Eur J Plant Pathol (2012) 133:841–855
DOI 10.1007/s10658-012-0006-5

Phytophthora bilorbang sp. nov., a new species associated with the decline of *Rubus anglocandicans* (European blackberry) in Western Australia

Sonia Aghighi · Giles E. St. J. Hardy · John K. Scott · Treena I. Burgess

Accepted: 16 May 2012 / Published online: 1 June 2012

Abstract A new homothallic *Phytophthora* species, isolated from rhizosphere soil and roots of declining or dead *Rubus anglocandicans* (European blackberry) in south-west Western Australia, is described as *Phytophthora bilorbang* sp. nov. It produces non-papillate sporangia, smooth-walled oogonia containing thick-walled oospores, and paragynous antheridia. Although morphologically similar to several species within ITS Clade 6 and sub-clade II, namely *P. gibbosa*, *P. gregata* and *P. megasperma,* phylogenetic analyses of the ITS, *cox*1, HSP90, BT and NADH gene regions demonstrate that *P. bilorbang* sp. nov. is a distinct species. Additionally, *P. bilorbang* differs from these species in its growth and colony morphology on several media. Pathogenicity tests indicate that *P. bilorbang* could be responsible for the decline syndrome of blackberry within the Warren and Donnelly River catchments in the south-west of Western Australia.

Keywords Biological control · Weed decline · *Rubus* species · Soil pathogen · Multigene phylogeny · Riparian ecosystem

Electronic supplementary material The online version of this article (doi:10.1007/s10658-012-0006-5) contains supplementary material, which is available to authorized users.

S. Aghighi · G. E. S. J. Hardy · T. I. Burgess (✉)
Centre for Phytophthora Science and Management,
School of Biological Sciences and Biotechnology,
Murdoch University,
90 South Street,
Murdoch, WA 6150, Australia
e-mail: tburgess@murdoch.edu.au

J. K. Scott
CSIRO Ecosystem Science and Climate Change Adaptation Flagship,
Private Bag P.O.,
Wembley, WA 6913, Australia

Introduction

Rubus anglocandicans is the most widespread and invasive species in the *Rubus fruticosus* aggregate (European blackberry) found in Australia (Evans and Weber 2003) and one of the few weeds of national significance widespread in the south-west of Western Australia (WA). It is a major weed of conservation areas (particularly in wetter regions), forestry and agriculture because of its high degree of invasiveness, potential for spread, and economic and environmental impacts. Herbicides and cultural control methods are generally ineffective against blackberry, or require multiple applications and have proved to be expensive and difficult to apply in natural habitats and inaccessible areas invaded by blackberry (Amor et al. 1998; Bruzzese et al. 2000).

European blackberry in Australia has been targeted by means of biological control since the 1980s. Most of this effort has focused on introducing exotic strains of the host-specific leaf rust, *Phragmidium violaceum.* New strains of the rust selected for *R. anglocandicans*

and other European blackberries (Morin et al. 2011) were released in 2004 and 2005 in WA. In some areas the levels of rust developed on blackberry was high, at least initially, but it seems that it does not have enough potential to control the weed, and is possibly limited by climatic factors (Morin and Evans 2011).

During surveys established to assess the releases of the rust fungus in 2005, dead and diseased blackberry plants were found at two locations along the Warren and Donnelly Rivers in WA (P. Yeoh personal communication). However, the disease could not be attributed to the release of the rust fungus as a biological control agent. Over the next few years, the extent of the disease increased within the Warren and Donnelly river catchments, with noticeable landscape changes due to disappearance of dense blackberry thickets. This has lead to the disease being called "blackberry decline". The disease appears to be due to root pathogen/s and during preliminary sampling several *Phytophthora* species were isolated (G. Hardy unpubl. data).

In order to investigate the cause(s) of blackberry decline and the potential role of *Phytophthora* species in the disease, field surveys were carried out over 2010 and 2011 in the decline and non-decline sites along the Warren and Donnelly Rivers. During these surveys, isolates with identical ITS sequence to *P.* taxon oaksoil were recovered and are described here as *Phytophthora bilorbang* sp. nov., a new taxon within the ITS Clade 6 of *Phytophthora*.

Materials and methods

Isolation procedure

Rhizosphere soil and roots were collected from five sites with dying *Rubus anglocandicans* and three non-decline sites with apparently healthy blackberry in the south-west of Western Australia. Both soil and root samples were baited with *R. anglocandicans* and *Alnus* sp., *Quercus suber*, *Q. ilex* juvenile leaves, *Rosa* and *Hibbertia* petals and *Eucalyptus sieberi* cotyledons following the modified method of Rea et al. (2010). After 3–7 days, baits with brownish lesions were blotted dry, and the lesions cut into 1–2 mm sections and plated onto a *Phytophthora* selective medium including NARPH (Hüberli et al. 2000) and a modified recipe of PARPHN (Jung et al. 2000) from which pentachloronitrobenzene was excluded. Plates were incubated in the dark at 20 °C and checked regularly for *Phytophthora* hyphae. Colonies growing from the plated lesion sections were transferred to V8 agar (0.1 l filtered V8 juice, 0.1 g $CaCo_3$ and 0.9 l distilled water and pH adjusted to 7) for confirmation of hyphae typical of *Phytophthora* species. Cultures were maintained under long-term storage in water at the Murdoch University Culture Collection.

DNA isolation, amplification and sequencing

The *Phytophthora* isolates were grown on half-strength potato dextrose agar PDA (Becton, Dickinson and Company, Sparks, USA, 19.5 g PDA, 7.5 g of agar and 1 l of distilled water) at 20 °C for 2 weeks 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. Harvested mycelium was frozen in liquid nitrogen and ground to a fine powder. Genomic DNA was extracted according to the method of Andjic et al. (2007). 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). The PCR reaction mixture and PCR conditions were as described previously (Andjic et al. 2007). The mitochondrial gene *cox*1 was amplified with primers FM84 and FM83 (Martin and Tooley 2003). The PCR reaction mixture was the same as for the ITS region, but the PCR conditions were as described previously (Martin and Tooley 2003). Heat shock protein 90 (HSP90) was amplified with HSP90-F1 and HSP90-R2 primers (Blair et al. 2008). The PCR reaction mixture was the same as for the ITS region, but the PCR conditions were as described previously (Blair et al. 2008). β-tubulin (BT) was amplified with primers TUBU-F2 and TUBU-R1 and NADH dehydrogenase subunit 1 was amplified with NADH-F1 and NADH-R1 primers according to Kroon et al. (2004).

For all gene regions except HSP templates were sequenced in both directions with primers used in amplification. Additionally, for *cox*1 templates were also sequenced with primers FM 85 and FM 50 (Martin and Tooley 2003). For HSP templates were sequenced in both directions with primers HSP90-F1int and HSP90-R1.

The clean-up of products and sequencing were performed as described previously (Sakalidis et al. 2011). All sequences derived in this study were deposited in GenBank and accession numbers are given in Table 1.

Table 1 Identity, host, location, isolation information and GenBank accession numbers for *Phytophthora* isolates considered in the phylogenetic study

Reference collection no.[1,2]	Other collection no. [1,2,]	Identity	Substrate	Host	Location	Isolated by	Date	GenBank accession no[2].				
								ITS	*Cox*I	HSP90	BT	NADH
	VHS17644	*P. asparagi*	Soil	*Lomandra sonderi*	Australia, WA, Murdoch	VHS	2007	EU301168	HQ012845	HQ012891	***JN547592***	***JN547680***
	SA92	*P. bilorbang*	Soil	*Rubus anglocandicans*	Australia, WA, Warren River	S Aghighi	2010	***JN547621***	***JN547643***	***JN547654***	***JN547582***	***JN547670***
	SA142	*P. bilorbang*	Soil	*R. anglocandicans*	Australia, WA, Warren River	S Aghighi	2010	***JN547622***	***JN547644***	***JN547655***	***JN547583***	***JN547671***
	SA143	*P. bilorbang*	Soil	*R. anglocandicans*	Australia, WA, Warren River	S Aghighi	2010	***JN547623***	***JN547645***	***JN547656***	***JN547584***	***JN547672***
	SA146	*P. bilorbang*	Soil	*R. anglocandicans*	Australia, WA, Warren River	S Aghighi	2010	***JN547624***	***JN547646***	***JN547657***	***JN547585***	***JN547673***
CBS 161653	SA262	*P. bilorbang*	Soil	*R. anglocandicans*	Australia, WA, Warren River	S Aghighi	2010	***JQ256377***	***JQ256375***	***JQ256376***	***JQ256374***	***JQ256378***
HSA1959		*P. lacustris*	Water	Road drainage sump baiting	Australia, WA, Welshpool	R Hart	1994	HQ012956	HQ012880	HQ012924	***JN547618***	***JN547706***
IMI 389725	P245	*P. lacustris*	Root	*Salix matsundana*	UK, Kent, Bexley Heath	CM Brasier	1972	AF266793	AY564181	EU080534	AY564066	AY564008
	VHS17350	*P. fluvialis*	Water	native vegetation	Australia, WA, Badgingarra	VHS	2007	EU593261	JF701440	JF701437	***JN547593***	***JN547681***
CBS 129424	DH086	*P. fluvialis*	Water	stream baiting, native vegetation	Australia, WA, Moore River	D Hüberli	2009	JF701436	JF701442	JF701439	***JN547595***	***JN547682***
CBS 127951	VHS21998	*P. gibbosa*	Soil	*Acacia pycnantha*	Australia, WA, Scott River	VHS	2009	HQ012933	HQ012846	HQ012892	***JN547596***	***JN547683***
	VHS22008	*P. gibbosa*	Soil	*Grevillea* sp.	Australia, WA, Scott River	VHS	2009	HQ012936	HQ012849	HQ012895	***JN547597***	***JN547685***
MUCC761	SLPA72	*P. gonapodyides*	Water	*Eucalyptus obliqua* forest	Australia, VIC, Toolangi North	WA Dunstan	2008	HQ012937	HQ012850	HQ012896	***JN547598***	***JN547686***
MUCC776	TAS 35	*P. gonapodyides*	Water	stream baiting, native vegetation	Australia, Tas, Houn River	Y Ziqing	2009	***JN547620***	***JN547642***	***JN547653***	***JN547581***	***JN547669***
	VHS21961	*P. gregata*	Soil	*Hakea* sp.	Australia, WA, Busselton	VHS	2009	HQ012941	HQ012857	HQ012903	***JN547605***	***JN547693***
CBS 127952	VHS21962	*P. gregata*	Soil	*Patersonia* sp.	Australia, WA, Busselton	VHS	2009	HQ012942	HQ012858	HQ012904	***JN547606***	***JN547694***
WPC P6702		*P. humicola*		*Phaseolus* sp. *(*bean*)*	Taiwan	Ann Chia-Yi		FJ801938	***JN935957***	***JN935946***	***JN935975***	***JN935994***
IMI 390121	P894	*P. inundata*	Roots	*Olea* sp.	Spain, Seville, Ecija	E. Sanchez-Hernandez	1996	EF210201	EF210207	***JN935947***	EF210203	EF210205
	VHS17085	*P. litoralis*	Soil	*Banksia* sp.	Australia, WA, Hopetoun	VHS	2007	EU593262	HQ012864	HQ012909	***JN547609***	***JN547697***
CBS 127953	VHS20763	*P. litoralis*	Soil	*Banksia* sp.	Australia, WA, Ravensthorpe	VHS	2008	HQ012948	HQ012866	HQ012911	***JN547611***	
	DDS3432	*P. megasperma*	Soil	*Banksia* sp.	Australia, WA, North Dinninup	VHS	1992	HQ012949	HQ012867	HQ012906	***JN547608***	***JN547696***
IMI 389741		*P. megasperma*		*Malus sylvestris*	Australia, WA	HL Harvey	1968	AF266794	***JN935959***	***JN935949***	***JN935977***	***JN936029***
CBS 122924	CMW26668	*P. pinifolia*	Needles	*Pinus radiata*	Chile, Arauco, Llico plantation	MJ Wingfield	2007	EU725806	***JN935960***	***JN935950***	***JN935978***	***JN936030***
CBS 122922	CMW26669	*P. pinifolia*	Needles	*P. radiata*	Chile, Arauco, Llico plantation	MJ Wingfield	2007	EU725807	***JN935961***	***JN935951***	***JN935979***	***JN936031***
CBS 127954	VHS13530	*P. thermophila*	Soil	*E. marginata*	Australia, WA, Dwellingup	VHS	2004	EU301155	HQ012872	HQ012916	***JN547613***	***JN547700***
	VHS16164	*P. thermophila*	Soil	*B. grandis*	Australia, WA, Pemberton	VHS	2006	EU301158	HQ012875	HQ012919		***JN547701***
	WPC P1410	P. taxon cranberry						AY964102				
CBS 117381	H-7/02	P. taxon hungarica	Root	*Alnus glutinosa*	Hungary, Földsziget	J Bakonyi and ZÁ Nagy	2002	EF452179				
	H-8/02	P. taxon hungarica	Soil	*A. glutinosa*	Hungary, Földsziget	J Bakonyi and ZÁ Nagy	2002	EF452180				
IMI 389733	P1055	P. taxon oaksoil	Soil	*Quercus robur*	France, Alsace, Illwald Forest	EM Hansen	1998	AF541906				
	GD38a	P. taxon oaksoil	Soil		Poland	L Belbahri		EU240038			EU795882	
	GD4b	P. taxon oaksoil				L Belbahri		EF152512				
	P11652	P. taxon oaksoil				L Belbahri		FJ801622				

Table 1 (continued)

Reference collection no.[1,2]	Other collection no. [1,2,]	Identity	Substrate	Host	Location	Isolated by	Date	GenBank accession no[2].				
								ITS	*Cox*I	HSP90	BT	NADH
	GD4f	P. taxon oaksoil	Soil		Poland	L Belbahri		EF152510				
	GD2f	P. taxon oaksoil	Soil		Poland	L Belbahri		EF152514				
	UASWS0281	P. taxon oaksoil	Soil		Poland	L Belbahri		DQ528752				
	WA46.3	P. taxon oaksoil	Water	stream bait	USA, Oregon, Curry County	P Reeser		HM004234				
P12645	P1044	P. taxon riversoil	Soil	riverbank	UK, Worcestershire	J Delcan	1997	AF541907				
MUCC765	SLPA166	P. taxon paludosa	Water	Pond baiting, native forest	Australia, VIC, Sugarloaf Reservoir Reserve	W Dunstan	2008	HQ012953	HQ012876	HQ012920	***JN547615***	***JN547702***
	DDS3753	P. taxon PgChlamydo	Soil	Native forest	Australia, WA, Manjimup	VHS	1995	EU301160	HQ012878	HQ012922	***JN547616***	***JN547703***
	VHS6595	P. taxon PgChlamydo	Soil	Native forest	Australia, WA, Manjimup	VHS	1999	EU301159	HQ012879	HQ012923	***JN547617***	***JN547704***
IMI 389746	P1050	P. taxon raspberry	Root	*Rubus idaeus*	Sweden, Scania	CHB Olsson	1994	AF541905				
	RAS1	P. taxon raspberry	Soil	*Betula pendula*	Germany, Bavaria, Neuburg	T Jung	2006	HQ012964				
P12645	P1044	P. taxon riversoil	Soil	riverbank	UK, Worcestershire	J Delcan	1997	AF541907				

[1] Abbreviations of isolates and culture collections: *CBS* Centraalbureau voor Schimmelcultures Utrecht, Netherlands; *IMI* CABI Bioscience (Imperial Mycological Institute), UK; *VHS* Vegetation Health Service Collection, Department of Environment and Conservation, Perth, Australia; *DDS* earlier prefix of VHS Collection; *TCH* TC Hill, in VHS Collection; *MJS* MJC Stukely, in VHS Collection; *HSA* Hart, Simpson and Associates, in VHS Collection; *DCE* EM Davison, in VHS Collection; *MUCC* Murdoch University Culture Collection

[2] isolates in bold, italics were sequenced during this study

Phylogenetic analysis

The data set comprised of sequences of *P. bilorbang* sp. nov. and those of closely related species in ITS Clade 6, sub-clade II, that were either sequenced for this study or obtained from GenBank (http://www.ncbi.nlm.nih.gov/). ITS sequence data were assembled and manually edited as described previously (Jung and Burgess 2009). There were no gaps in the *cox*1, NADH, BT, and HSP90 alignments. Trees were rooted to species from ITS Clade 6, sub-clade I (*P. inundata* and *P. humicola*) and sub-clade III (*P.* taxon asparagi).

Parsimony analysis was performed in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) and Bayesian analysis with MrBayes v. 3.1 (Ronquist and Heuelsenbeck 2003) as described previously (Jung and Burgess 2009). Alignment files and trees can be viewed on TreeBASE (http://www.treebase.org/).

Colony morphology

Colony growth patterns were described from 7-day-old cultures grown at 20 °C in the dark on V8A, 2 % malt-extract agar MEA, half-strengthen PDA (19.5 g PDA, 7.5 g agar and 1 l distilled water), carrot agar (CA) (0.1 l filtered carrot juice, 17 g agar and 1 l distilled water) and corn-meal agar (CMA) (17 g CMA and 1 l distilled water) (all media and agar were sourced from Becton Dickinson Co. Sparks MD 21152 USA). Colony morphologies were described according to the patterns described previously (Erwin and Ribeiro 1996; Jung et al. 2011).

Growth rates and cardinal temperature

All isolates were sub-cultured onto V8A plates and incubated at 20 °C to initiate growth for 24 h. Three replicate plates for each isolate were then transferred to incubators set at 4, 10, 15, 20, 25, 30, 32.5, 35 and 37.5 °C (± 0.5 °C), and radial colony growth was measured after 7 days. Max/min thermometers were placed in all the incubators. Plates showing no growth above 30 °C were returned to 20 °C to determine isolate viability.

Morphology of sporangia and gametangia

Sporangia, hyphal swellings and gametangia of five isolates of *P. bilorbang* sp. nov. produced on V8A were measured using the methods described in Jung et al. (1999). Sporangia were produced by flooding 15×15 mm agar squares taken from growing margins of 7-day-old colonies, so that their surfaces were covered with distilled water in 90 mm Petri dishes which were incubated at room temperature around 22 °C in natural daylight. The water was decanted and replaced again after 2 and 8 h. Two ml of diluted non-sterile soil extract was added at 8 h. The soil extract was made from 100 g of pine bark potting mixture suspended in 1 l distilled water, incubated for 24 h at 20 °C, filtered through cheesecloth and refiltered through Whatman no. 1 paper. After 15–24 h, dimensions and characteristic features of 50 mature sporangia, 25 exit pores and zoospore cysts per isolate were chosen at random and measured. Likewise, after 3–7 days, 25 hyphal swellings were also measured. All measurements were made at x400 magnification (BX51, Olympus). Isolates grown in the dark on V8A and CA plates at 20 °C for 14–28 d were examined and 50 mature oogonia, and oospores together with 35 antheridia per isolate chosen at random were measured at x400 magnification (BX51, Olympus).

Glasshouse pot trial

In order to investigate pathogenicity of *P. bilorbang* sp. nov., three isolates (SA092, SA142 and CBS 161653) were tested in a soil-infestation pot trial. *Rubus anglocandicans* daughter plants (less than 1 month old) were collected from sites that have remained disease-free in the Manjimup region and placed into 130 mm free-draining polyurethane pots containing a commercial bark based substrate (Soils Aint Soils, Perth, Western Australia). The plants were grown in an evaporatively-cooled glasshouse (11–25 °C) for 5 months prior to use.

Two different types of inocula were made. Firstly, plugs were prepared as described previously (Butcher et al. 1984; Rea et al. 2010) except that tree lucerne (*Cytisus palmensis*) was used for the plugs instead of *Pinus radiata*. Secondly, vermiculite inoculum (vermiculite 1 l, millet seeds 10 g and 600 ml V8 broth) was produced as described previously (Jung et al. 1996)

The vermiculite inoculum was mixed with pasteurized and washed river sand in a ratio of 40 g/l sand and healthy blackberry plants and attached soil were transferred from the 130 mm pots into 170 mm free-draining pots filled with 1 l of the infested sand (control pots received non-inoculated vermiculite). Each

pot also received four colonised or non-colonised (control) tree lucerne plugs. There were 11 replicate pots for each isolate and the pots were placed in a randomized design in a glasshouse (12 °C min–25 °C max). The plants were watered to container capacity daily, and fertiliser (water soluble, Thrive®, Yates

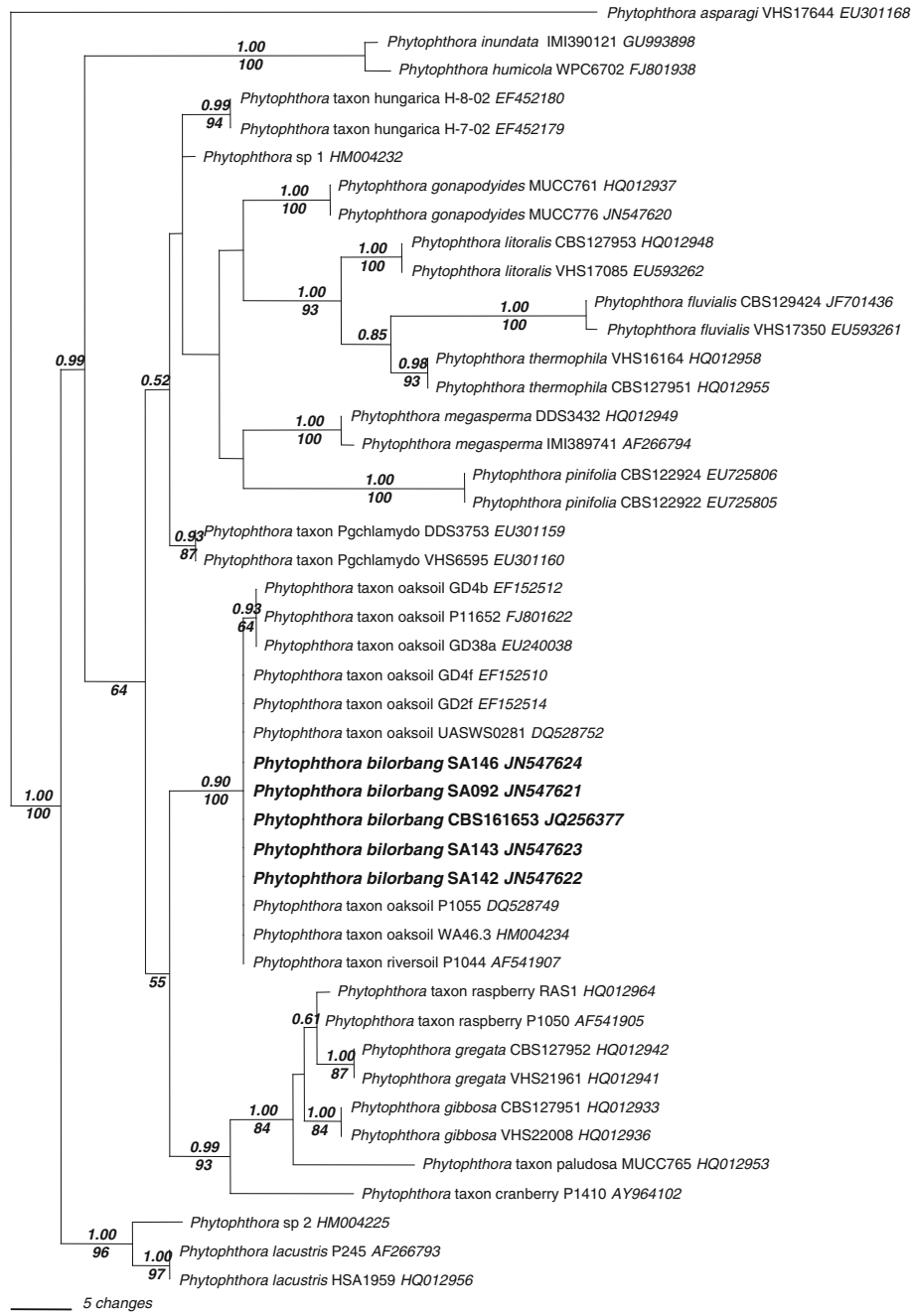


Fig. 1 Bayesian inference tree based on rDNA ITS sequences showing phylogenetic relationships between *Phytophthora bilorbang* and other species in ITS Clade 6. Numbers above the branches represent posterior probability based on Bayesian analysis; numbers below the branches represent the bootstrap support based on parsimony analysis. *P.* taxon asparagi was used as outgroup taxa

Company, Australia) was applied twice in the second and third weeks after inoculation. The pots were flooded in 9-l buckets twice at 2 and 7 weeks after inoculation for 15–17 h. After 4 months, infested soil was baited to recover *Phytophthora* species, and the plants were rated as asymptomatic, declining and dead. Necrotic roots were plated directly on PARPHN. Symptomatic roots were baited with *Q. suber* and *Q. ilex* juvenile leaves, and baits with lesions were plated onto PARPHN as described previously.

Results

Recovery of *P. Bilorbang* sp. nov.

Fourteen isolates of *P. bilorbang* sp. nov. were isolated from four decline sites and it was not recovered from non-decline sites. Juvenile leaves of *R. anglocandicans, Alnus* sp., *Quercus suber* and *Q. ilex,* but not petals, were effective at recovering this species.

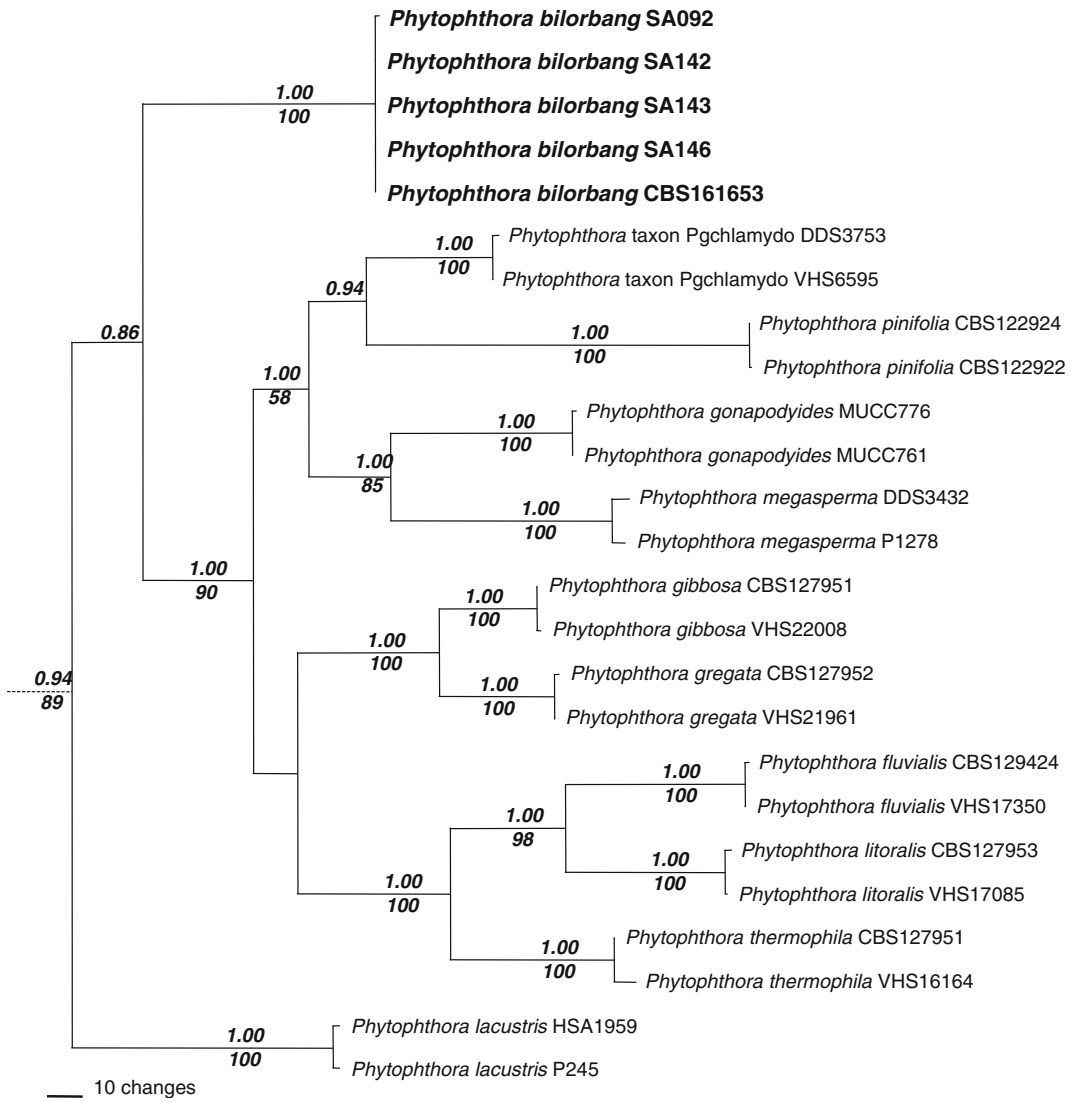


Fig. 2 The most parsimonious tree based on the concatenated ITS, HSP90, BT, *cox*I and NADH dataset showing phylogenetic relationships between *Phytophthora bilorbang* and other species in ITS Clade 6. Numbers above the branches represent posterior probability based on Bayesian analysis, numbers below the branches represent the bootstrap support based on parsimony analysis. *P. inundata, P. humicola* and *P.* taxon asparagi were used as outgroup taxa (not shown)

Phylogenetic analysis

The aligned datasets for ITS, HSP90, BT, *cox*1 and NADH consisted of 846, 895, 914, 1192 and 813 characters, respectively. Based on partition homogeneity tests in PAUP, the datasets were congruent (P=0.12) and were concatenated, resulting in a combined dataset of 4,646 characters. Additional relevant sequences of the ITS region were available on GenBank and are presented in addition to the concatenated dataset.

There were 103 informative characters in the ITS dataset and significant phylogenetic signal (P<0.01, g1=−0.75). Heuristic searches resulted in 39 most parsimonious trees of 191 steps (CI=0.67, RI=0.86). The trees from the Bayesian analysis had similar topology to those from the parsimony analysis, but generally provided greater support for deeper branches (Fig. 1, TreeBASE 12248). Isolates of *P. bilorbang* sp. nov. reside in a strongly supported terminal clade together with several isolates designated as *P.* taxon oaksoil in Europe and northern America and an isolate designated as *P.* taxon riversoil. There is weak support for the clustering of the *P. bilorbang* sp. nov. isolates to the *P. gregata—P. gibbosa* species group. The other cluster contains the remaining species within subclade II with the exclusion of *P. lacustris* and *Phytophthora* sp 2 (HM004225) which are the basal species.

There were 580 informative characters in the concatenated ITS, HSP90, BT, *cox*I and NADH dataset and significant phylogenetic signal (P<0.01, g1=−0.98). Heuristic searches resulted in a single most parsimonious tree of 1,115 steps (CI=0.66, RI=0.83). The tree from the Bayesian analysis had similar topology to the ITS dataset (Fig. 1), but generally provided greater support for deeper branches (Fig. 2, TreeBASE 12248). The isolates of *P. bilorbang* sp. nov. were identical and reside in a strongly supported terminal clade, basal to the majority of described species and designated taxa in ITS Clade 6, sub-clade II with the exclusion of P. taxon salixsoil which is the basal species to the whole sub-clade.

Taxonomy

Phytophthora bilorbang sp. nov.

MycoBank no. MB563863; Figs. 3 and 4.

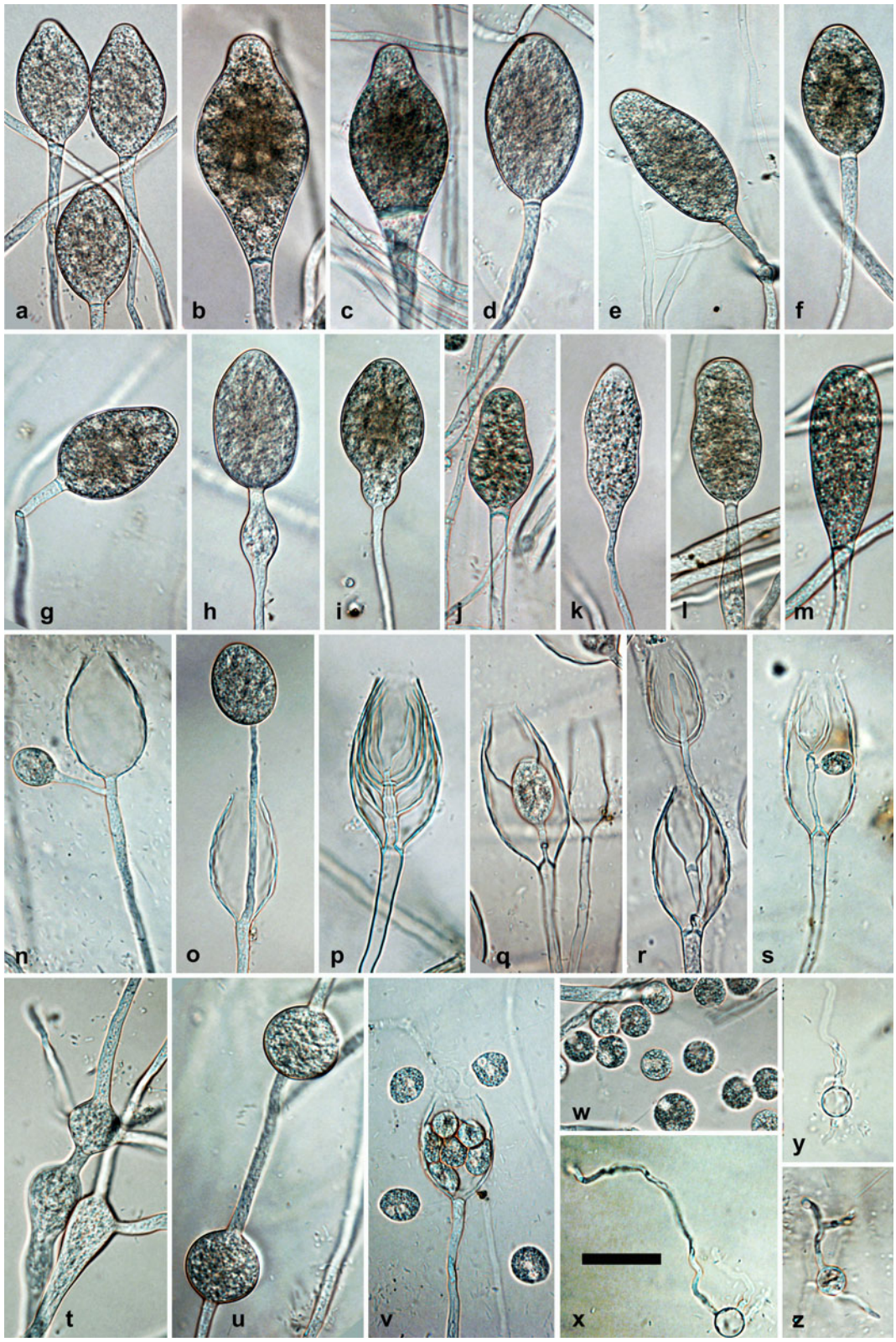
Etymology: The chosen name of the new species refers to a Noongar (southwest Australian Aboriginal) word for a person living on the banks of a river.

Fig. 3 Morphology of asexual structures of *Phytophthora bilorbang* on V8 agar flooded with diluted soil extract. **a**-**c**. limoniform sporangia with (**b**) tapering base and (**c**) conspicuous basal plug: **d**. an ellipsoid sporangium; **e**-**i**. ovoid sporangia with (**h**) hyphal swelling on sporangiophore or (**i**) a swollen base; **j**. an obpyriform sporangium; **k**-**l**. peanut shaped sporangia with (**k**) tapering base; **m**. elongated club shaped sporangium; **n**-**s**. an empty sporangium with (**n**) a short external proliferation, (**o**) extended internal proliferation (**p**) nested internal proliferations (**q**-**r**) two extended internal proliferating sporangiophores in just one empty sporangium (**s**) an external proliferation arising from the internally proliferating sporangiophore; **t**-**u**. hyphal swellings (**t**) elongated and radiate and (**u**) catenulate and globose; **v**. sporangium releasing zoospores; **w**. encysted zoospores; **x**-**z**. encysted zoospores germinating through up to three germ tubes. Scale bar=50 μm

P. bilorbang is homothallic. Oogonia globose and averaged 33.5±4.4 μm. Oospores highly plerotic to slightly aplerotic, averaged 31.3±4.1 μm. Oospore wall, thick with a mean of 3.0±08 μm. Antheridia paragynous and globose to cylindrical (12.4±2.9× 14.1±2.9 μm). Sporangia abundant in liquid cultures and also in V8A, persistent, terminal, nonpapillate, limoniform, limoniform with a tapering base, ellipsoid, ovoid and less obpyriform, peanut shaped and club shaped (51.6±6.4×29.0±4.6 μm) with a length/breadth ratio 1.8±0.3 μm. Conspicuous basal plug common. Sporangiophores simple, sometimes with basal swelling or swelling along the sporangiophore. Sporangial proliferation external and internal observed both in liquid culture and V8A. Hyphal swellings globose to ellipsoid, catenulate or angular with radiating hyphae. Chlamydospore not formed. Colony in V8A stellate to petaloid (carnation shape). Optimum temperature on V8A 25 °C, average radial growth rate 4.2±0.1, maximum temperature 32.5 °C.

Typus: **Western Australia**, Pemberton, banks of Warren River, from rhizosphere soil of dying *Rubus anglocandicans*, 2010, collected by S. Aghighi, **holotypus** MURU 470; cultures ex-type CBS 131653; ITS, *cox*I, HSP90, BT and NADH sequence JQ256377, JQ256375, JQ256376, JQ256374 and JQ256378, respectively.

Sporangia, proliferations and hyphal swellings: Sporangia of *P. bilorbang* were observed on solid agar media including V8A, CA and CMA and were produced abundantly in V8A when flooded with non-sterile soil extract. Sporangia were borne terminally on unbranched sporangiophores, often in chains of internally extended or non-extended proliferating


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u
v
w
x
y
z

sporangia. They were non-caducous and non-papillate (Fig. 3), although in all isolates a fragile twist was infrequently observed in the pedicle (Fig. 3e, g). Sporangial shape was variable ranging from limoniform (41.6 %; Fig. 3a-c) to ellipsoid (27.6 %; Fig. 3d), ovoid (23.2 %; Fig. 3e-i), and less frequently was obpyriform (3.6 %; Fig. 3j), peanut shaped (3.2 %; Fig. 3k-l) and club shaped (0.8 % Fig. 3m). Features such as a tapering base (Fig. 3b, k) or a conspicuous basal plug were common (Fig. 3c). Sporangia usually proliferated internally in both a nested and extended way (Fig. 3o-s). External proliferation was observed as a short extension just behind the primary sporangium (Fig. 3n). In all isolates, two extended internal proliferating sporangia were sometimes observed (Fig. 3q-r). Occasionally an externally proliferating

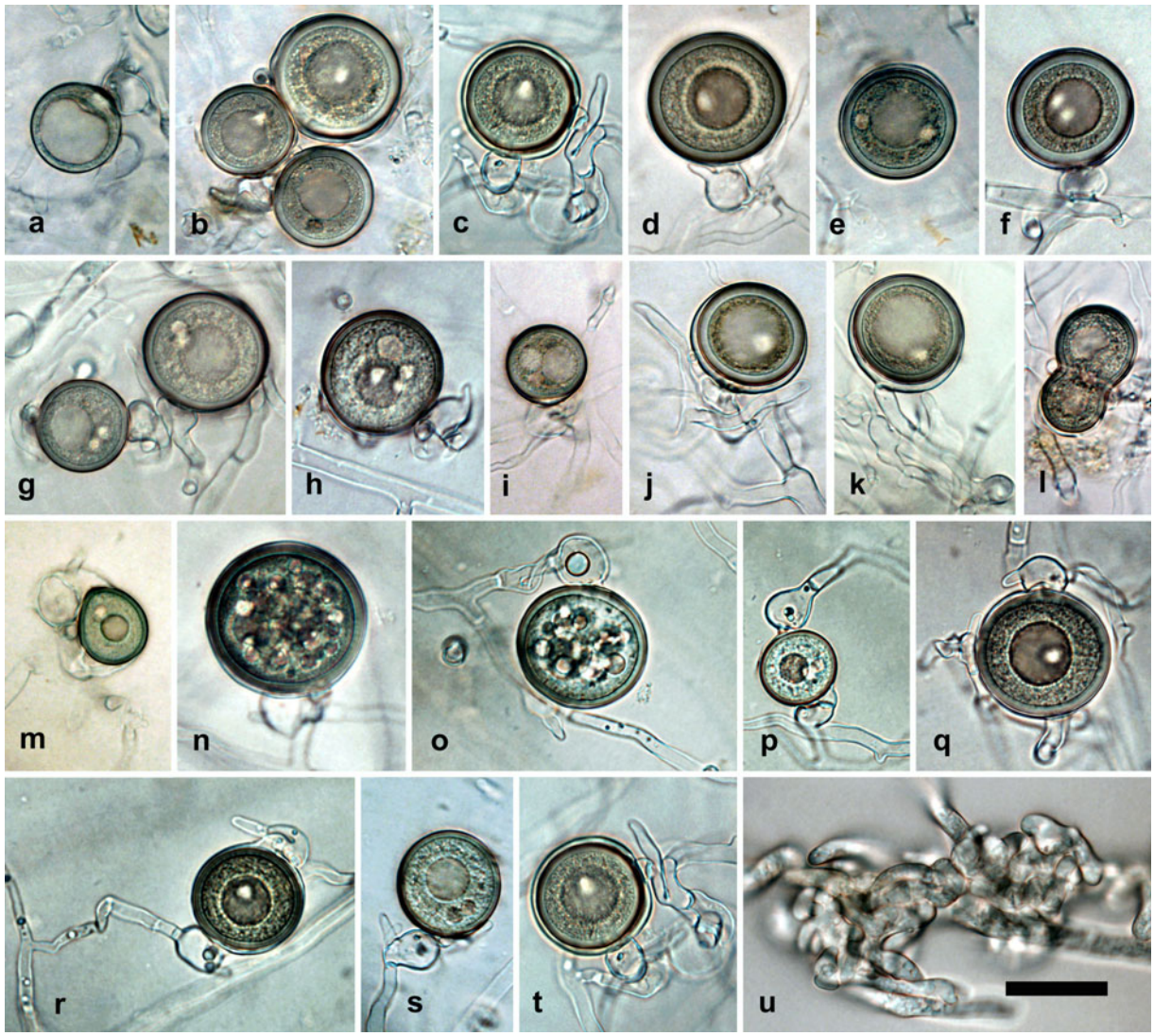


Fig. 4 Morphology of *Phytophthora bilorbang* sexual structures (**a**-**t**) on carrot agar and hyphal aggregation (**u**) of malt extract agar. **a**. young oogonium; **b**. three oospores with large ooplast bodies and thin walls; **c**. an apleorotic oospore and paragynous antheridium with finger like projections; **d**. a pleorotic oospore and paragynous and spherical antheridium with a short projection; **e**-**g**. oospores with two nuclei; **h**-**i**. oospores with two ooplast bodies (**h**-**i**) and three nuclei (**h**); **j**. oospore and antheridium with several projections; **k**. oospore with a long oogonium stalk; **l**-**m**. abnormal shaped oospores; **n**-**o**. aborted oospores after fertilisation; **p**. oospore with twisted oogonium stalk and paragynous antheridium; **q**-**t**. paragynous antheridia with short finger-like projections; **u**. large size aggregation. Scale bar=50 μm

sporangium was produced from an internal extended proliferation while was still in the primary sporangium (Fig. 3s). Direct germination of sporangia and germination of zoospores inside sporangia were observed on agar media.

Sporangial dimensions of the five *P. bilorbang* isolates averaged 50.3±10.6×27.3±5.5 μm with a range of 43.8–59.6×25.0–30.0 μm. The length/breadth ratio of the sporangia averaged 1.87±0.34. Zoospores were discharged through exit pores 10.4–13.4 μm wide (av. 11.6±2.5 μm) (Fig. 3n-s). They were limoniform, ovoid to reniform whilst motile, becoming spherical (av. diam=10.9±1.7 μm) on encystment (Fig. 3w). Cysts often germinated with up to three hyphae (Fig. 3x-z). In V8A flooded with non-sterile soil extract, globose, ellipsoid or angular hyphal swellings, frequently catenulate and with radiating hyphae or forming branching points, were regularly formed (Fig. 3t-u). Hyphal swellings had a mean diameter of 18.6±4.8 μm. Chlamydospores were not seen. Coiled hyphae were observed on agar media.

Oogonia, oospores, antheridia and aggregations: P. bilorbang is homothallic and all the five isolates readily produced oogonia in single culture on CA and V8A and oospores matured within 3–5 weeks. Oogonial

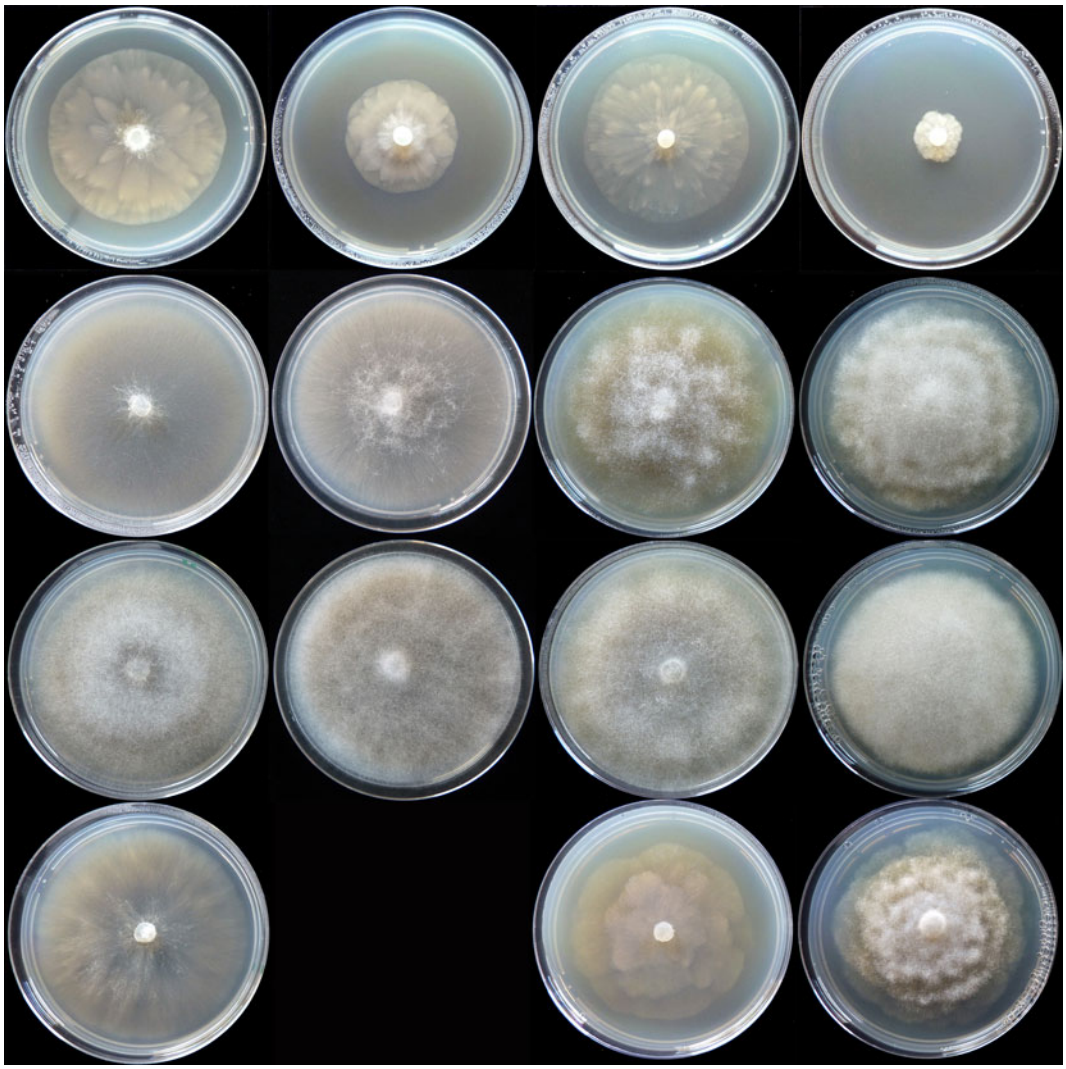


Fig. 5 Colony morphology of (*top to bottom*) *Phytophthora bilorbang* SA262, *P. gregata*, *P. gibbosa* and *P. megasperma* after 7 days growth at 20 °C on different agar (*left to right*); V8A, CA, MEA and half strength PDA. *P. megasperma* was not grown on CA

shape was globose with smooth walls (Fig. 4a-k). Oogonial stalks were sometimes long (Fig. 4i-j). Oogonial diameters averaged 33.9±6.0 μm with a range of 26.5–37.4 μm. Most oogonia contained oospores with a large ooplast (Fig. 4b-g) (aborted oospores=3.4 %) (Fig. 4n-o) or with two ooplasts (Fig. 4h-i). Oospores were highly plerotic (Fig. 4b, d and g-h), slightly aplerotic (Fig. 4e-f) to aplerotic (Fig. 4j-k, t) and averaged 32.2±6.1 μm in diameter with relatively thick oospore walls (average 2.7±0.7 μm; range 2.0–3.0 μm) and a high mean oospore wall index of 0.42±0.07. Abnormal shaped oospores were rarely formed (Fig. 4m).

Antheridia were one-celled, hyaline and globose to cylindrical, with a range of isolate means of 11.00–12.43×12.19–14.09 (av. 11.4±2.28×13.0±2.51). They were paragynous (Fig. 4g) and up to six finger-like projections were observed (Fig. 4c-d, h, j, q-t). Generally, there was more than one antheridium attached per oogonium (Fig. 4c, r, t). Small hyphal aggregations were produced on CMA. Large aggregations were formed on malt extract agar and on selective media (Fig. 4u).

Colony morphology, growth rates and cardinal temperatures: All five *P. bilorbang* isolates formed stellate to petaloid (carnation shape) colonies with sparse to limited aerial mycelium on V8A, carrot agar and malt extract agar, and petaloid, dense-felty and dome shape at the centre on half-strengthen PDA (Fig. 5). Colonies on corn meal agar were sparse and almost invisible.

Among the temperatures tested, *P. bilorbang* had an optimum and maximum temperature for growth of 25 °C and 32.5 °C, respectively (Fig. 6). All isolates failed to grow at 32.5 °C, and did not resume growth when plates incubated for 7 d at 32.5 °C were transferred to 20 °C. isolates grew slowly at 4 °C. The average radial growth rate on V8A at 25 °C was 4.17±0.21 mm d^{-1}.

Additional specimens examined: WESTERN AUSTRALIA, Manjimup, from rhizosphere soil of dying *Rubus anglocandicans*, 2010, collected by S. Aghighi SA92, SA142, SA143, SA146.

Notes: P. bilorbang most closely resembles other homothallic species in Clade 6; *P. gregata, P. gibbosa* and *P. megasperma*, but can be easily distinguished based on a combination of molecular and morphological differences (online resource 1). In a multigene phylogeny of the ITS, HSP90, BT, NADH and *cox*I gene regions, *P. bilorbang* differs from *P. gregata* by 183 steps (3.23 %), *P. gibbosa* by 178 steps (3.14 %), *P. megasperma* by 199 steps (3.51 %). All four species have been isolated from soil in Western Australia; *P. gregata* and *P. gibbosa* are only known from WA, while *P. megasperma* and *P. bilorbang* have been isolated elsewhere. In comparison to these species, *P. bilorbang* has the ability to produce chains of nested and extended proliferating sporangia, external proliferation, production of secondary lateral sporangia and forming branched sporangiophores in primary sporangia. *P. bilorbang* produces smooth-walled oogonia, whilst *P. gibbosa* forms ornamented oogonia. Moreover, *P. bilorbang* has the ability to form aggregations on agar media but *P. gibbosa* and *P. megasperma* do not. Additionally, zoospores of *P. bilorbang* can germinate with up to three germ tubes. In comparison with *P. gregata, P. gibbosa* and *P. megasperma, P. bilorbang* grows very slowly on PDA and moderately

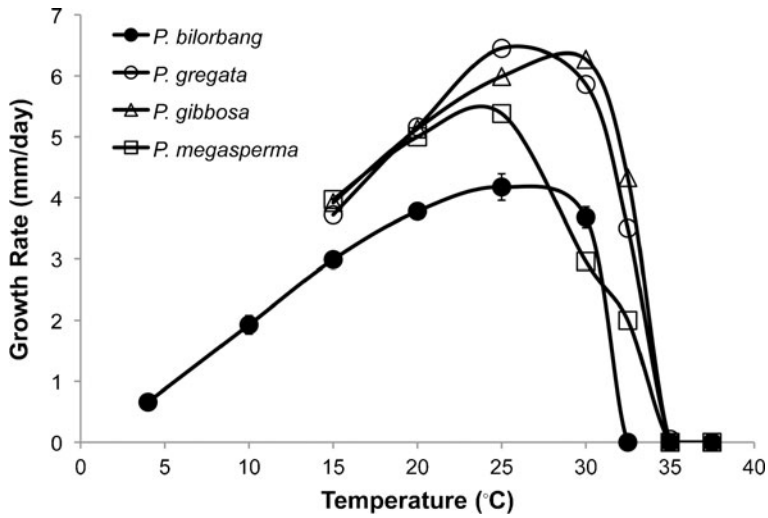


Fig. 6 Mean radial growth rates of *Phytophthora bilorbang* (five isolates) compared to *P. gregata, P. gibbosa* and *P. megasperma* on V8 agar at different temperatures. Standard error bars for *P. bilorbang* were smaller than the symbols

slowly on other media (Fig. 5), and unlike these species it produces stellate colonies on carrot and V8A.

Pathogenicity

The three *P. bilorbang* isolates differed in their aggressiveness to *R. anglocandicans*. Those plants inoculated with isolates SA092 and CBS 161653 showed severe wilting and extensive root lesions; whereas plants inoculated with SA142 revealed only moderate symptoms of wilting. Also, 54.5 %, 18.2 % and 45.4 % of plants inoculated with isolates SA092, SA142 and CBS 161653, respectively were dead or with severe symptoms of decline by the end of the trial, while non-inoculated control plants showed no symptoms. *P. bilorbang* was recovered from plated necrotic lesions on fine roots and also from baited roots of inoculated blackberry plants satisfying Koch's postulates.

Discussion

A new homothallic species from *Phytophthora* Clade 6 with identical ITS sequence to the undescribed *P.* taxon oaksoil was described here as *P. bilorbang.* This new species has been isolated from baited rhizosphere soil and roots of dead and dying European blackberry (*R. anglocandicans*) and not from healthy plants at four out of five decline sites, in the south-west of Western Australia. Phylogenetic analysis shows *P. bilorbang* to be a unique species residing in subclade II of ITS Clade 6. *P. bilorbang* is easily distinguished from related species by a range of morphological criteria. In a soil infestation glasshouse trial, *P. bilorbang* was reisolated from necrotic diseased roots on selective medium indicating that it is a pathogen of *R. anglocandicans*.

A single unique *Phytophthora* isolate was obtained in 1998 from soil under oak trees in Illwald Forest of Alsace, France (Hansen and Delatour 1999). In 2003, P1055 was informally designated as *P.* taxon oaksoil by Brasier et al. (2003a) and was reported to be sexually sterile. Isolates with identical sequence to P1055 were more recently identified from stream water in Oregon (Reeser et al. 2011a; b). Additionally, there are ITS sequence data on GenBank for several isolates obtained in Poland designated as *P.* taxon oaksoil. These are not linked to any formal publication and no more information is available for these isolates. In addition to isolates designated as *P.* taxon oaksoil, the study of Brasier et al. (2003a) also reported a single isolate, P1044 of *P.* taxon riversoil isolated from a riverbank in Worcestershire, UK which also has identical ITS sequence to *P.* taxon oaksoil. However, these two species were reported as sexually sterile (Brasier et al. 2003a), whereas all the isolates recovered in WA were homothallic. On carrot agar *P.* taxon oaksoil isolates formed flat featureless colonies (Brasier et al. 2003a), while *P. bilorbang* produced stellate colonies.

During a re-evaluation of the *Phytophthora* collection maintained by the Vegetation Health Service of the Department of Environment and Conservation in WA, many new undescribed taxa and unique isolates were identified (Burgess et al. 2009) within what is known as ITS Clade 6 (Cooke et al. 2000). Many of Clade 6 taxa are associated with riparian ecosystems, or with forest soils prone to waterlogging. Those species known to cause disease, such as *P. inundata*, only do so when sites are flooded (Brasier et al. 2003a, b; Jung et al. 2011). During the winter rainfall months in the south west of Western Australia both the Warren and Donnelly rivers are subjected to periods of flooding. These flooding events are likely to provide the conductive conditions that favour *P. bilorbang* and potentially cause the massive decline events being observed for European blackberry in this region. These field observations were supported by the glasshouse trial where flooding led to the mortality of plants.

Phytophthora root rot can be an extremely destructive disease on susceptible cultivars of *Rubus* spp. such as red, purple and black raspberries, where conditions favour its development. *Phytophthora* species reported on raspberry include *P. cactorum*, *P. citricola*, *P. gonapodyides*, *P.* taxon raspberry, *P. megasperma*, *P. cryptogea*, *P. rubi*, *P. idaei* and *P. bisheria* (Abad et al. 2008; Brasier et al. 2003a; Duncan and Kennedy 1987; Ellis et al. 1991; Jung et al. 2011; Kennedy and Duncan 1995; Washington 1988; Wilcox et al. 1993; Wilcox and Latorre 2002). In contrast, many blackberry cultivars appeared to be highly tolerant to *Phytophthora*. However, a *Phytophthora* root rot in blackberries in Kentucky has been recorded by Ellis et al. (2004) but further information is unavailable. The association of *P. bilorbang* with the decline of European blackberry in the south west of WA appears to be the first formally documented *Phytophthora* disease of blackberries.

The known distribution of *P. bilorbang* in Western Australia is limited to the Warren and Donnelly river catchments. *P. bilorbang* was recovered from damp regions along the banks of these rivers. According to the climate statistics for Australian locations, the long term mean monthly maximum temperature in this region is 20.3 °C in summer and 9.7 °C in winter (Bureau of Meteorology of Australian Government, http://www.bom.gov.au/). These temperatures are within the range favourable for growth of *P. bilorbang* and if it was considered as a biological control agent it could be active throughout the year.

In conclusion, a novel taxon, *P. bilorbang* is described. Based on GenBank accession data for ITS gene region, this species has been isolated from other parts of the world, and may have been introduced to the south-west of WA. Further investigations are required to better understand the epidemiology of the disease in the field and other possible factors which are involved in the decline. A host-range study of *P. bilorbang* is recommended to evaluate the biological control potential of this new species as a part of integrated management strategy of the noxious *R. anglocandicans*.

Acknowledgements The authors are grateful to the Western Australian Department of Environment and Conservation for funding, the Ministry of Science, Research and Technology of Iran for financially supporting the senior author, Paul Yeoh (CSIRO Ecosystem Science, WA) and Lee Fontanini (Warren Catchments Council, Manjimup, WA) for field help, Diane White (CPSM, Murdoch University, WA) for technical support, Thomas Jung (Phytophthora Research and Consultancy, Brannenburg, Germany) for assistance with morphology and József Bakonyi (Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences) for providing sequence data for several species.

References

Abad, Z. G., Abad, J. A., Coffey, M. D., Oudemans, P. V., Man in't Veld, W. A., de Gruyter, H., et al. (2008). *Phytophthora bisheria* sp. nov., a new species identified in isolates from the Rosaceous raspberry, rose and strawberry in three continents. *Mycologia, 100*, 99–110.

Amor, R., Richardson, R., Pritchard, G., & Bruzzese, E. (1998). *Rubus fruticosus* L. Agg. In F. Panetta, R. Groves, & R. Shepherd (Eds.), *The biology of Australian weeds* (pp. 225–246). Melbourne: RG & FJ Richardson.

Andjic, V., Cortinas, M. N., Hardy, G. E. St. J., Wingfield, M. J., & Burgess, T. I. (2007). Multiple gene genealogies reveal important relationships between species of *Phaeophleospora* infecting *Eucalyptus* leaves. *FEMS Microbiology Letters, 268*, 22–33.

Blair, J. E., Coffey, M. D., Park, S.-Y., Geiser, D. M., & Kang, S. (2008). A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genetics and Biology, 45*, 266–277.

Brasier, C. M., Cooke, D. E. L., Duncan, J. M., & Hansen, E. M. (2003a). Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides–P. megasperma* ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. *Mycological Research, 107*, 277–290.

Brasier, C. M., Sanchez-Hernandez, E., & Kirk, S. A. (2003b). *Phytophthora inundata* sp. nov., a part heterothallic pathogen of trees and shrubs in wet or flooded soils. *Mycological Research, 107*, 477–484.

Bruzzese, E., Mahr, F., & Faithfull, I. (2000). Best practice management guide for environmental weeds: Blackberry, *Rubus fruticosus* aggregate, pp. 1–6. The Cooperative Research Centre for Weed Management Systems: Australia. Retrieved May 20, 2012, from http://www.weeds.org.au/WoNS/blackberry/docs/Blackberry_BPMG.pdf

Burgess, T. I., Webster, J. L., Ciampini, J. A., White, D. W., Hardy, G. E. St. J., & Stukely, M. J. C. (2009). Re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in Western Australia using molecular techniques. *Plant Disease, 93*, 215–223.

Butcher, T. B., Stukely, M. J. C., & Chester, G. W. (1984). Genetic variation in resistance of *Pinus radiata* to *Phytophthora cinnamomi*. *Forest Ecology and Management, 8*, 197–220.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G., & Brasier, C. M. (2000). A molecular phylogeny of *Phytophthora* and related Oomycetes. *Fungal Genetics and Biology, 30*, 17–32.

Duncan, J. M., & Kennedy, D. M. (1987). Identities and pathogenicities of *Phytophthora* spp. causing root rot of red raspberry. *Plant Pathology, 36*, 276–289.

Ellis, M. A., Converse, R. H., Williams, R. N., & Williamson, B. (1991). *Compendium of raspberry and blackberry diseases and insects*. St. Paul: APS Press.

Ellis, M. A., Doohan, D., Bordelon, B., Welty, C., Williams, R. N., Funt, R. C., et al. (2004). Midwest Small Fruit Pest Management Handbook. *The Ohio State University Bulletin, 861,* 70. Retrieved March, 2012, from http://ohioline.osu.edu/b861/pdf/ch03_70-73.pdf

Erwin, D. C., & Ribeiro, O. K. (1996). *Phytophthora diseases worldwide*. St. Paul: APS Press.

Evans, K. J., & Weber, H. E. (2003). *Rubus anglocandicans* (Rosaceae) is the most widespread taxon of European blackberry in Australia. *Australian Systematic Botany, 16*, 527–537.

Hansen, E. M., & Delatour, C. (1999). *Phytophthora* species in oak forests of north-east France. *Annales des Sciences Forestières, 56*, 539–547.

Hüberli, D., Tommerup, I. C., & Hardy, G. E. St. J. (2000). False-negative isolations or absence of lesions may cause misdiagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology, 29*, 164–169.

Jung, T., & Burgess, T. I. (2009). Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in

Europe and North America reveals a new species. *Phytophthora plurivora sp. nov. Persoonia, 22*, 95–110.

Jung, T., Blaschke, M., & Neumann, P. (1996). Isolation, identification and pathogenicity of Phytophthora species from declining oak stands. *European Journal of Forest Pathology, 26*, 253–272.

Jung, T., Cooke, D. E. L., Blaschke, H., Duncan, J. M., & Oβwald, W. (1999). *Phytophthora quercina* sp. nov., causing root rot of European oaks. *Mycological Research, 103*, 785–798.

Jung, T., Blaschke, H., & Oβwald, W. (2000). Involvement of soilborne *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. *Plant Pathology, 49*, 706–718.

Jung, T., Stukely, M. J. C., Hardy, G. E. St. J., White, D., Paap, T., Dunstan, W. A., & Burgess, T. I. (2011). Multiple new *Phytophthora* species from ITS clade 6 associated with natural ecosystems in Australia: evolutionary and ecological implications. *Persoonia, 26*, 13–39.

Kennedy, D. M., & Duncan, J. M. (1995). A papillate *Phytophthora* species with specificity to *Rubus*. *Mycological Research, 99*, 57–68.

Kroon, L. P. N. M., Bakker, F. T., van den Bosch, G. B. M., Bonants, P. J. M., & Fliera, W. G. (2004). Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology, 41*, 766–782.

Martin, F. N., & Tooley, P. W. (2003). Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. *Mycologia, 95*, 269–284.

Morin, L., & Evans, K. J. (2011). *Rubus fruticosus* L. aggregate - European blackberry. In M. Julien, R. McFadyen, & J. Cullen (Eds.), *Biological control of weeds in Australia* (pp. 499–509). Melbourne: CSIRO Publishing.

Morin, L., Evans, K., Jourdan, M., Gomez, D. R., & Scott, J. K. (2011). Sourcing additional genetically distinct isolates of the rust fungus *Phragmidium violaceum* to enhance biological control of European blackberry in Australia. *European Journal of Plant Pathology, 131*, 289–303.

Rea, A., Jung, T., Burgess, T. I., Stukely, M. J. C., & Hardy, G. E. St. J. (2010). *Phytophthora elongata* sp. nov. a novel pathogen from the *Eucalyptus marginata* forest of Western Australia. *Australasian Plant Pathology, 39*, 477–491.

Reeser, P., Sutton, W., & Hansen, E. (2011a). *Phytophthora* species in tanoak trees, canopy-drip, soil, and streams in the sudden oak death epidemic area of south-western Oregon, USA. *New Zealand Journal of Forestry Science, 41S*, S65–S73.

Reeser, P. W., Sutton, W., Hansen, E. M., Remigi, P., & Adams, G. C. (2011b). *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia, 103*, 22–35.

Ronquist, F., & Heuelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics, 19*, 1572–1574.

Sakalidis, M. L., Hardy, G. E. St. J., & Burgess, T. I. (2011). Endophytes and potential pathogens of the baobab species *Adansonia gregorii*; a focus on the Botryosphaeriaceae. *Fungal Ecology, 4*, 1–14.

Swofford, D. L. (2003). *Phylogenetic analysis using parsimony (*and other methods)*. Version 4. (eds). Sunderland, Massachusetts: Sinauer Associates.

Washington, W. S. (1988). *Phytophthora cryptogea* as a cause of root rot of raspberry in Australia; resistance of raspberry cultivars and control by fungicides. *Plant Pathology, 37*, 225–230.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innes, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). San Diego: Academic.

Wilcox, W. F., & Latorre, B. A. (2002). Identities and geographic distributions of *Phytophthora* spp. causing root rot of red raspberry in Chile. *Plant Disease, 86*, 1357–1362.

Wilcox, W. F., Scott, P. H., Hamm, P. B., Kennedy, D. M., Duncan, J. M., Brasier, C. M., et al. (1993). Identity of a *Phytophthora* species attacking raspberry in Europe and North America. *Mycological Research, 97*, 817–831.