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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.* 'personii', 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. condilina*, *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 oogonial 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 (Buisman 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* (Granke 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.* 'personii' 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. condilina*, *P. cooljarloo*, *P. kwongonina* and *P. pseudorosacearum.*

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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 $CaCO_3$, 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

Table 1 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.				
						ITS	TUB	HSP	COX	NADH
CBS 143058[1]	*P. balyanboodja*	Soil	Native vegetation	Australia, WA, Alfred Cove	2011	KJ372258	MF326806	MF326892	MF326862	MF326927
VHS25675 R3	*P. balyanboodja*	Soil	Native vegetation	Australia, WA, Alfred Cove	2011	KJ372259	MF326807	MF326893	MF326863	MF326926
MUCC768	*P. condilina*	Water	Native vegetation	Australia, WA, Esperance	2008	HQ012959	MF326808	HQ012927	HQ012883	MF326923
MUCC769[2]	*P. condilina*	Water	Native vegetation	Australia, WA, Esperance	2008	HQ012960	MF326809	HQ012928	HQ012884	MF326924
MUCC806	*P. condilina*	Soil	*Casuarina obesa*	Australia, WA, Alfred Cove	2011	KC748465	MF326810	MF326867	MF326839	MF326917
MUCC807	*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	MF326841	MF326920
VHS25241	*P. condilina*	Soil	*Casuarina obesa*	Australia, WA, Alfred Cove	2011	KJ372263	MF326813	MF326868	MF326842	MF326919
CBS 143059[1]	*P. condilina*	Soil	*Casuarina obesa*	Australia, WA, Alfred Cove	2011	KJ372262	MF326814	MF326869	MF326843	MF326915
VHS28614[2]	*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[1]	*P. cooljarloo*	Soil	*Hibbertia* sp.	Australia, WA, Cooljarloo	2008	HQ012957	MF326816	HQ012925	HQ012881	MF326910
CBS123381[1]	*P. gemini*	Seed	*Zostera marina*	The Netherlands, Zealand		FJ217680	MF326818	MF326891	MF326859	MF326932
CBS200.81[1,2]	*P. humicola*		*Citrus*	Taiwan	1981	AF266792 GU259087	AY564069	EU080172	AY564184	AY564011
WPC P6702	*P. humicola*		*Phaseolus sp.*	Taiwan		FJ801938	JN935975	JN935946	JN935957	JN936027
DDS3481	*P. inundata*	Soil	Native vegetation	Australia, WA, Northern Sandplains	1991	KJ372261	MF326819	MF326864	MF326845	MF326921
IMI 390121[1]	*P. inundata*	Roots	*Olea* sp.	Spain, Seville, Ecija	1996	EF210201	EF210203	JN935947	EF210207	JN936043
VHS16836	*P. inundata*	Soil	*Xanthorrhoea preissii*	Australia, WA, Boyup Brook	2007	HQ012944	MF326820	MF326865	HQ012860	MF326925
VHS19081[2]	*P. inundata*	Soil	*Banksia attenuata*	Australia, WA, Bold Park	2008	HQ012945	MF326821	MF326866	HQ012861	MF326922
DDS3599	*P. kwongonina*	Soil	*Xanthorrhoea platyphylla*	Australia, WA, Fitzgerald River NP	1993	EU593258	MF326822	MF326875	MF326846	MF326913
IMI 329669	*P. kwongonina*	Roots	*Banksia prionotes*	Australia, WA, Cervantes	1986	EU593265	MF326823	HQ012932	HQ012889	MF326912
CBS 143060[1]	*P. kwongonina*	Soil	*Banksia grandis*	Australia, WA, Bunbury	2010	JN547636	MF326824	MF326876	MF326847	MF326914
HSA1959[2]	*P. lacustris*	Soil	Native vegetation	Australia, Wa, Welshpool	1994	HQ012956	JN547618	HQ012924	HQ012880	JN547706
HSA2530	*P. pseudorosacearum*	Swamp	Native vegetation	Australia, WA, Cooljarloo	1998	HQ012963	MF326825	HQ012931	HQ012887	MF326908
VHS24266	*P. pseudorosacearum*	Soil	*Xanthorrhoea platyphylla*	Australia, WA, Albany	2010	JN547637	MF326826	MF326877	MF326857	MF326909
CBS 143061[1]	*P. pseudorosacearum*	Soil	*Persoonia longifolia*	Australia, WA, Jarrahdale	2013	KJ372267	MF326827	MF326878	MF326858	MF326907
CBS 124696[1]	*P. rosacearum* I		*Malus domestica*	USA, California		EU925376	MF326832	MF326885	MF326859	MF326904
HSA1658	*P. rosacearum* I	Swamp	Native vegetation	Australia, WA, Cooljarloo	1993	KJ372274	MF326830	MF326884	MF326851	MF326906
IMI 389749	*P. rosacearum* I		*Malus domestica*	USA, California, Sonoma County	1979	AF541911	JN935980	JN935952	JN935962	JN936032
OSU55	*P. rosacearum* I		*Prunus armeniaca*	USA, Maryland		KJ372271	MF326833	MF326882	MF326854	MF326902
OSU62	*P. rosacearum* I		*Prunus avium*	USA, California		KJ372273	MF326834	MF326887	MF326856	MF326903
OSU63	*P. rosacearum* I		*Prunus avium*	USA, California		KJ372272	MF326835	MF326883	MF326855	MF326901
OSU65	*P. rosacearum* I		*Malus domestica*	USA, California		KJ372270	MF326836	MF326886	MF326853	MF326905
DDS2909	*P. rosacearum* II	Soil	*Pinus radiata*	Australia, WA, Albany	1989	HQ012958	MF326828	HQ012926	HQ012882	MF326898
HSA1650	*P. rosacearum* II	Swamp	Native vegetation	Australia, WA, Cooljarloo	1993	KJ372268	MF326829	MF326880	MF326850	MF326896
HSA2529	*P. rosacearum* II	Swamp	Native vegetation	Australia, WA, Cooljarloo	1998	HQ012962	MF326831	HQ012930	HQ012886	MF326899
VHS25476	*P. rosacearum* II	Soil	*Banksia repens*	Australia, WA, Wellstead	2011	KJ372269	MF326838	MF326881	MF326850	MF326897
VHS6186	*P. rosacearum* II	Soil	Native vegetation	Australia, WA, Manjimup	1999	JN547638	MF326837	MF326879	MF326849	MF326900
CBS127954[2]	*P. thermophila*	Soil	*Eucalyptus marginata*	Australia, WA, Dwellingup	2004	EU301155	JN547613	HQ012916	HQ012872	JN547700
MUCC767	*P.* 'personii'	Water	Native vegetation	Australia, VIC, Ti-Tree Creek	2008	HQ012954	MF326804	MF326889	MF326861	MF326930
SA278	*P.* 'personii'	Soil	*Rubus anglocandicans*	Australia, WA, Walpole	2012	MF326894	MF326803	MF326888	MF326860	MF326929
VHS14801	*P.* 'personii'	Soil	*Grevillea mccutcheonii*	Australia, WA, Busselton	2005	EU301169	MF326805	MF326890	HQ012877	MF326928
IMI 389735	*P.* 'walnut'		*Juglans hindsii*	USA, California, Merced County	1988	AF541910	JN935990	JN935956	JN935971	JN936042

[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 int 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 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 lacustris* (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. cinnamomi* (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

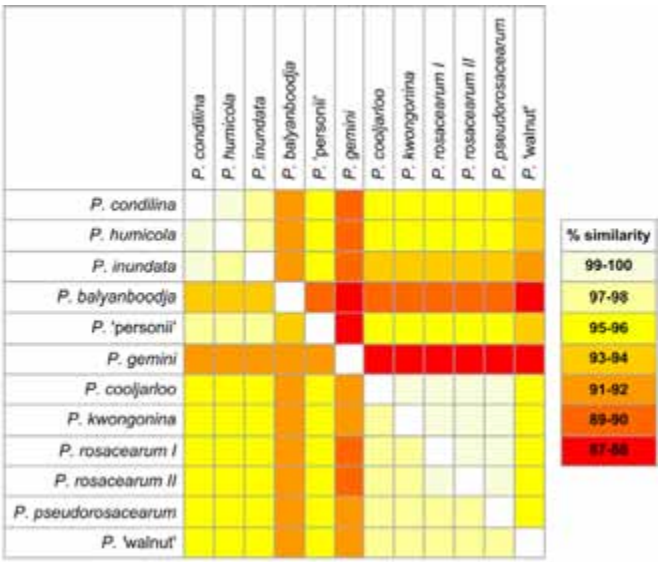
Phylogenetic analysis

The alignments for TUB, HSP, ITS, COX and NADH were consisted of 1 187, 957, 826, 1 196 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 balyanboodja* 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

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.



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.

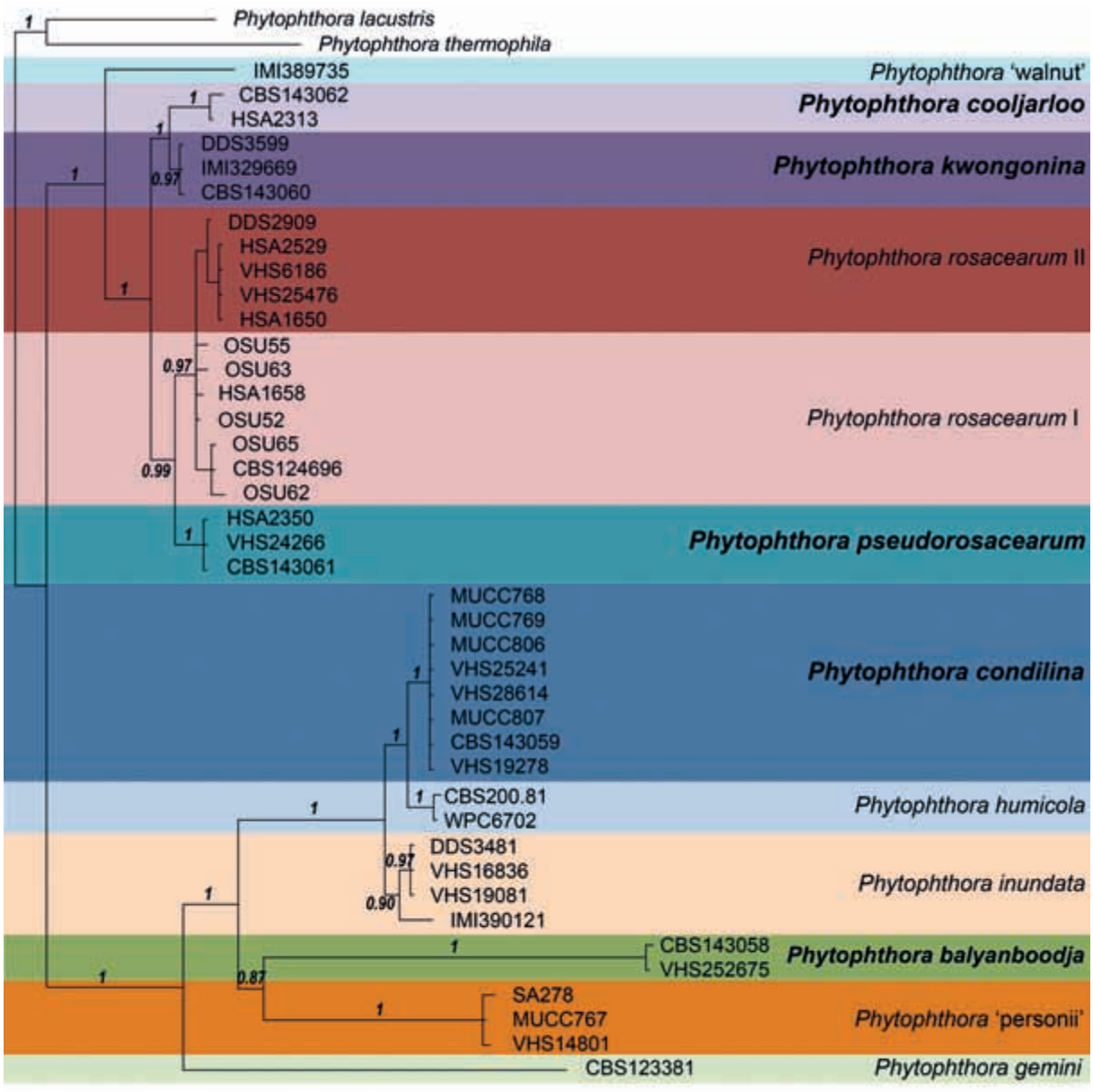


Fig. 1 Bayesian inference tree based on concatenated sequence data from nuclear genes regions, ITS, TUB and HSP, generated in MrBayes using the GTR + G substitution model showing relationship between all Clade 6a. The posterior probability is shown at the nodes. *Phytophthora lacustris* and *P. thermophila* were used as outgroup taxa.

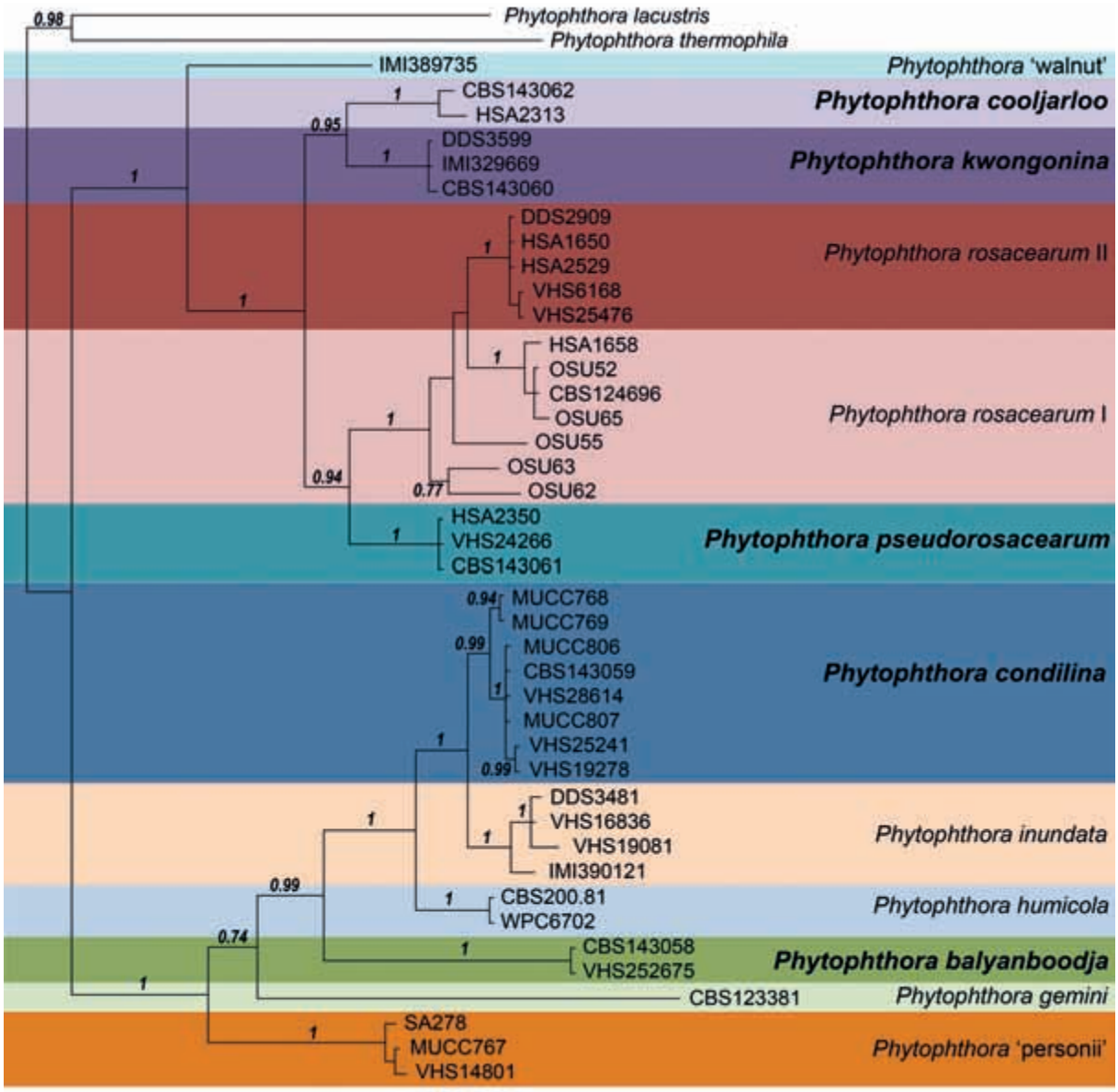


Fig. 2 Bayesian inference tree based on concatenated sequence data from mitochondrial gene regions, COX and NADH, generated in MrBayes using the GTR + G substitution model showing relationship between all Clade 6a. The posterior probability is shown at the nodes. *Phytophthora lacustris* and *P. thermophila*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

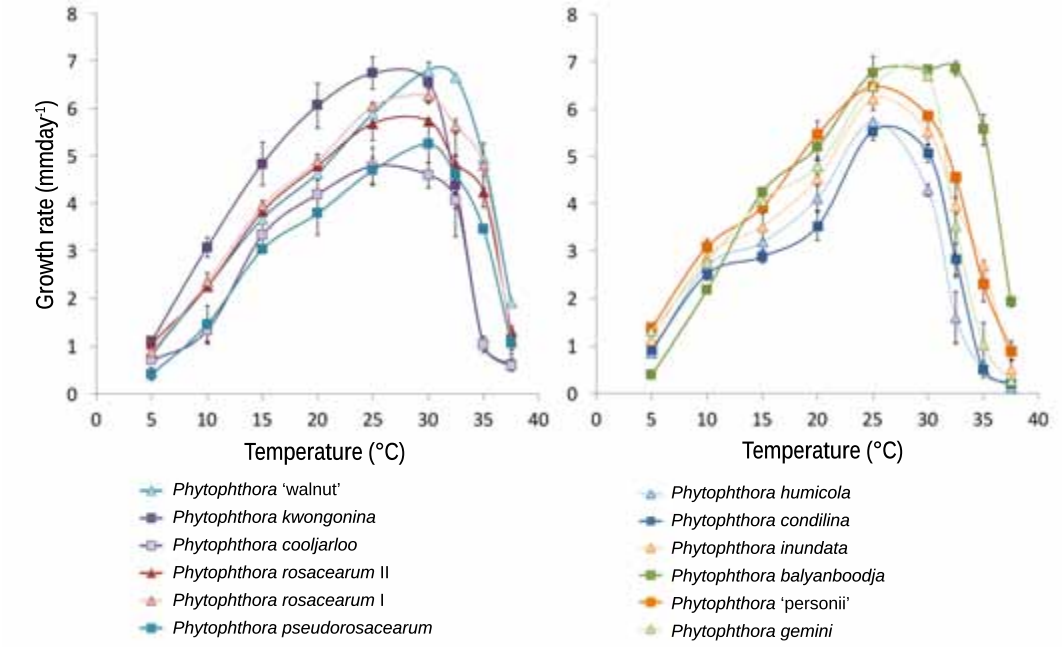


Fig. 3 Average radial growth rate (mm/d ± SE) of all Clade 6a species on V8 agar across the temperature range from 5–37.5 °C.

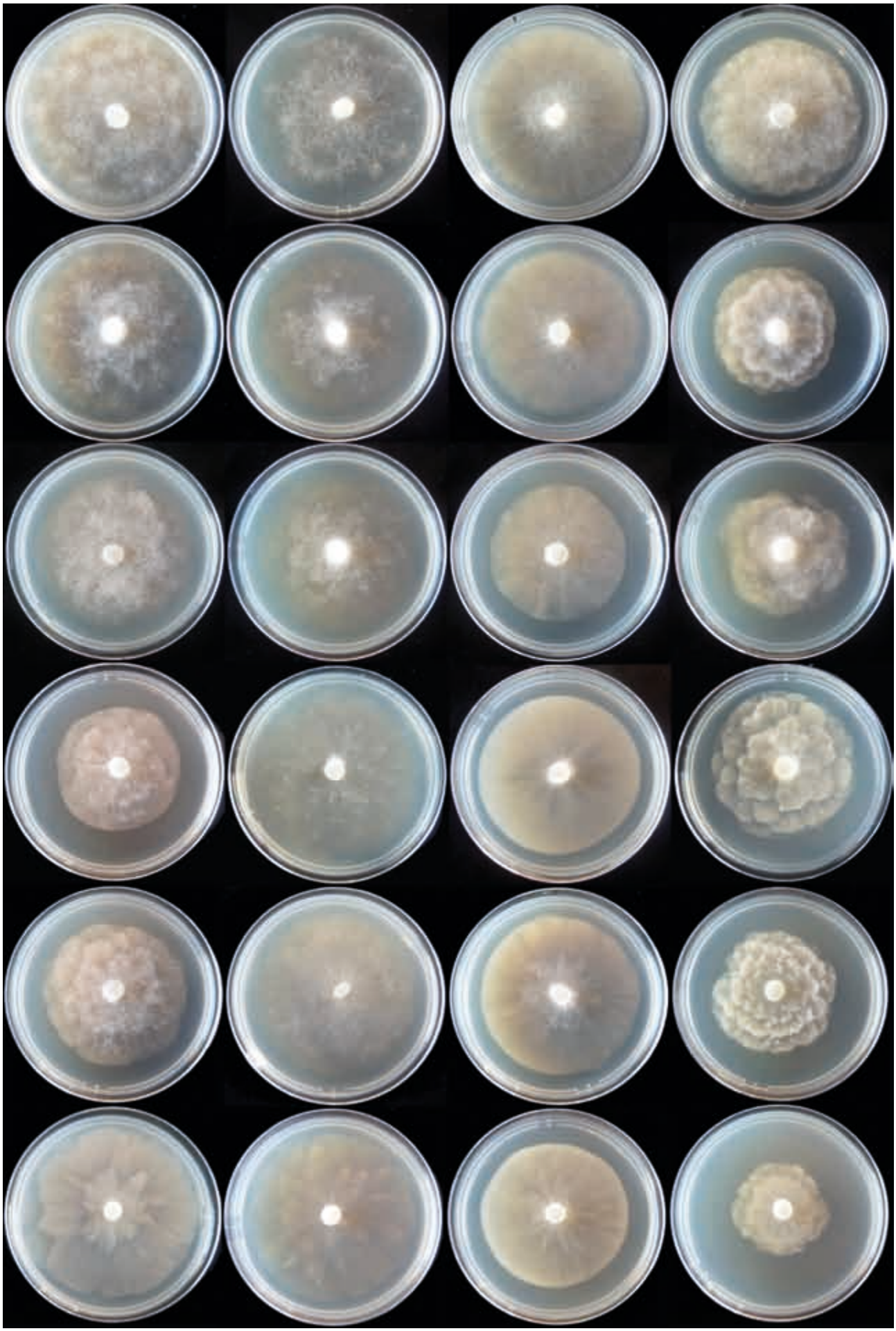


Fig. 4 Colony morphology of *Phytophthora kwongonina*, *P. cooljarloo*, *P. pseudorosacearum*, *P. rosacearum* I, *P. rosacearum* II and *P.* 'walnut' (from top to bottom) after 5 d growth at 20 °C on carrot agar, V8 agar, malt extract agar and potato-dextrose agar (from left to right).

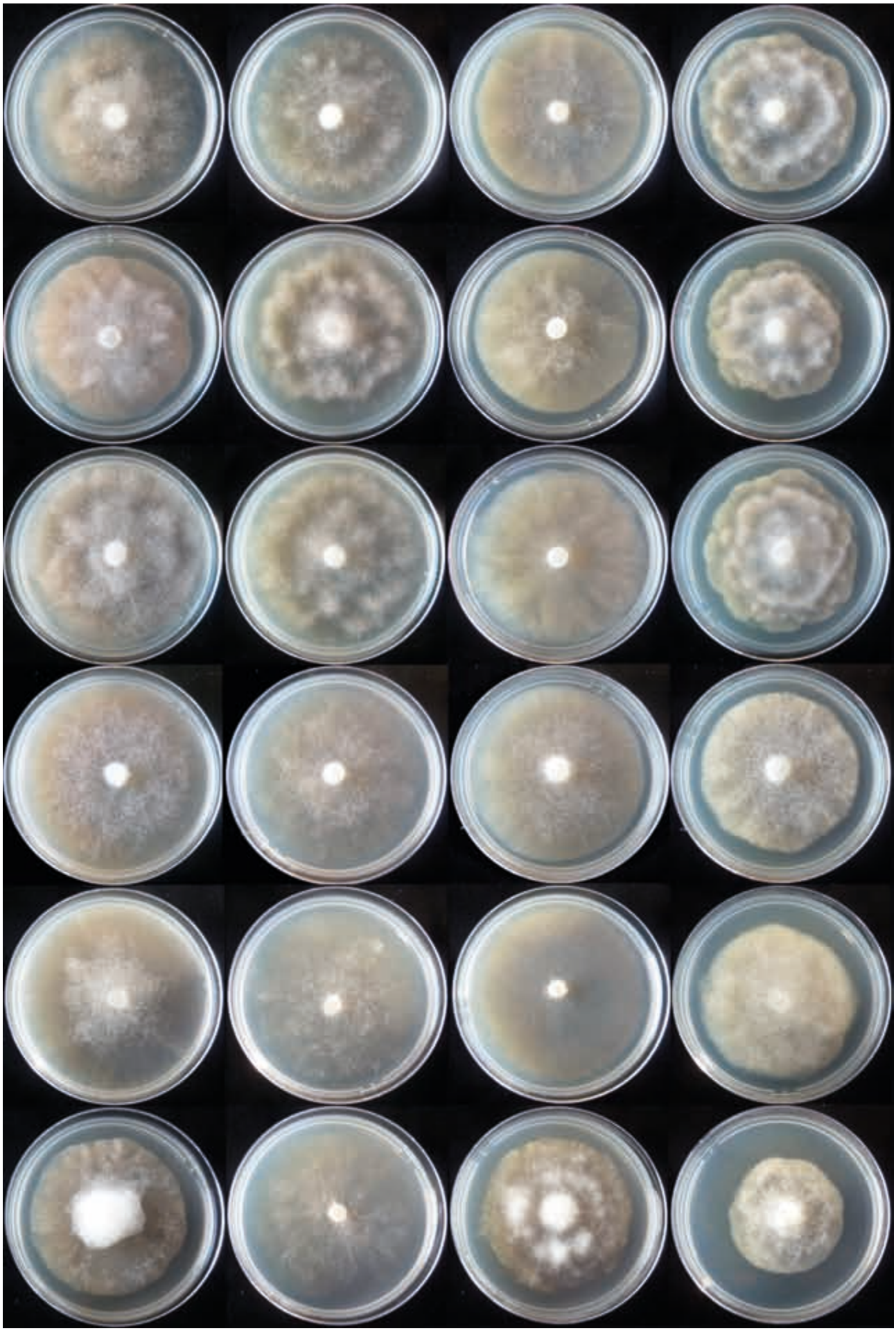


Fig. 5 Colony morphology of *Phytophthora condilina*, *P. humicola*, *P. inundata*, *P. balyanboodja*, *P.* 'personii' and *P. gemini* (from top to bottom) after 5 d growth at 20 °C on carrot agar, V8 agar, malt extract agar and potato-dextrose agar (from left to right).

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 rosacaceous 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 and MF326927, respectively).

Sporangia, chlamydospores and hyphal swellings (Fig. 6a–h) — 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 woolly 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).

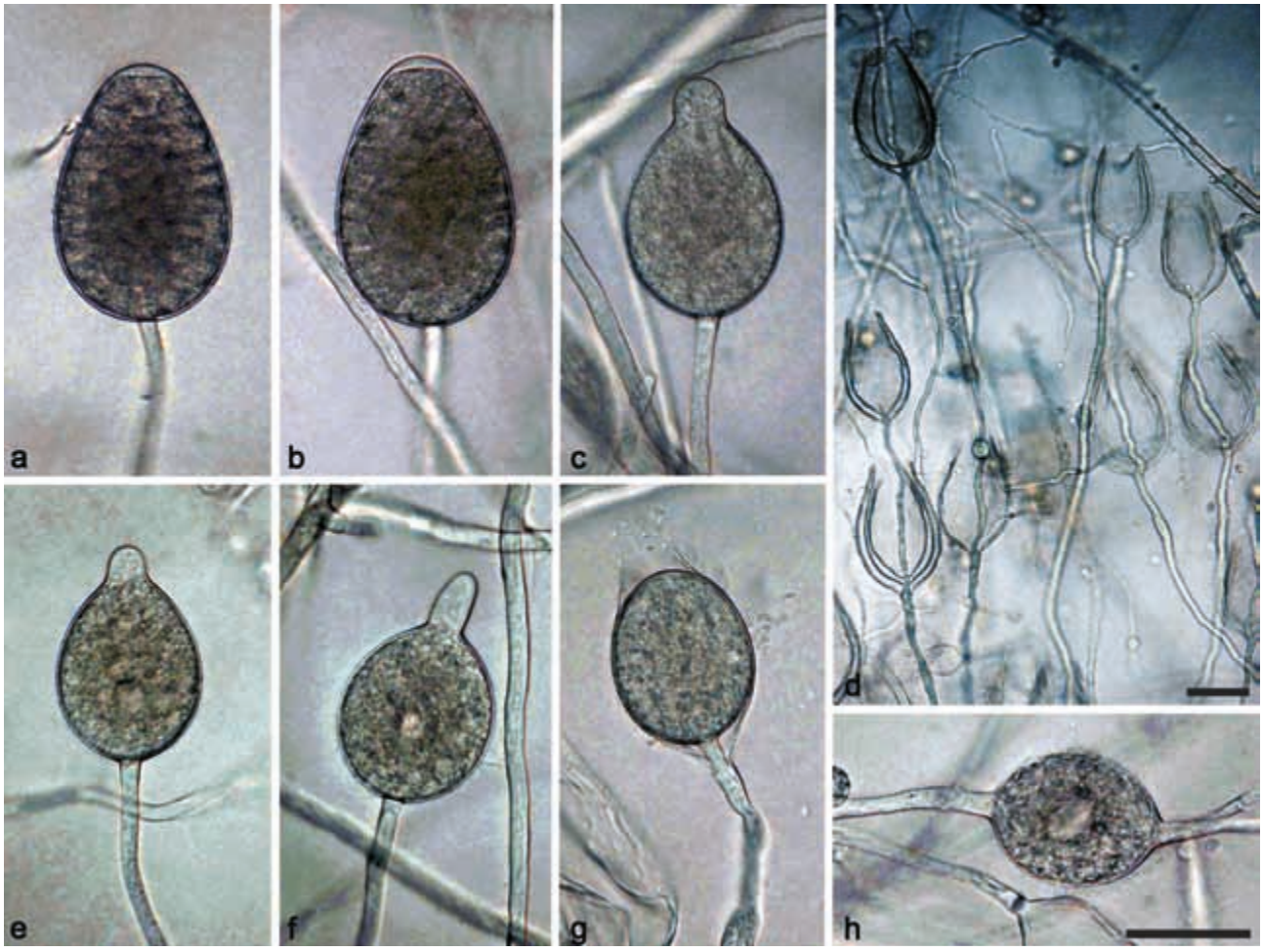


Fig. 6 *Phytophthora balyanboodja*. a–g. Persistent sporangia formed on V8 agar flooded with soil extract. a–b. ovoid with flat apex; c, e–f. ovoid with a pointed apex giving the appearance of papilla; d. chains of empty ovoid sporangia with internal nested and extended proliferation; h. direct germination 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).

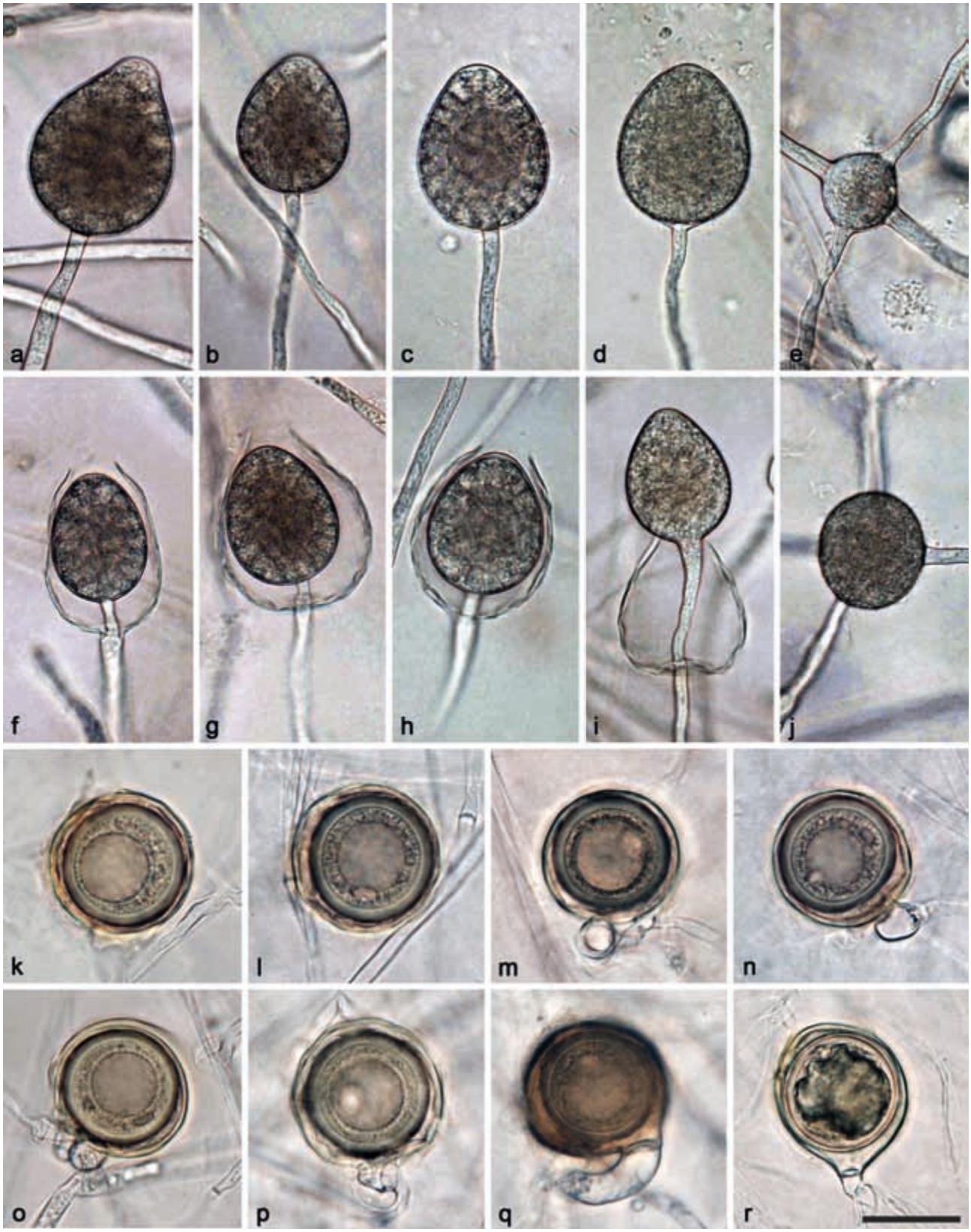


Fig. 7 *Phytophthora condilina*. a–d, f–i. Persistent, non-papillate, ovoid sporangia formed on V8 agar flooded with soil extract. f–h. empty sporangia with internal nested proliferation; i. empty sporangium with internal extended proliferation; e. spherical hyphal swellings with radiating hyphae; j. 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.

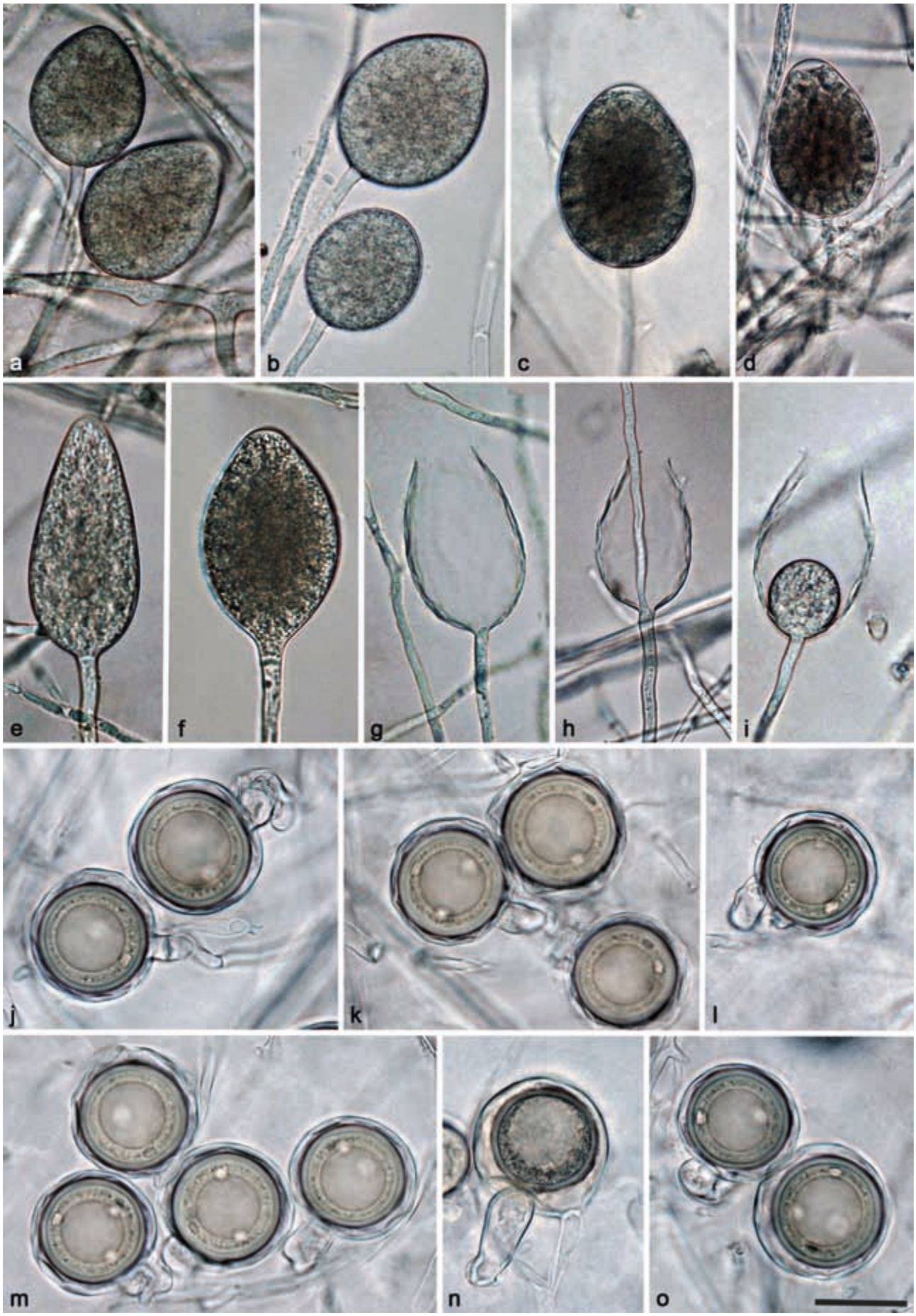


Fig. 8 *Phytophthora cooljarloo*. a–i. Persistent, non-papillate sporangia formed on V8 agar flooded with soil extract. a–d. ovoid; e. elongated ovoid; f. limoniform; g. empty ovoid sporangia; h. empty ovoid sporangium showing internal extended proliferation; i. empty ovoid sporangium showing internal nested proliferation. — j–o. Mature oogonia formed in single culture in V8 agar. j–m, o. oogonia with wavy walls containing aplerotic, pale brown oospores 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 regularly formed; 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 aplerotic, 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. Amphigynous antheridia were occasionally seen (p). This species is considered to be homothallic.

Colony morphology, growth rates and cardinal temperatures — Colonies on all media are woolly with a slight petaloid pattern on CA and V8A, striations on MEA and petaloid on PDA (Fig. 5). The minimum, maximum and lethal temperatures for growth were around 4, 37.5 and > 37.5 °C, respectively (Fig 3b). The average radial growth rate on V8A at the optimum temperature of 25 °C was 5.5 ± 0.19 mm/d (Fig. 3b).

Additional materials examined. Australia, Western Australia, Alfred Cove, from rhizosphere soil of dying *Casuarina obesa*, isolated by *VHS*, 2011, VHS25241, MUCC806, MUCC807; Esperance, from stream baiting within native vegetation, 2008, *D. Hüberli*, MUCC768 and MUCC769; Ravensthorpe from rhizosphere of mixed native vegetation, *VHS*, 2008, VHS19278.

Phytophthora cooljarloo T.I. Burgess, *sp. nov*. — MycoBank MB822011; Fig. 8

Etymology. Refers to the location where the isolates were recovered.

Typus. Australia, Western Australia, Cooljarloo, from rhizosphere soil of dying *Hibbertia* sp., *W.A. Dunstan*, 2008 *(*holotype MURU 479, dried culture on V8A, Herbarium of Murdoch University, Western Australia, culture ex-types CBS 143062. ITS, TUB, HSP, COX and NADH sequences GenBank HQ012957, MF326816, HQ012925, HQ012881 and MF326910, respectively).

Sporangia, chlamydospores and hyphal swellings (Fig. 8a–i) — Sporangia of *P. cooljarloo* 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 (a–d) to elongated ovoid (e) in shape although limoniform (f), ellipsoid and broad ovoid shapes were observed. Both nested and extended internal proliferation (g–i) of sporangia was observed. Exit pores were 11.5–22.5 µm wide (av. 17.5 ± 2.5 µm), zoospore cysts were spherical and 9–15 µm diam (av. = 11.7 ± 1.6 µm). Sporangial dimensions of two isolates of *P. cooljarloo* averaged 55.0 ± 9.5 × 37.6 ± 5.5 µm (overall range 30.5–79 × 25–49.5 µm). The length/breadth ratio ranged from 1.10–2.18 (av. = 1.47 ± 0.24). Chlamydospores were absent. Hyphal swellings were absent.

Oogonia, oospores and antheridia (Fig. 8j–o) — Gametangia were produced in single culture within 14 d. Oogonia were generally borne terminally ranging from 32–48.5 µm diam (av. = 41.9 ± 4.0). Oogonia had wavy walls. Oospores were aplerotic, globose, and pale on maturity, ranging in size from 26–40 µm diam (av. = 35.1 ± 3.5). The oospore walls were moderately thick (2.76 ± 0.59 µm), with a mean oospore wall index of 0.40 ± 0.07. The antheridia were exclusively paragynous, averaging 26.1 ± 8.4 × 13.1 ± 2.5 µm, terminal, round- to club-shaped and situated adjacent to the oogonial stalk. 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 rosacaceous 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 kwongonina T.I. Burgess, *sp. nov.* — MycoBank MB822012; Fig. 9

Etymology. Refers to association with the kwongon vegetation in the southwest of Western Australia.

Typus. 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 VHS23298. ITS, TUB, HSP, COX and NADH sequences GenBank JN547636, MF326824, MF326876, MF326847 and MF326914, respectively).

Sporangia, chlamydospores and hyphal swellings (Fig. 9a–i) — Sporangia of *P. kwongonina* 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, i) 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. kwongonina* 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) — 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. Oospores were highly aplerotic, 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 (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 materials 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.*

Typus. 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 VHS29592. ITS, TUB, HSP, COX and NADH sequences GenBank KJ372267, MF326827, MF326878, 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

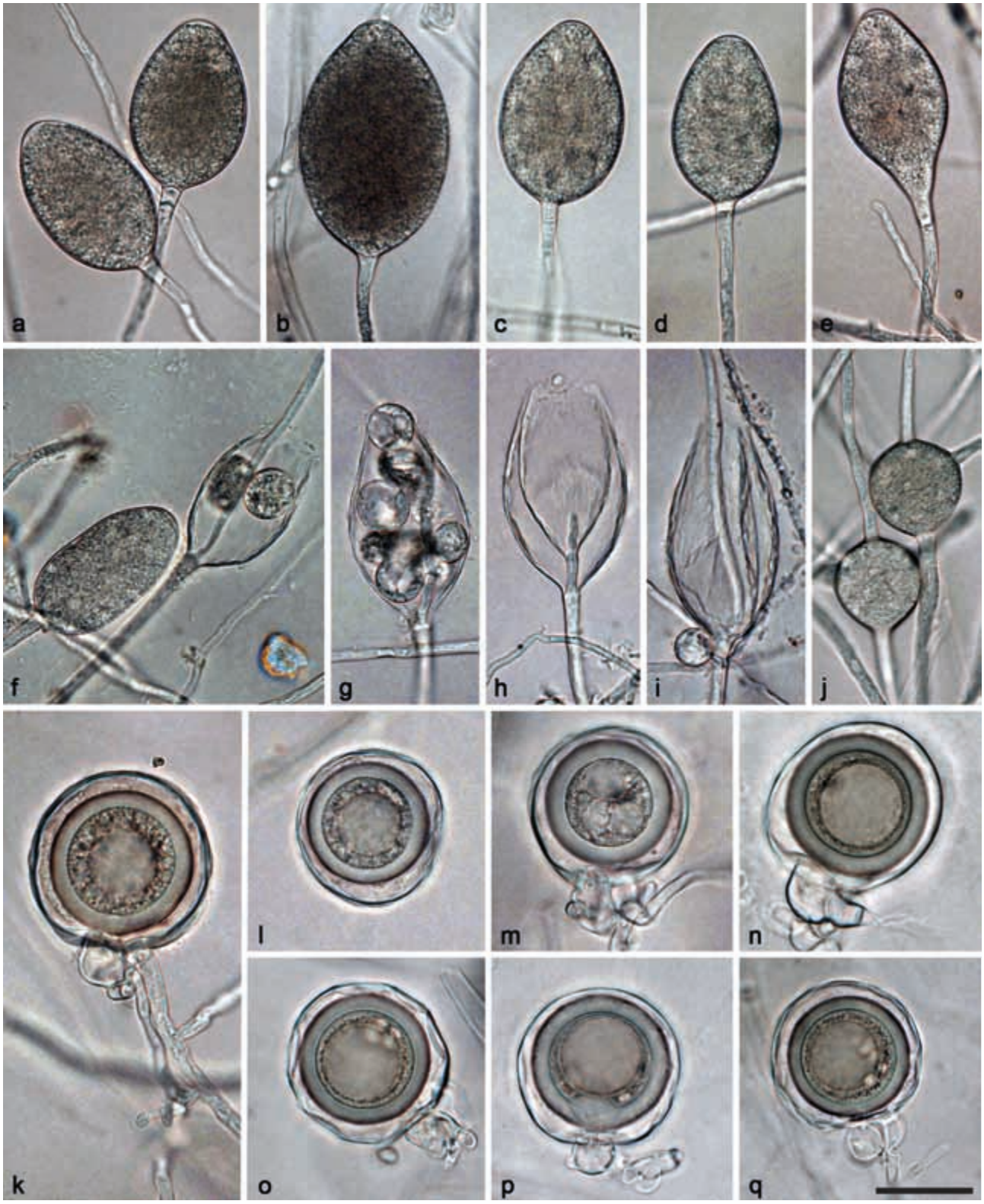


Fig. 9 *Phytophthora kwongonina*. a–i. Persistent, non-papillate sporangia formed on V8 agar flooded with soil extract. a, c–d. ovoid; b. ellipsoid; e. limoniform; f. empty ovoid sporangium with internal extended proliferation; g. ovoid sporangium releasing zoospores; h. empty sporangium showing internal nested proliferation; i. empty elongated ovoid sporangium with internal nested and extended proliferation; j. spherical hyphal swellings with radiating hyphae. — k–q. Mature wavy-walled oogonia containing thick walled, aplerotic, pale oospores with large ooplasts, formed in single culture in V8 agar; k, m–q. paragynous unicellular antheridia were situated adjacent to the oogonial stalk. — Scale bar = 25 µm.

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). Oospores 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

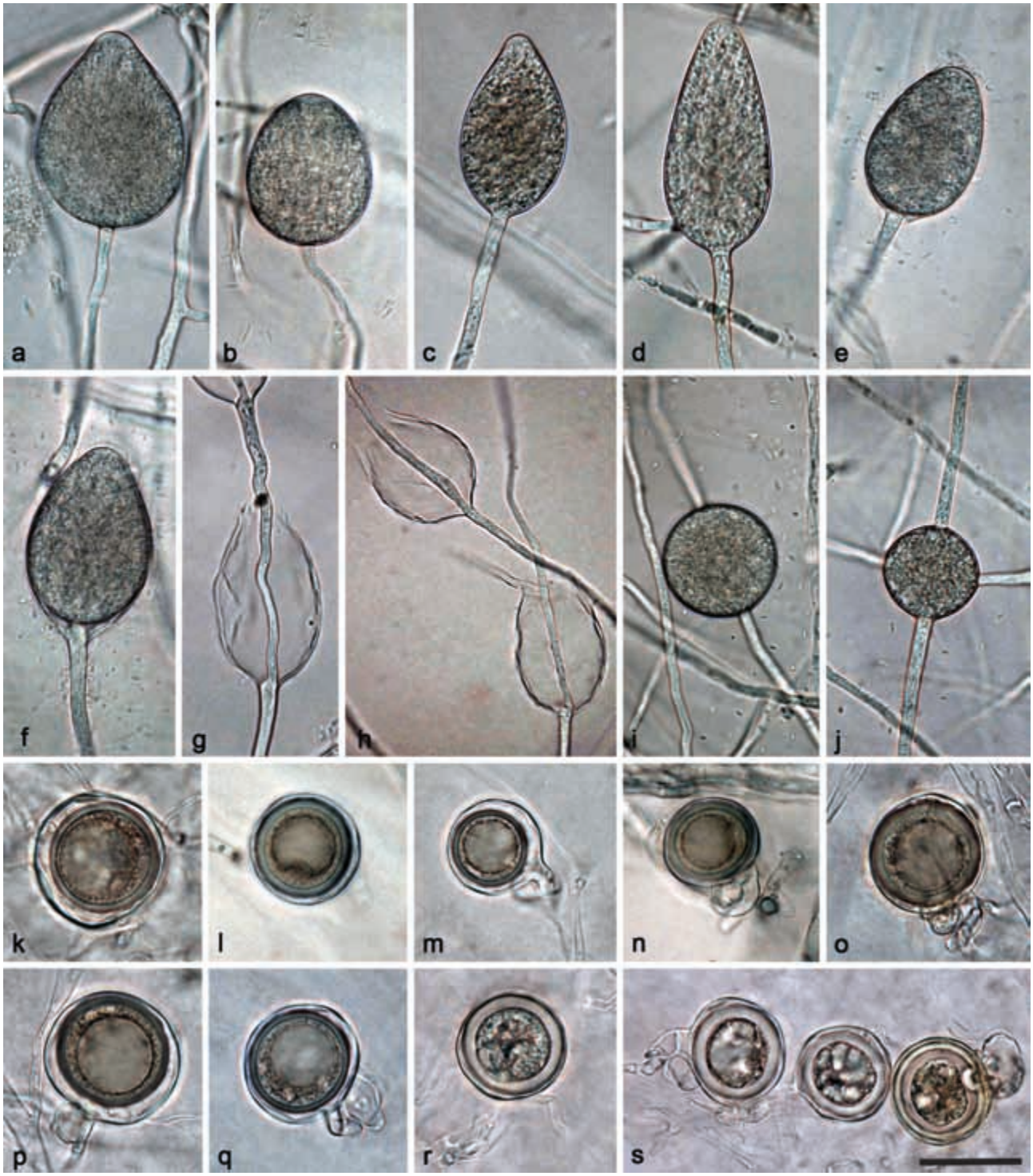


Fig. 10 *Phytophthora pseudorosacearum*. a–h. Persistent, non-papillate sporangia formed on V8 agar flooded with soil extract. a, e–f. ovoid; b. broad ovoid; c. limoniform; d. elongated ovoid; f. ovoid sporangium showing internal nested proliferation; g–h empty ovoid sporangia showing internal extended proliferation; i. intercalary chlamydospores; j. spherical hyphal swellings. — k–s. Mature oogonia formed in single culture in V8 agar. k–q. oogonia with pale walls containing aplerotic, pale brown oospores with large ooplasts; r–s. oospores aborted after the formation of the wall; n–q, s. paragynous unicellular antheridia situated adjacent to the oogonial stalk. — Scale bar = 25 µm.

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. rosacearum* 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						
L×B mean ± SD	44.8 ± 5.3 × 27.4 ± 5.0	47.6 ± 10.5 × 29.7 ± 4.5	52.7 ± 10.0 × 34.1 ± 5.6	57.5 ± 11.2 × 36.0 ± 6.9	55.0 ± 9.5 × 37.6 ± 5.5	59.5 ± 6.0 × 38.1 ± 4.8
Total range	32.0–59.3 × 16.9–38.3	22.5–73.4 × 16.7–40.1	32.7–59.3 × 19.4–38.3	34.6–87.0 × 23.2–56.5	30.6–79.1 × 25.1–49.8	43.2–68.4 × 30.8–57.3
Range of isolates means	43.7–47.9 × 23.7–31.9	36.2–57.8 × 24.7–31.4	49.4–56.0 × 30.7–37.8	53.8–60.3 × 31.9–38.3	51.7–57.8 × 37.2–37.9	
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
Sporangiophores	simple	simple	simple	simple	simple	simple
Shapes	ovoid 60 % elongated ovoid 20 % ellipsoid 20 %	ovoid 50 % elongated ovoid 34 % ellipsoid 12 % limoniform 4 %	ovoid 55 % elongated ovoid 30 % limoniform 5 % ellipsoid 5 % broad ovoid 5 %	ovoid 48 % elongated ovoid 20 % limoniform 25 % ellipsoid 5 % broad ovoid 2 %	ovoid 68 % elongated ovoid 12 % limoniform 4 % obpyriform 4 % broad ovoid 12 %	ovoid 90 %, elongated ovoid 10 %
Proliferation	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)
Zoospore cysts	12.3 ± 1.0 (10.0–14.8)	11.4 ± 1.1 (9.6–16.0)	11.6 ± 1.8 (8.0–19.9)	13.1 ± 1.5 (10.9–18.2)	11.7 ± 1.6 (9.2–15.1)	11.6 ± 1.1 (10.6–15.1)
Chlamydospores	absent	absent	present	absent	absent	absent
Diameter (range)			28.4 ± 5.3 (20.1–42.7)			
Hyphal swellings	present	absent	present	present	absent	absent
Features	predominantly spherical and intercalary with radiating hyphae		predominantly spherical and intercalary with radiating hyphae	predominantly spherical and intercalary with radiating hyphae		
Mean diam	17.6 ± 5.7 (9.0–27.8)		17.8 ± 6.0 (6.1–30.9)	21.5 ± 6.1 (12.2–46.4)		
Breeding system	homothallic	homothallic	homothallic	homothallic	homothallic	sterile in culture
Oogonia						
Features	slightly wavy walls	slightly wavy walls	wavy walls, sometimes with a slightly tapering base	wavy walls	wavy walls	
Mean diam	35.7 ± 3.7 (23.8–45.4)	36.6 ± 4.0 (25.3–47.3)	35.8 ± 4.9 (23.8–49.0)	45.4 ± 3.4 (36.7–52.4)	41.9 ± 4.0 (31.9–48.3)	
Range of isolates means	32.6–38.8	31.8–38.9	33.1–37.4	42.8–47.9	40.2–43.5	
Oospores						
Features	slightly aplerotic, pale on maturity	slightly aplerotic, pale on maturity	aplerotic, slightly golden on maturity and often slightly eccentric	aplerotic, pale on maturity	aplerotic, pale on maturity	
Abortion	90 %	50 %	20 %	0 %	0 %	
Mean diam	31.6 ± 3.4 (20.3–41.0)	30.8 ± 2.9 (22.8–38.8)	30.8 ± 3.3 (22.3–38.1)	37.1 ± 2.9 (31.9–44.1)	35.1 ± 3.5 (26.1–39.9)	
Range of isolates means	28.4–35.4	27.2–32.6	29.5–31.8	34.9–39.1	33.3–36.9	
Wall diameter	1.93 ± 0.43	2.21 ± 0.46	2.46 ± 0.47	4.89 ± 0.81	2.76 ± 0.59	
Oospore wall index	0.32 ± 0.05	0.37 ± 0.06	0.41 ± 0.06	0.60 ± 0.05	0.40 ± 0.07	
Antheridia						
Features	paragynous round-club shaped, predominantly adjacent to oogonial stalk, very few amphigynous in some isolates	paragynous round-club shaped, predominantly adjacent to oogonial stalk	paragynous round-club shaped, predominantly adjacent to oogonial stalk	paragynous round-club shaped, predominantly adjacent to oogonial stalk	paragynous round-club shaped, predominantly adjacent to oogonial stalk	
L×B mean	13.7 ± 2.7 × 9.5 ± 2.1	12.0 ± 2.1 × 9.4 ± 2.0	13.8 ± 3.9 × 11.4 ± 3.2	16.2 ± 3.5 × 11.8 ± 2.2	26.1 ± 8.4 × 13.1 ± 2.5	
L×B range	8.1–18.7 × 4.7–13.9	7.5–19.2 × 5.0–13.6	6.1–26.6 × 5.5–22.1	9.8–28.8 × 6.8–20.8	11.5–43.6 × 7.8–16.4	
Growth characteristics						
Max temp (°C)	37.5	37.5	37.5	35	35	37.5
Opt temp (°C)	25–30	25–30	30	25–30	25–30	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 (mmday^{-1})	5.8 ± 0.24	6.3 ± 0.15	5.2 ± 0.40	6.8 ± 0.32	4.8 ± 0.39	6.7

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*[1]	*P. humicola*	*P. condilina*	*P. balyanboodja*	*P. gemini*	*P.* 'personii'
No of isolates	3	1	6	2	1	3
Sporangia						
L×B mean ± SD	59.7 ± 13.3 × 44.2 ± 11.5	60.6 ± 6.8 × 43.1 ± 4.9	48.0 ± 7.4 × 36.3 ± 6.2	61.3 ± 8.3 × 39.7 ± 5.8	49.6 ±10.3 × 35.0 ± 5.0	62.9 ± 12.7 × 44.3 ± 9.9
Total range	31.4–84.5 × 20.6–63.5	43.3–72.1 × 29.0–57.3	29.8–69.3 × 20.1–51.4	40.9–75.7 × 21.2–51.1	35.3–72.0 × 25.7–43.1	43.3–72.1 × 29.0–57.3
Range of isolates means	54.4–65.3 × 38.9–49.4		44.4–49.8 × 32.6–38.8	61.1–61.6 × 38.6–40.7		58.5–67.8 × 42.0 × 46.8
L/B ratio (range)	1.38 ± 0.16 (1.03–1.90)	1.41 ± 0.15 (1.10–1.71)	1.33 ± 0.15 (1.00–1.86)	1.56 ± 0.17 (1.19–2.23)	142 ± 0.17 (1.15–1.73)	1.44 ± 0.15 (1.17–1.91)
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
Sporangiophores	simple	simple	simple	simple	simple	simple
Shapes	ovoid 80 % broad ovoid 10 % ellipsoid 5 % limoniform 5 %	ovoid 90 % limoniform 10 %	ovoid 70 % broad ovoid 25 % elongated ovoid 5 %	ovoid 80 % elongated ovoid 20 %	ovoid 100 %	ovoid 65 % ellipsoid 20 % elongated ovoid 5 % limoniform 5 % obpyriform 5 %
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 (10.2–22.4)	16.1 ± 2.9 (12.6–23.6)	13.6 ± 2.9 (6.7–21.2)	15.5 ± 2.0 (12.5–21.9)	15.6 ± 3.3 (10.2–22.0)	14.4 ± 2.5 (11.6–21.9)
Zoospore cysts	10.9 ± 0.9 (9.7–12.5)	11.8 ± 0.8 (10.6–12.9)	11.6 ± 1.5 (7.7–14.7)	10.9 ± 0.6 (9.9–12.5)	13.49 ± 1.4 (11.9–15.9)	11.1 ± 0.7 (9.7–12.3)
Chlamydospores	present	present	present	absent	absent	present
Diameter (range)	47.8 ± 8.6 (25.3–61.5)	36.8 ± 6.1 (19.3–45.4)	38.1 ± 10.6 (19.8–59.2)			54.5 ± 11.5 (29.9–78.5)
Hyphal swellings	present	present	present	absent	present	present
Features	predominantly spherical and intercalary with radiating hyphae	predominantly spherical and intercalary with radiating hyphae	predominantly spherical and intercalary with radiating hyphae		hyphal coils	predominantly spherical and intercalary with radiating hyphae
Mean diam	24.4 ± 4.9 (18.7–37.7)	20.3 ± 3.1 (12.7–32.7)	24.1 ± 7.2 (11.7–44.7)			38.9 ± 9.9 (27.8–49.4)
Breeding system	mixed	homothallic	homothallic	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 (32.9–49.5)	42.0 ± 4.7 (26.9–57.5)			
Range of isolates means	34.7–43.4		39.2–45.9			
Oospores						
Features	aplerotic, slightly golden on maturity, often slightly eccentric	aplerotic, slightly golden on maturity	aplerotic, golden on maturity, often slightly eccentric			
Abortion		10 %	80 %			
Mean diam	37.5 ± 2.8	32.9 ± 3.7 (26.4–38.7)	35.6 ± 3.8 (23.3–42.5)			
Range of isolates means	31.4–38.2		33.2–37.9			
Wall diameter	5.4 ± 0.9	4.02 ± 0.78	3.31 ± 0.72			
Oospore wall index	0.64	0.57 ± 0.07	0.46 ± 0.07			
Antheridia						
Features	amphigynous	predominantly paragynous, round-club shaped, often multiple	paragynous round-club shaped, attached on side of oogonia			
L×B mean	16.5 ± 1.2 × 15.9 ± 0.5	17.2 ± 1.8 × 14.4 ±1.9	15.6 ± 3.3 × 10.7 ± 2.0			
L×B range		13.1–19.9 × 10.5–17.6	9.0–22.4 × 6.0–14.2			
Growth characteristics						
Max temp (°C)	37.5	35	35	37.5	35	37.5
Opt temp (°C)	25	25	25–30	32.5	30	25
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 (mmday^{-1})	6.2 ± 0.22	5.8 ± 0.02	5.6 ± 0.19	6.8 ± 0.15	6.7 ± 0.03	6.5 ± 0.09

[1] Measurements for oospores and oogonia from Brasier et al. (2003b).

and situated adjacent to the oogonial stalk, averaging 13.8 ± 3.9 × 11.4 ± 3.2 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 (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 platyphylla*, 2010, *VHS*, 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* sharing 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 (Brasier 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 (Brasier 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. lacustris* 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. mississippiae*, *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 dataset on the distribution of *Phytophthora* in natural ecosystems and has not been replicated to the same extent elsewhere (except maybe 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 kwongon 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 kwongon heathlands (Laliberté 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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