistent, non-papillate | terminal, persistent, non-papillate | terminal, persistent, non-papillate | terminal, persistent, non-papillate |
| Shapes | ovoid 80% | ovoid 90% | limoniform 10% | broad ovoid 70% | broad ovoid 25% | oviod 80% |
| Proliferation | internal, both nested and extended | internal, both nested and extended | internal, both nested and extended | internal, both nested and extended | external | internal, both nested and extended |
| Exit pores Width (range) | 17.1 ± 3.2 | 16.1 ± 2.9 | 13.6 ± 2.9 | 15.5 ± 2.0 | 15.8 ± 3.3 | 14.4 ± 2.5 |
| Zoospore cysts | 10.9 ± 0.9 | 11.8 ± 0.8 | 11.6 ± 1.5 | 10.9 ± 0.6 | 10.9 ± 0.6 | 11.1 ± 0.7 |
| Chlamydospores Diameter (range) | 47.8 ± 8.6 | 36.8 ± 6.1 | 38.1 ± 10.6 | 38.1 ± 10.6 | 38.1 ± 10.6 | 54.5 ± 11.5 |
| Hyphal swellings Features | predominantly spherical and intercalary with radiating hyphae | predominantly spherical and intercalary with radiating hyphae | predominantly spherical and intercalary with radiating hyphae | predominantly spherical and intercalary with radiating hyphae | predominantly spherical and intercalary with radiating hyphae | predominantly spherical and intercalary with radiating hyphae |
| Mean diam | 24.4 ± 4.9 | 20.3 ± 3.1 | 24.1 ± 7.2 | 24.1 ± 7.2 | 24.1 ± 7.2 | 39.9 ± 9.9 |
| Breeding system | mixed | homothallic | sterile in culture | sterile in culture | sterile in culture | sterile in culture |
| Oogonia Features | wavy wall, often with a slightly tapering base | | | | | |
| Mean diam | 41.1 ± 3.5 | 39.5 ± 4.3 | 42.0 ± 4.7 | 39.2 ± 4.9 |
| Range of isolates | 34.7–43.4 | 32.9–49.5 | 25.3–49.5 | 32.9–49.5 |
| Oospores Features | aplerotic, slightly golden on maturity, often slightly eccentric | aplerotic, slightly golden on maturity, often slightly eccentric | aplerotic, golden on maturity, often slightly eccentric | aplerotic, golden on maturity, often slightly eccentric | aplerotic, golden on maturity, often slightly eccentric | aplerotic, golden on maturity, often slightly eccentric |
| Abortion | 10% | 80% | 80% | 80% | 80% | 80% |
| Mean diam | 37.5 ± 2.8 | 32.9 ± 3.7 | 33.2 ± 3.2 | 31.4 ± 3.2 | 33.2 ± 3.2 | 31.4 ± 3.2 |
| Range of isolates | 29.6–39.2 | 25.8–39.5 | 27.7–36.9 | 26.4–39.7 | 27.7–36.9 | 26.4–39.7 |
| Wall diameter | 5.4 ± 0.9 | 4.02 ± 0.78 | 3.31 ± 0.72 | 3.31 ± 0.72 | 3.31 ± 0.72 | 3.31 ± 0.72 |
| Oospore wall index | 0.64 | 0.57 ± 0.07 | 0.46 ± 0.07 | 0.46 ± 0.07 | 0.46 ± 0.07 | 0.46 ± 0.07 |
| Antheridia Features | amphigynous | predominantly paragynous, round-club shaped, often multiple | predominant round-club shaped, attached on side of oogonia | predominant round-club shaped, attached on side of oogonia | predominant round-club shaped, attached on side of oogonia | predominant round-club shaped, attached on side of oogonia |
| L:B mean | 16.5 ± 5.9 | 17.2 ± 5.4 | 15.6 ± 3.3 | 15.6 ± 3.3 |
| L:B range | 15.9–17.3 | 14.4–15.4 | 10.7–12.0 | 10.7–12.0 |
| Growth characteristics | Max temp (°C) | 37.5 | 35 | 35 | 35 | 35 |
| Opt temp (°C) | 25 | 25 | 25–30 | 25–30 | 25–30 | 25–30 |
| Min temp (°C) | 4 | 4 | 4 | 4 | 4 | 4 |
| Lethal temp (°C) | > 37.5 | > 37.5 | > 37.5 | > 37.5 | > 37.5 | > 37.5 |
| Growth rate on V8A at optimum (mm/day) | 6.2 ± 0.22 | 5.8 ± 0.02 | 5.6 ± 0.19 | 5.6 ± 0.19 | 6.8 ± 0.15 | 6.7 ± 0.03 |

*Measurements for oospores and oogonia from Brasier et al. (2003b).
and situated adjacent to the oogonial stalk, averaging 13.8 ± 3.9 \times 11.4 ± 3.2 \mu m. This species is considered to be homothallic.

Colony morphology, growth rates and cardinal temperatures — Colonies on V8A, CA and PDA were cottony with a slight petaloid pattern, growth was appressed with striations on MEA (Fig. 4). The minimum, maximum and lethal temperatures for growth were around 4, 37.5 and > 37.5 °C, respectively. The average radial growth rate on V8A at the optimum temperature of 30 °C was 5.2 ± 0.40 mm d⁻¹ (Fig. 3a).

Additional materials examined. Australia, Western Australia, Cooljarloo, from water baiting in native vegetation, 1998, R. Hart, HSA2350; Albany, from rhizosphere soil of dying Xanthorrhoea platypylhia, 2010, VHS24266.

Comparison of Clade 6a species

Phytophthora condilina, P. balyanboodja, P. pseudorosacearum, P. kwongonina and P. cooljarloo can easily be separated from each other and other related species in Clade 6a by differences in their ITS, BT, HSP, COX and NADH sequences (Table 2), and by a combination of morphological and physiological characters (Table 3—4). In all gene trees, the species fall into two strongly supported groups. The first group contains P. pseudorosacearum as a sister species to P. rosacearum having a common ancestor with P. cooljarloo, P. kwongonina and P. ’walnut’ (Fig. 1–2). The second group contains P. condilina as a sister species to P. inundata and P. humicola sharing a common ancestor with P. balyanboodja, P. ’personii’ and P. gemini (Fig. 1–2). All species have high temperature optima and most grow at 37.5 °C (Fig. 3, Table 3–4).

Species in the P. rosacearum group share many morphological features (Table 3). Phytophthora kwongonina and P. cooljarloo have larger oospores with thicker walls than the other species. Within P. rosacearum itself, morphological features of USA and Australian isolates overlapped completely, and the only observed difference was the lack of hyphal swellings for the Australian isolates. In both the nuclear and mitochondrial gene phylogenies the isolates were clustered separately, however the support for this was not strong enough to consider a new species description, and the differences are thought to reflect intraspecific variation. Phytophthora pseudorosacearum can be separated from its sister species, P. rosacearum, by its larger sporangia, the presence of chlamydospores and aplerotic oospores which were golden brown on maturity. Phytophthora cooljarloo and P. kwongonina are also sister species and their features overlap, the only difference is the abundance of hyphal swellings found in cultures of P. kwongonina, the thicker oospore walls of P. kwongonina, and the much larger antheridia of P. cooljarloo. Phytophthora ’walnut’ differs from the other species in this cluster in that it appears to be sterile.

Species in the P. inundata group also share many morphological features (Table 4). Phytophthora balyanboodja, P. gemini and P. ’personii’ are all considered to be sterile species, but can be separated based on the presence of chlamydospores in P. ’personii’, and the absence of both chlamydospores and hyphal swellings in P. balyanboodja. Phytophthora inundata, P. humicola and P. condilina are sister taxa and share many features. Of the three species, P. condilina has the smallest sporangia and has oogonia with slightly tapering bases. Phytophthora inundata is defined by having a mixed mating system with homothallic, sterile and heterothallic isolates (Brazier et al. 2003b).

DISCUSSION

Five new species have been described from Clade 6a, which is now represented by nine species and two designated taxa. All species are morphologically similar, with predominantly ovoid sporangia and nested and extended internal proliferation. If oospores are present, they tend to be aplerotic with paragynous antheridia mostly attached adjacent to the oogonial stalk. They can all grow at 35 °C and have a fast growth rate on most agar media. With the exception of P. gemini and P. humicola, all these species have been recovered from natural ecosystems in SWWA, often from water gaining sites and often from very isolated areas. The radiation, origin and potential ecological role of these species will be discussed.

In a phylogenetic revision of relationships between Clade 6 species, Brasier et al. (2003a) observed that Clade 6b species were characterised by multiple short branches with weak support for higher level clustering, while Clade 6a was characterised by relatively long branch lengths. Such a pattern was considered indicative of recent divergence in Clade 6b and ancient divergence in Clade 6a. Subsequent descriptions of new species have reinforced this observation for Clade 6b (Jung et al. 2011). However, with the addition of the new species described here, Clade 6a now also contains two clusters of species separated by smaller genetic distances representing more recent divergence. In particular, the cluster containing P. rosacearum, P. pseudorosacearum, P. cooljarloo and P. kwongonina and that with P. humicola, P. inundata and P. condilina. There is even some evidence for additional cryptic species within the P. rosacearum complex, but more isolates are required to elucidate this. We also have evidence for cryptic speciation within P. inundata as Australian isolates differ by several base pairs to those from the northern hemisphere.

Hybridisation is common among species in Clade 6b (Nagel et al. 2013, Parke et al. 2014, Burgess 2015). This is considered to be a consequence of their predominantly aquatic lifestyle (Jung et al. 2011), and perhaps the reuniting of related, but formerly geographically isolated species through global trade (Burgess 2015). To date, the same cannot be said of Clade 6a species. While most of the nuclear gene regions contained some polymorphic positions in some species, these were not consistent across isolates or loci and were considered to represent intraspecific variation.

Historical global movement of Phytophthora species during European settlement associated with the establishment of agriculture and horticulture, and contemporary movement in the trade of plants-for-planting is well documented (Brazier 2008, Scott et al. 2013). Even so, there are clearly species within Clade 6b with either a northern (NH) or southern (SH) hemisphere distribution. For example, P. thermophila and P. amnicola are common in streams in the SH, while P. gonapodyides and P. laevisus dominate in the NH. Phytophthora chlamydospora appears to originate in the NH, but has been detected in South Africa, Argentina, Australia and New Zealand, but at much lower frequency than the local species. Similarly, Clade 6a species have patchy distribution. Phytophthora humicola is restricted to Taiwan, and P. gemini has only been recovered from estuaries in the Netherlands. Phytophthora rosacearum was first recovered from orchards in California, but is common in native ecosystems in SWWA. Phytophthora inundata has a global distribution and is of unknown origin. The remaining species in Clade 6a have, to our knowledge, only been recovered from predominantly dry kwongon heathlands in SWWA.

Of the 28 formerly described species in Clade 6, 13 have been described based on recoveries from natural vegetation in SWWA, and only seven (P. riparia, P. gonapodyides, P. borealis, P. mississippiipae, P. pinifolia, P. gemini and P. humicola) have not been recovered from this region. Due to the devastating impact of P. cinnamomi in natural ecosystems in Western Australia and the subsequent legislative requirement to map its distribution, the Vegetation Health Service of the Department of Parks and Wildlife has been receiving samples from suspect
dying plants for over 35 years. This is an unprecedented data-set on the distribution of *Phytophthora* in natural ecosystems and has not been replicated to the same extent elsewhere (except perhaps the Pacific northwest of USA). As such, the incredible diversity found in SWWA could just be an artefact of sampling intensity. Indeed, in a recent survey across Australia where *Phytophthora* was detected directly from soils using high throughput sequencing (HTS) technology, the number of species detected in the SWWA was almost equivalent to the number of species isolated and reported in databases (Burgess et al. 2017). While elsewhere in Australia, where sampling intensity has been much less, the numbers of species known from databases were much lower than those detected by HTS. In particular, only 9 *Phytophthora* species had been previously reported for Tasmania, but 49 were detected with HTS. Many Clade 6 species first described in WA were detected using HTS in other states of Australia (Burgess et al. 2017). However, there is an alternate explanation for the incredible species diversity observed in SWWA; it could be seen as a reflection of the plant species diversity of this biodiversity hot-spot. Until more data become available for surveys of natural ecosystems worldwide, the SWWA could be considered as either the origin of Clade 6a, or a region where significant radiation has occurred.

The Clade 6a *Phytophthora* species in SWWA have been isolated from within natural vegetation located in national parks and reserves, often in water gaining areas. The SWWA is a harsh environment with long dry summers and often the annual rainfall in the region dominated by the northern kwongan vegetation can be less than 200 mm (Bureau of Meteorology, http://www.bom.gov.au/climate/change/acorn-sat/), and the water gaining areas could remain dry for several years. The high temperature optima of the species and the relatively thick-walled oospores of many of the species may assist with their survival in these conditions. However, while the summers are hot and dry, the winter and spring temperatures and moisture availability are suitable for growth and proliferation of *Phytophthora*. All experimental data to date has found these species to be non-pathogenic (Albornoz et al. 2017), or to cause only minimal fine root damage (unpubl. data). These species, if endemic, could have evolved with specific hosts (or related hosts) in a way that could enhance co-existence of a wide diversity of plant species in the dry kwongan heathlands (Labbé et al. 2015). Negative density dependence is the phenomenon whereby soil-borne pathogens build up in the root zone of mature plants leading to poor conspecific seed germination, growth and survival. Thus, seeds will perform better the further they are from a conspecific adult plant. This theory has not as yet been demonstrated for Clade 6a species. However, in another scenario in a mixed host trial with non-mycorrhizal Proteaceae and mycorrhizal Myrtaceae, the presence of Clade 6a *Phytophthora* species equalised the competition by reducing the dominance of the *Proteaceae* (Albornoz et al. 2017). Further experiments are currently underway to test these hypotheses.

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