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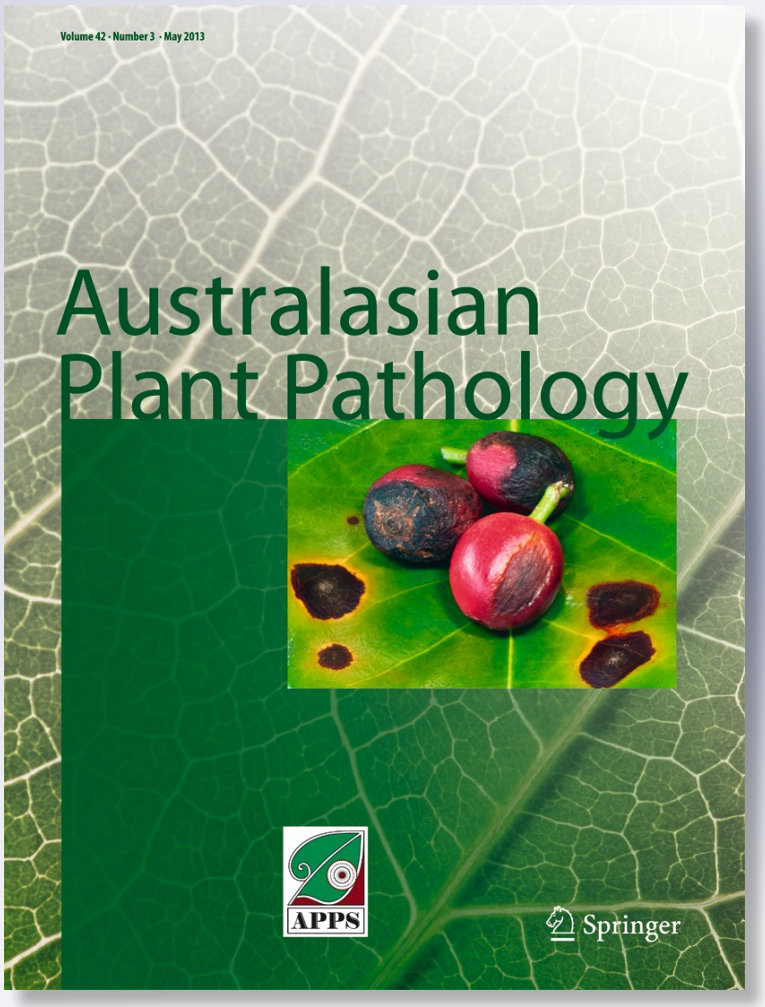
Australasian Plant Pathology
Journal of the Australasian Plant Pathology Society

ISSN 0815-3191
Volume 42
Number 3

Australasian Plant Pathol. (2013) 42:251-260
DOI 10.1007/s13313-012-0195-6



Springer

Australasian Plant Pathol. (2013) 42:251–260
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Fishing for *Phytophthora* from Western Australia's waterways: a distribution and diversity survey

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Received: 3 August 2012 / Accepted: 20 December 2012 / Published online: 24 January 2013

Abstract During one spring season, 12 *Phytophthora* species, two *Phytophthora* hybrids, three *Halophytophthora* species and three *Phytopythium* species, were isolated from 48 waterways across Western Australia. The waterways were sampled using nylon mesh bags containing leaf baits of up to six different plant species and were isolated by plating necrotic lesions on these onto *Phytophthora*-selective agar media. *Phytophthora* species were isolated from all except one waterway. Of the *Phytophthora* species isolated, eight are known while the remaining four are undescribed taxa. Six of the *Phytophthora* species and the two hybrids are from clade 6. The two hybrids and *P. inundata* were the predominant species recovered. Recoveries from different plant leaf baits varied with the best two baits being *Pittosporum undulatum* and *Banksia attentuata*; and from these two combined all *Phytophthora* species were isolated. There was a marked difference in the *Phytophthora* diversity in the waterways from different regions. This is the first comprehensive study from Australia to examine the *Phytophthora* communities in waterways, and advances our understanding of the role of these oomycetes in natural and anthropized ecosystems.

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Keywords *Halophytophthora* · Oomycetes · Leaf bait · *Phytopythium* · River · Stream baiting

Introduction

The genus *Phytophthora* comprises a large number of plant pathogens that are renowned for their devastating economic and ecological impacts on agricultural and natural ecosystems. As a stramenopile, *Phytophthora* requires aquatic environments to maintain a major part of its asexual lifecycle and to aid its dispersal. The devastation caused by two very recent exotic *Phytophthora* species, *P. alni* and *P. ramorum*, in Europe and in north western USA has elevated the importance of detection of these and other *Phytophthora* species from plant, soil and water samples. Water surveys have been popularised across the world, particularly in regions where early detection of an infested area is important to the success of containment and eradication efforts. In Oregon, Washington and California, the USDA conducts annual surveys of *Phytophthora* species from soil and waterways to ascertain if *P. ramorum* has escaped into native ecosystems (Frankel 2008; Sutton et al. 2009).

Over 20 *Phytophthora* species have been isolated from water around the world, including several new species still undescribed (e.g. Brasier et al. 2003a; Hong et al. 2008; Hulvey et al. 2010; Reeser et al. 2011; Scibetta et al. 2012). Among this list are the notorious plant pathogens, *P. ramorum* and *P. cinnamomi* (Palzer 1980; Von Broembsen 1984; Smith et al. 2009; Sutton et al. 2009). Also commonly recovered are Clade 6 *Phytophthora* species which are hypothesised as having a prevalently saprophytic lifestyle (Brasier et al. 2003a; Hansen et al. 2012) as supported by their presence and dominance in water surveys (e.g. Hansen and Delatour 1999; Reeser et al. 2011). Under favourable conditions some of these Clade 6 species, such as *P. inundata*, *P.* taxon

PgChlamydo, *P. gonapodyides* and *P. lacustris* (e.g. Hansen and Delatour 1999; Greslebin et al. 2005; Smith et al. 2009; Randall 2011; Reeser et al. 2011; Nechwatal et al. 2012), can be aggressive tree pathogens (Brasier et al. 2003b; Brown and Brasier 2007; Jung 2009). *P. inundata* has been isolated from dying native vegetation in Western Australia (WA) (Stukely et al. 2007).

In Australia, most *Phytophthora* surveys in native ecosystems have focused primarily on isolations from samples of soil and symptomatic plant tissues including the extensive vegetation health surveys conducted in Western Australia (WA) (Burgess et al. 2009). A few surveys have examined the spread of particular species, such as *P. cinnamomi* or a range of species, in relationship to a current disease risk (Palzer 1980; Davison et al. 2005). Davison et al. (2005) found *P. cinnamomi* var. *parvispora* and two unknown species related to *P. insolita* in the Ord River Irrigation Scheme (ORIA), Kununurra, an important horticultural region in WA.

Since the *P. ramorum* outbreaks in the northern hemisphere, Australasian waterway surveys have focused towards a "perceived" disease risk from new invasive *Phytophthora* species including categorising and studying the species present in these important ecosystems, and the development of a tool to monitor waterways effectively for potential new invasive species (Smith et al. 2009; Randall 2011). Nine different *Phytophthora* species have been isolated from four Victorian streams and their recovery differed between the summer and winter sampling times (Smith et al. 2009). Here we describe the diversity and distribution of *Phytophthora* and some related oomycetes from waterways across WA during one spring season.

Methods

Collection of *Phytophthora* species

A total of 48 waterways in WA, including streams, lakes, ponds and estuaries, were baited 1-4 times during October to early December of 2008 at one or more locations (Table 1). Sites were selected across regional locations from Kununurra (most northern site) to Esperance (most south eastern site), and locations in the Perth metropolitan area (Fig. 1, Table 1). Bait bags were made from nylon fly-wire shaped into an A4 envelope divided into four pouches and a flap at the top to close the pouches. The bottom and the top ends of the bait bags contained carpet underlay to allow the bags to float just below the water surface. A nylon rope was tied through the top of the bag so that it could be tied to the bank of a waterway. Bag pouches were filled with two to three leaves of each plant species; *Banksia attenuata*, *Pittosporum undulatum*, *Hakea laurina* and *Quercus robur*, and germinated lupin (*Lupinus angustifolius*) seedlings. These were sent via overnight-post to 16 volunteers across WA who deployed the bags in waterways, and retrieved and returned the baits to the laboratory via overnight post or delivered by hand after 7–10 days in the water. In some cases, volunteers added additional leaf baits to the bags, such as *Eucalyptus* and *Kennedia* species.

Isolation of *Phytophthora* species

In the laboratory, leaves were cleaned with DI water, blotted dry and examined for necrosis. Five to 10 rectangular sections (~5–8 mm by 1–2 mm in size) were cut from nectrotic tissue and plated onto NARPH agar plates (Hüberli et al. 2000), a medium selective for *Phytophthora*. Plates were incubated in darkness for up to 2 weeks at 20 °C and were checked every second day for *Phytophthora* colonies under the microscope at 4x magnification with special attention paid to exclude *Pythium*-like colonies. Colonies were counted and representative colonies were transferred onto fresh selective agar medium under a dissecting microscope using a sterile Ophthalmic knife. After 1 week growth at 20 °C, secondary cultures were checked for purity and subcultured (single hyphal tips) to corn meal agar (CMA) for characterization and molecular identification.

DNA isolation, amplification and sequencing

From each location, all isolates were grouped according to macroscopic colony morphology and any microscopic evidence of spores (sporangia and chlamydospores) and hyphae. One representative morph-type from each site per sampling was identified using the sequence of the ITS region of the rDNA.

The *Phytophthora* isolates were grown on half-strength potato dextrose agar (1/2 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 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. Harvested mycelium was frozen in liquid nitrogen, ground to a fine powder and genomic DNA was extracted according to Andjic et al. (2007).

For each isolate the region spanning the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal DNA was amplified using the primers ITS-6 (Cooke et al. 2000) and ITS-4 (White et al. 1990). The PCR reaction mixture and conditions were as described by Andjic et al. (2007). For identification purposes templates were sequenced with ITS-4. For some isolates the mitochondrial gene *cox*I was amplified with forward primer FM84 and as a reverse primer either FM83 or FM77 (Martin and Tooley 2003). The PCR reaction mixture was the same as for the

Table 1 Number of sites and times sampled, and number of *Phytophthora* species identified from 48 waterways in Western Australia which were baited for *Phytophthora* during October to early December 2008. No *Phytophthora* species was recovered from Peenebup Creek in the southwest. Note that identification of *Halophytophthora* and *Phytopythium* species are not included here

Region	Waterway	Sites sampled (n=48)	No. of times sampled (n=1 to 4)	*Phytophthora* species identified[a] (n=14)
Perth metropolitan	Bibra Lake	2	2	3
	Bickley Brook	1	2	1
	Canning River	4	3	5
	Churchman's Brook	1	2	2
	Cookes Brook	2	4	3
	Jarrah Creek	2	4	2
	Lake Coogee	2	2	1
	Lake Jualbup	1	3	2
	Lake Mabel Talbot	1	3	1
	Little Rush Lake	2	2	2
	MGS-Kotisinia Lake	1	2	2
	Neerigin Brook	1	2	3
	Poison Gully Creek	4	4	4
	Subiaco Common	1	3	1
	Wooroloo River	4	2	5
	Yangebup Lake	2	2	4
	Yule Brook	2	3	2
North	Ord River Irrigatiation Channel	2	1	3
Northwest	Bella Vista Nature Reserve	1	2	2
	Chapman River	2	2	3
	Howatharra Road Creek	1	2	4
Southwest	Alexander River	1	1	1
	Bremer River	2	2	2
	Calyerup Creek	1	2	2
	Coramup Creek	2	2	4
	Dalyup River	2	2	1
	Dam	2	1	1
	Denmark River	2	2	3
	Fitzgerald River	1	2	1
	Gairdner River	2	2	0[b], 1
	Gnowangerup Creek	1	1	2
	Goonanugo Creek	1	4	1
	Hay River	2	2	2
	Jerdacuttup River	2	2	1
	Little River	1	2	2
	Monjebup Creek	1	1	2
	Moore River (north arm)	1	4	1
	Pallingup Creek	4	1	0[b], 1
	Peenebup Creek	1	1	0
	Robbies Creek	1	2	1
	Steere River	2	2	3
	Stockyards Creek	1	1	3
	Suzetta River	2	2	2
	Thomas River	2	1	0[b], 1
	Warperup Creek	1	1	1
	Wellstead Estuary	2	2	2
	West River	2	2	1
	Young River	1	1	1

[a]Total number of *Phytophthora* species, including hybrids, identified across all sites sampled in a waterway and at all sampling times

[b]No *Phytophthora* species were isolated from one site of a waterway

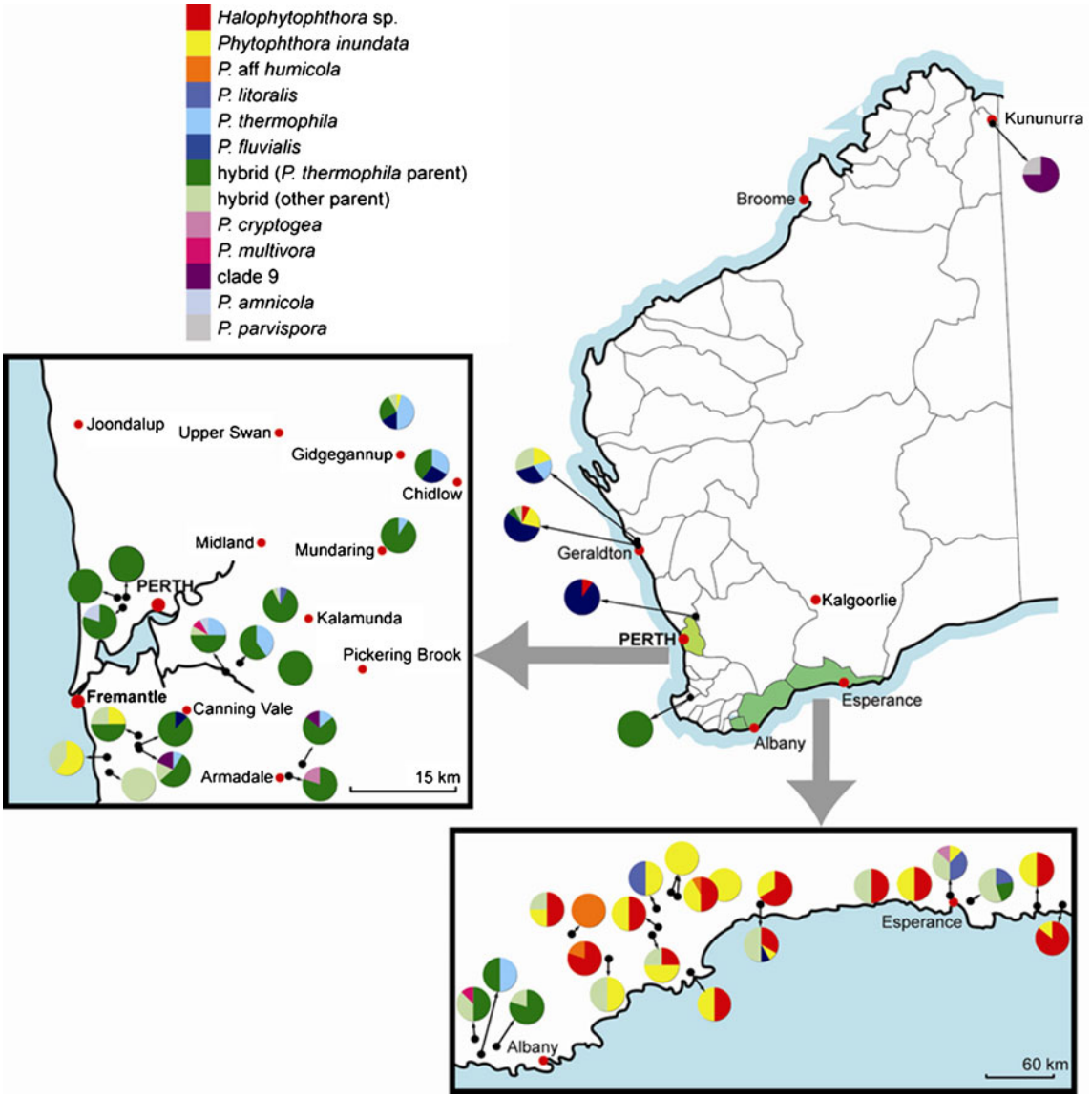

Fig. 1 Distribution of *Phytophthora* and *Halophytophthora* species recovered from 47 out of 48 waterways across Western Australia during spring (October to early December) 2008. River basins are shown. Some waterways that were close together were combined

ITS region, but the PCR conditions were as described previously (Martin and Tooley 2003). Templates were sequenced with FM77 and FM50 (Martin and Tooley 2003). The clean-up of products and sequencing were as described by Sakalidis et al. (2011).

Isolate identification and phylogenetic analysis

Sequence data for the ITS region were initially cleaned and subsequent manual adjustments made in Geneious Pro 5.4.4 (Drummond et al. 2011). Ambiguous bases were coded according to the IUPAC nucleotide code. Identification was initially based on blast searches in GenBank (http://blast.ncbi.nlm.nih.gov), but in all cases, sequence data from closely related species were obtained from GenBank or from our own previous studies and alignments were constructed in Geneious and exported as nexus files. Parsimony analysis was performed in Phylogenetic Analysis Using Parsimony (PAUP) version 4.0b10 (Swofford 2003). Ambiguous positions were coded and included in analyses. All datasets and trees arriving from parsimony analyses are available from TreeBASE.

Results

Phytophthora species identification

In one season, 360 isolates were recovered from the 48 sampled waterways. Isolates obtained from each site were sorted based on colony morphology and 249 of the 360 isolates were

selected for molecular identification. Based on ITS sequence data, 107 isolates were identified by comparison to known species in the phylogenetic analysis (Table 2, Fig. 1). Of these, 18 isolates were identified as *Phytopythium* and 26 as *Halophytophthora.* A total of 85 *Phytophthora* isolates were identified, and in order of frequency these were *P. inundata*, *P. fluvialis*, *P. thermophila*, *P. litoralis*, *P. amnicola*, *P. cryptogea*, *P. multivora* and *P. cinnamomi* var. *parvispora* (Fig. 2, Table 2). Eight isolates were a close (but not exact) phylogenetic match to several known species in ITS clade 6 (*P.* aff. *humicola*) and clade 9 (*P.* aff. *insolata*, *P.* aff. Kununurra and *P.* aff. *hydropathica*) (Fig. 2, Table 2). The remaining 112 *Phytophthora* isolates produced ITS sequence with high levels of polymorphism including indels and could not be used to identify isolates to a species level. Therefore, many of these isolates are putative hybrids (Burgess et al. 2012a,b). To facilitate identification of these 'putative' hybrid isolates the *cox*I region was sequenced and the identity determined by phylogenetic comparison to all known clade 6 species (Table 2, Fig. 3). As *cox*I is from the mitochondrial genome only one of the putative parents can be identified. The isolates fell into two groups; 78 isolates of hybrid A had a *cox*I allele of *P. thermophila* (Fig. 3). The remaining 34 isolates had a *cox*I allele corresponding to *P. amnicola*, *P. litoralis*, *P. fluvialis*, sub-clade 2.1, 2.2 or 2.3 (Fig. 3). As this group of hybrid isolates has not been fully characterized they are grouped as hybrid B.

Phytophthora diversity

A total of 12 *Phytophthora* species and two hybrids were isolated during the October to December 2008 survey from 48 waterways across Western Australia. Only from one waterway (Peenebup Creek, southwest WA) was no *Phytophthora* isolated (Table 1). From the remaining waterways at least one *Phytophthora* species was recovered. *Halophytophthora* species and *Phytopythium* species were isolated from 20 and 15 waterways, respectively.

The most frequently isolated *Phytophthora* species in the southern waterways was *P. inundata*. Hybrid B was also common in this region (Fig. 1). Additionally, most of the *Halophytophthora* isolates were recovered from 12 waterways. *P.* aff. *humicola* was isolated from only three waterways.

In the Perth metropolitan area, *P. thermophila* and both hybrids A and B were commonly recovered from waterways (Fig. 1). *P. amnicola* and *P.* aff. *hydropathica* were each isolated three times and only from Perth. *P. cryptogea* (two waterways), *P. multivora* (two waterways) and *P. litoralis* (six waterways) were infrequently isolated from Perth and the southwest of WA only.

The most frequently recovered species from the northern waterways was *P. fluvialis* (Fig. 1). From the most northern location, three unique species were isolated from an irrigation channel (ORIA, Kununurra) that were not recovered elsewhere in WA. These were *P. cinnamomi* var. *parvispora*, *P.* aff. *insolata* and *P.* aff. Kununurra (Table 2).

Comparison of plant baits

Most species were recovered from *B. attenuata* and *P. undulatum* leaves (Table 2). In combination, both these baits recovered all 12 of the *Phytophthora* species and Clade 6 hybrids. Volunteers were given the opportunity to add one of their own plant baits to the bags, which predominately were *Eucalyptus* species leaves, but these isolated *Phytophthora* infrequently. In most cases, isolations were obtained from necrotic regions on the leaves, but occasionally also from non-symptomatic tissue. The durability of the bait was found to be the important factor for isolations from the different baits. Both *B. attenuata* and *P. undulatum* remained intact and were not degraded. Lupin seedlings and oak leaves were often decomposed and were not suitable for isolations.

Discussion

This is the first comprehensive Australasian waterway survey to investigate the diversity and distribution of *Phytophthora* species and some related oomycetes in 48 waterways across the vast state of WA. The survey encompassed both natural and man-made waterways, many of which ran through ecosystems invested with Phytophthora dieback that is generally attributed to *P. cinnamomi*. Several of the *Phytophthora* species isolated, including *P. inundata*, *P. thermophila* and the two hybrids, are widespread in waterways and most species recovered have known associations with dying native and/or horticultural important plant taxa.

P. inundata is widespread in the south coast and some wheatbelt regions and has been associated with dying native vegetation including the grass tree (*Xanthorrhoea preissii*) in several southwest locations of WA (Stukely et al. 2007). Additionally, *P. inundata* has also been associated with dying horticultural shrubs and trees including *Aesculus*, *Olea*, *Salix*, *Prunus* and *Vitis* (Brasier et al. 2003b; Cunnington et al. 2006). *P. multivora* and *P. cryptogea* were rarely encountered in waterways in this study, but both species are commonly associated with numerous dead and dying native hosts (Burgess et al. 2009; Scott et al. 2009). This is the first report of *P. multivora* in water in Australia, but it has been recorded in New Zealand waterways (Randall 2011). *P. thermophila*, *P. litoralis*, *P. fluvialis* and *P. amnicola* are four newly described species from ITS Clade 6, all found exclusively in Western Australia (Jung et al. 2011; Crous et al. 2011, 2012). These four species have been found in waterways but also, with the exception of *P. fluvialis*, they have been found associated with dying

Table 2 Summary of the incidence and molecular identification of *Phytophthora* (n=12), *Phytophthora* hybrids (n=2), *Halophytophthora* (n=3) and *Phytopythium* (n=3) species recovered with the proportions obtained from each of the bait leaves from 48 waterways across Western Australia during October to early December 2008

Species	ITS Clade	No. ITS ID[a]	No. *cox*I ID[a]	No. of waterways (n=48)	Incidence (% total recoveries)[b]	Plant bait recovery (%)					
						Banksia. attenuata	*Pittosporum undulatum*	*Hakea laurina*	Other[c]	*Quercus. robur*	*Lupinus angustifolius* seedling
Halophytophthora avicenniae		12		9	3.9	21.4	7.1	14.3	14.3	28.6	14.3
Halophytophthora polymorphica		8		8	4.2	20.0	20.0	13.3	20.0	20.0	6.7
Halophytophthora aff. polymorphica		6		5	1.9	57.1	0	42.9	0	0	0
Phytopythium aff. helicoides		5		5	1.7	50.0	16.7	0	16.7	16.7	0
Phytopythium aff. *litorale*		11		8	4.2	40.0	20.0	20.0	6.7	0	13.3
Phytopythium citrinum		2		2	0.8	0	33.3	0	0	0	66.7
Phytophthora multivora	2	2		2	0.6	50.0	50.0	0	0	0	0
Phytophthora aff. *Kununurra*	9	1		1	0.3	0	100.0	0	0	0	0
Phytophthora aff. *hydropathica*	9	3		3	0.8	0	33.3	0	66.7	0	0
Phytophthora aff. *insolata*	9	1		1	0.6	100.0	0	0	0	0	0
Phytophthora cryptogea	8	2		2	0.6	0	100.0	0	0	0	0
P. cinnamomi var. *parvispora*	7	1		1	0.3	0	100.0	0	0	0	0
Phytophthora aff. *humicola*	6	3		3	0.8	66.7	0	33.3	0	0	0
Phytophthora amnicola	6	3		3	0.8	33.3	33.3	33.3	0	0	0
Phytophthora fluvialis	6	19		12	8.3	30.0	30.0	3.3	20.0	13.3	3.3
Phytophthora inundata	6	32		28	11.7	45.2	11.9	21.4	4.8	4.8	11.9
Phytophthora litoralis	6	7		6	2.8	30.0	30.0	20.0	10.0	10.0	0
Phytophthora thermophila	6	19		14	7.5	14.8	29.6	33.3	0	3.7	18.5
Clade 6 hybrids[d]		112									
Hybrid A	6		78	36	34.7	19.2	30.4	23.2	14.4	8.8	4.0
Hybrid B	6		34	22	12.8	45.6	30.4	4.3	13.0	4.3	2.2
(*P. amnicola)*			5								
(P. fluvialis)			6								
(P. litoralis)			3								
(sub-clade 2.1)			3								
(sub-clade 2.2)			5								
(sub-clade 2.3)			12								
	TOTAL	249		47	100.0	29.4	25.6	18.3	11.9	8.1	6.7

[a] Number of isolates that were identified using ITS and coxI sequences. Note a representative isolate was selected for molecular identification from groups of morph-types from each waterway for each sampling

[b] Incidence is based on total number of isolates recovered (n=360) of which 249 were identified using ITS and coxI sequences

[c] Selection of plant species used infrequently, including *Eucalyptus* and *Kennedia* species, which were added to the bait bag by the volunteer prior to their deployment in the waterway

[d] Based on cox1 sequence data hybrids were divided into two groups; Hybrid A had *P. thermophila* as a parent, all other isolates were placed in Hybrid B and had a range of parents (*P. amnicola*, *P. litoralis*, *P. fluvialis*) and three alleles that do not correspond to any known species

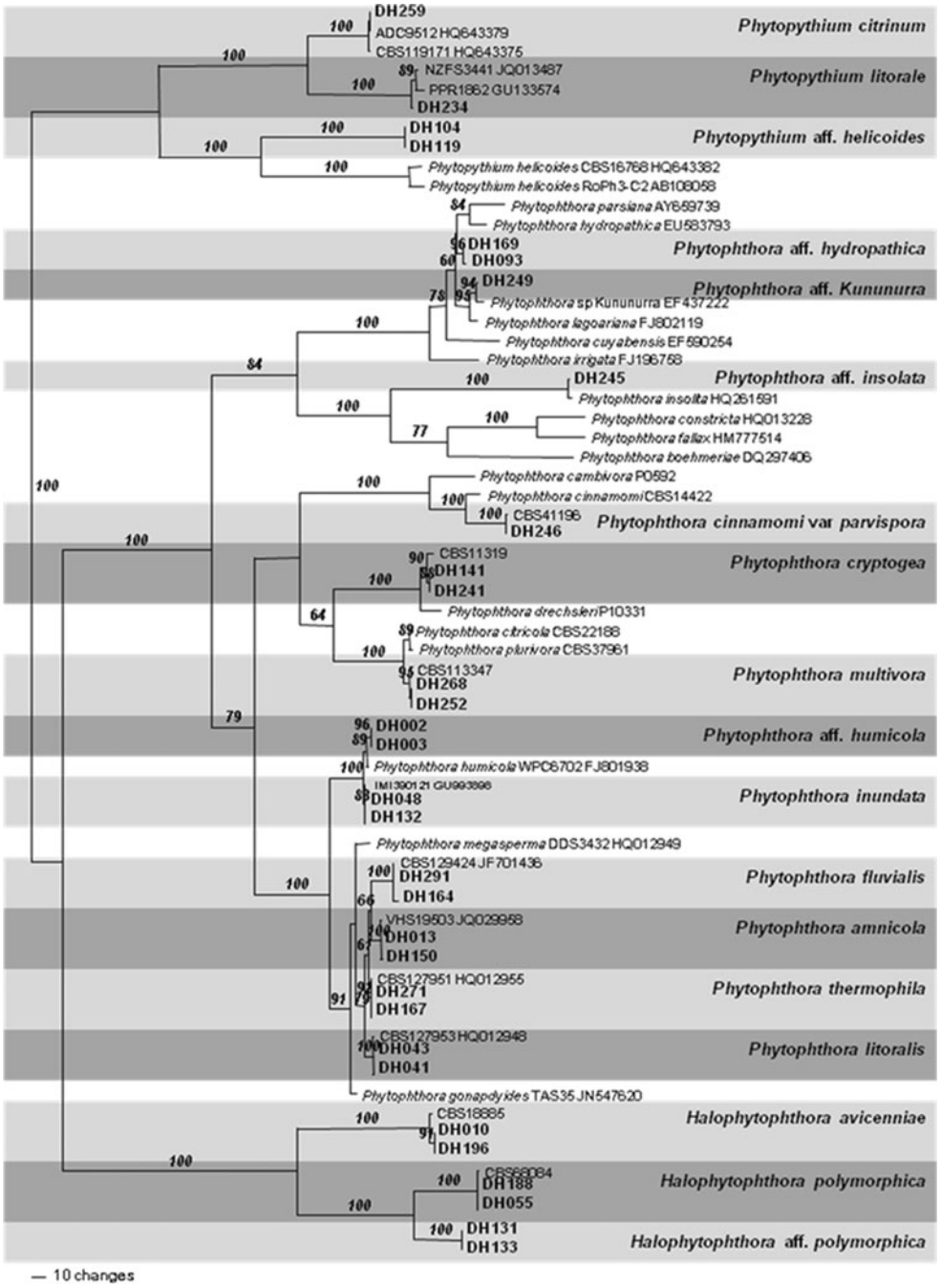

Fig. 2 One of four most parsimonious trees of 2603 steps (CI=0.60, RI=0.91) based on ITS sequence data illustrating the phylogenetic placement of the *Phytophthora, Halophytophthora* and *Phytopythium* species obtained in this study

plants. Studies are currently underway to characterise the Clade 6 hybrids, but to date very little is known about their biology and the role they play in the environment.

Three of the undescribed taxa were from ITS Clade 9. These and *P. cinnamomi* var. *parvispora* were found in the north of WA in Kununurra. Several new species have recently been described from ITS Clade 9 from irrigation water (Hong et al. 2008, 2010), however, the pathogenicity of these species is unknown and they may fill a similar ecological niche to that proposed for Clade 6 with a predominance in native riparian, forest and woody ecosystems (Brasier et al. 2003a; Jung et al. 2011; Nechwatal et al. 2012). Davison et al. (2005) had previously isolated *P. cinnamomi* var. *parvispora* and two undescribed Clade 9 taxa from irrigation channels in Kununurra. One of which, designated as *P.* sp. Kununurra was very similar to an isolate found in the current study.

In our study, the distribution of *P. inundata* mirrored that of the *Halophytophthora* species being found in estuaries or saline waterways. Also enoucountered in these waterways (although less frequently) were *P. fluvialis*, *P.* aff *humicola* and hybrids A and B. *Halophytophthora* shares a common

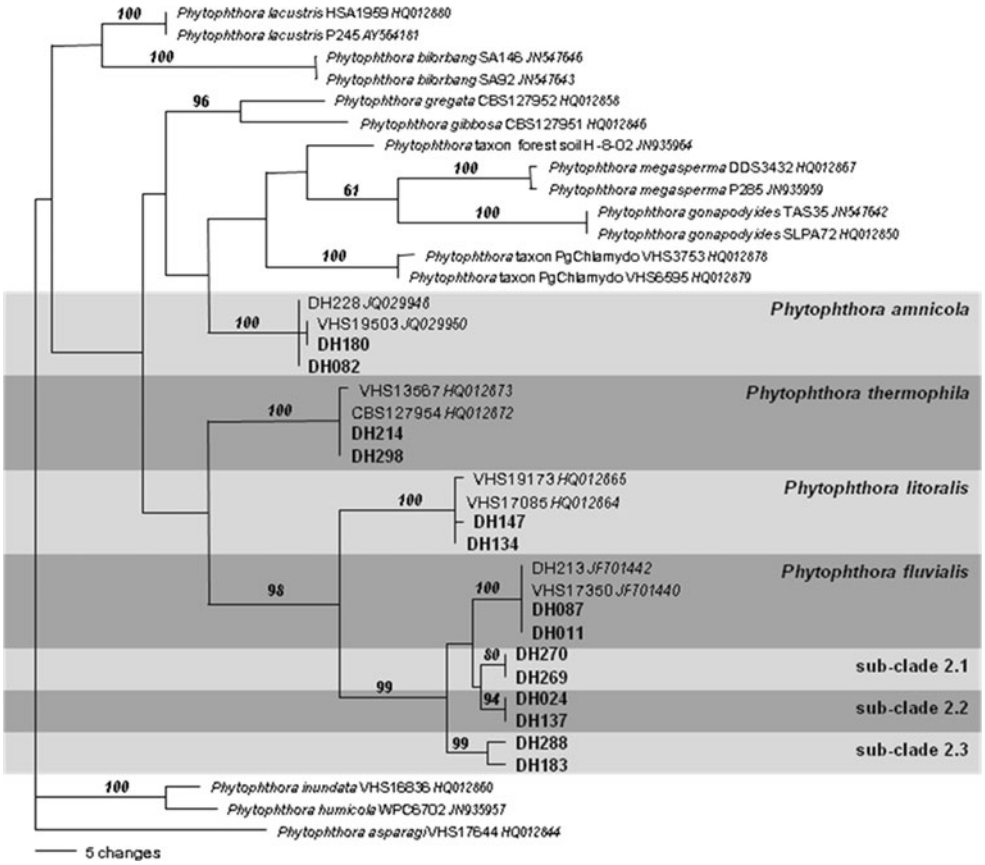

Fig. 3 One of 27 most parsimonious trees of 334 steps (CI=0.55, RI=0.84) illustrating the phylogenetic placement of the *cox*I allele obtained from the *Phytophthora* hybrid isolates

ancestor with *Phytophthora* (Robideau et al. 2011) and its presence is indicative of brackish water with its role in decomposing leaf litter having been extensively studied in mangroves ecosystems (Hyde et al. 1998). Reeser et al. (2011) also found *H. avicenniae* in surveys of streams in Oregon. Although *Phytophthora* species are known to differ in their sensitivity to salinity, few species have been reported from brackish or saline water bodies (Man in't Veld et al. 2011). However, recently two clade 6 species were recovered from declining seagrass (*Zostera marina*) beds in the Netherlands; *P. inundata* and the newly described species *P. gemini* (Man in't Veld et al. 2011).

Phytopythium is a recently described genus (Bala et al. 2010) which contains all species formerly designated in *Pythium* Clade K (Lévesque and de Cock, 2004). In phylogenetic analyses, this genus sits basal to *Phytophthora* and *Halophytophthora.* Their recovery reflects the similarity of their hyphal growth patterns in young colonies to that of *Phytophthora.* Very little is known about the role of the species recovered in this study.

The plant bait used influenced the diversity of *Phytophthora* species isolated. No single plant bait isolated all 12 *Phytophthora* species and two *Phytophthora* hybrids; however, *B. attenuata and P. undulatum,* did so when used in combination. In many studies, one plant bait or recovery method is used (e.g. Palzer 1980; Von Broembsen 1984; Davison et al. 2005; Hulvey et al. 2010). Studies where more than one plant species are used, as in our study, have shown that the *Phytophthora* species isolated varied with baits and that this variability may have been due to changes in phenological processes of one plant species which compromised the recoveries (Smith et al. 2009; Sutton et al. 2009). Additionally, some *Phytophthora* species are attracted to certain baits and not others. Whilst Reeser et al. (2011) found no differences in isolation between their two baits, they indicate that durability and handling of the baits might be important. From the plant baits we used, it is evident that a plant species could be selective for certain *Phytophthora* species emphasizing the importance of including several plant species as baits in *Phytophthora* diversity studies. For example, *P.* aff. *insolata* was only ever recovered from *B. attenuata* leaves and *P.* aff. *parsiana*, *P. cinnamomi* var. *parvispora* and *P. cryptogea* were only recovered from *P. undulatum*.

The absence of *P. cinnamomi* from any waterways in WA is noteworthy and somewhat surprising given sampling was conducted in areas of the southwest of WA known to be infested by *P. cinnamomi.* However, it is rare to isolate *P. cinnamomi* from rivers, dams or other water sources in WA across all seasons (Hardy and Williams, unpublished results). Other studies in other states of Australia and overseas have isolated this species and did so during the summer months (Von Broembsen 1984; Smith et al. 2009). In WA, a possible hypothesis for not recovering *P. cinnamomi* from

waterways may be that the frequently recovered *Phytophthora* species, *P. inundata* and/or hybrid B, from the southwest region have a competitive advantage by producing more sporangia and zoospores than *P. cinnamomi* in waterways which reduce the success of its recovery.

In the single spring/early summer season surveyed, we found regional differences in the *Phytophthora* species that occur in waterways in WA. The regional differences observed require further verification in light of the fact that variation in seasonal and geographic differences in *Phytophthora* species isolations have been reported previously (Smith et al. 2009; Reeser et al. 2011). Further sampling in all seasons and from multiple locations to get a complete species composition for an area would provide conclusive evidence of regional specificity in species.

The current study provides the results of an extensive survey from waterways across WA from which 12 *Phytophthora* species and two hybrid species were isolated, with distinct regional variation in species diversity being observed. What does this finding mean for natural ecosystems, land managers and the horticultural industry? Until such time that more is known about the role of these newly described and yet to be described *Phytophthora* species, along with the hybrids, a proactive approach in their management using the precautionary principle (Deville and Harding 1997) is prudent. Those who utilize water from these waterways should be aware that the water they use potentially contains pathogens of phytosanitary significance to native and agricultural plant systems in addition to the commonly acknowledged *P. cinnamomi* presence throughout many of these systems. Therefore, for horticultural businesses that source their water from these waterways it would be a prudent measure to incorporate appropriate chemical and filtration treatments before applying the water to plants, and that any runoff water is treated to prevent contamination of waterways with new species and/or additional strains of a species. For land managers, water used for road building and firefighting should be collected from within a catchment where it will be used or the water may need treatment prior to application particularly where the water will be applied to new regions beyond the waterway's catchment. Possibly little can be done to prevent the further spread of *P. inundata* which is widely distributed across WA. But its known potential as a plant pathogen on several native as well as horticultural crops means that land managers should be aware of its presence in their water source and use appropriate measures to prevent infection of plants.

Acknowledgements We thank C. Fletcher and T. Paap for providing assistance in the laboratory. The diverse range of waterways baited across the large State of Western Australia would not have been possible without the help from more than 16 community groups and volunteers who deployed, recovered and returned bait bags. The project website www.f4p.murdoch.edu.au was funded by the WWF.

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