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Phytophthora elongata sp. nov., a novel pathogen from the *Eucalyptus marginata* forest of Western Australia

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Abstract. A novel homothallic species of *Phytophthora* producing semipapillate sporangia on sympodially branching sporangiophores, thick-walled oospores in smooth-walled oogonia, and paragynous antheridia is described here as *Phytophthora elongata* sp. nov. DNA sequencing of the internal transcribed spacer (ITS) DNA and *cox*I gene confirm *P. elongata* as a distinct species within ITS clade 2. It has been isolated in the northern jarrah forest of Western Australia (WA) from the roots and collars of dead and dying *Eucalyptus marginata* and occasionally *Corymbia calophylla* in rehabilitated bauxite mine pits. It has also been associated with dead and dying plants of several mid- and understorey species in the northern and southern jarrah forest – *Banksia grandis*, *Leucopogon propinquus*, *Dryandra squarrosa* and an *Andersonia* sp., as well as the monocotyledonous *Xanthorrhoea preissii*, *X. gracilis* and *Patersonia xanthina. P. elongata* has also been isolated from sandy soils and loams in Victoria in eastern Australia. The pathogenicity of *P. elongata* to *E. marginata* and *Banksia* spp. has been shown in this and earlier studies. Due to the uniformity of the ITS DNA and *cox*1 sequence data in WA, *P. elongata* may be the result of a recent clonal introduction. More pathogenicity tests on a wider range of native plant species are needed to assess the host range of *P. elongata* and its invasive potential in WA.

Additional keywords: biosecurity, natural ecosystems, phylogenetics.

Introduction

The genus *Phytophthora* comprises a multitude of devastating plant pathogens responsible for both historical and current tree and ecosystem declines across the globe (Erwin and Ribeiro 1996). Most destructive *Phytophthora* epidemics are caused by introduced species, impacting on non-adapted ecosystems which lack host-pathogen coevolution (Shearer and Tippett 1989; Hansen *et al*. 2000; Rizzo *et al*. 2002; Brasier 2008; Jung 2009). In Western Australia (WA) the introduction of *Phytophthora cinnamomi* has had, and continues to have, a devastating effect on the floristically rich South-west Botanical Province, which is home to some 5700 endemic plant species, of which ~41% are susceptible (Shearer *et al*. 2004).

Increasingly, gene phylogenies are used to distinguish morphologically cryptic *Phytophthora* species. As a result, many new *Phytophthora* species have been identified and described. In recent years many of these new species have been found in association with tree declines (Jung *et al*. 1999, 2003; Man in't Veld *et al*. 2002; Brasier *et al*. 2003; de Cock and Lévesque 2004; Jung and Burgess 2009; Scott *et al*. 2009). A molecular reevaluation of a large number of isolates from the collection of the Vegetation Health Service (VHS) of the Western Australian Department of Environment and Conservation has revealed nine potentially new taxa, some of which were previously assigned to known morpho-species such as *P. citricola*, *P. drechsleri* and *P. megasperma* (Burgess *et al*. 2009). *P. multivora*, the first of these species to be described, had previously been identified as *P. citricola* (Scott *et al*. 2009). It has been isolated in WA from natural forest and heathland stands for the last 30 years from beneath dead and dying plants of 16 species from seven families. *P. multivora* is very widespread in south-west WA with a distribution similar to *P. cinnamomi*, but is also active on calcareous soils, whereas *P. cinnamomi* is not.

Between late spring 1992 through to mid summer 2006, 136 isolates of another new taxon, previously known as *Phytophthora* Psp2 (Burgess *et al*. 2009), *Phytophthora* sp. WA2 (Stukely *et al*. 2007) and *P. citricola* isozyme subgroup 1 (Bunny 1996) were recovered from dead and dying plants and rhizosphere soil from rehabilitated bauxite mine pits and adjacent intact jarrah forest around Dwellingup in the northern jarrah forest of the south-west of WA. These isolates were predominantly associated with dead or dying *Eucalyptus marginata* (jarrah) saplings and trees, and to

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a much lesser extent *Corymbia calophylla* (marri), growing on restored mine sites (Fig. 1). This taxon has also been isolated from the rhizosphere soil of dead or dying understorey species including *Andersonia* sp. (in 1989) and *Patersonia xanthina* (1999 and 2008) in the southern jarrah forest in the Bridgetown-Nannup area, *Banksia grandis* between Manjimup and Pemberton (2006), *Xanthorrhoea preissii* near Manjimup (2008) and *X. gracilis* west of Collie (2008). In the northern forest it has been isolated from the rhizosphere soil of dying *Leucopogon propinquus* (in 2000) near Dwellingup, and

Fig. 1. Wilting and dieback of young *Eucalyptus marginata* and *Corymbia calophylla* trees in a mining rehabilitation site near Dwellingup, WA due to root and collar rot caused by *Phytophthora elongata*.

Table 1. Isolates of *Phytophthora* spp. considered in this study

Species	Isolate number[A]	Isolation location	Isolation date	Host association	ITS	*cox*I
Phytophthora bisheria	VPRI 21375	Knoxfield, Victoria	n.a.[C]	*Rubus idaeus*	DQ298447	GQ847760
P. cinnamomi	MU 9448[B]	Willowdale, Western Australia	1994	*Eucalyptus marginata* (collar)	n.a.	n.a.
P. frigida	CMW 19433	Lions River, KwaZulu-Natal	2000	*E. smithii*	DQ988179	GQ847759
P. elongata	VHS 6362[B]	Bridgetown, Western Australia	12.vii.99	*Patersonia xanthina* (soil)	GQ847751	GQ847761
P. elongata	VHS 7134	Dwellingup, Western Australia	25.i.00	*Leucopogon propinquus* (soil)	GQ847752	GQ847762
P. elongata	VHS 12732	Dwellingup, Western Australia	16.xii.03	*E. marginata* (root)	GQ847753	GQ847763
P. elongata	VHS 13482, CBS 125799 (type)[B]	Dwellingup, Western Australia	18.xi.04	*E. marginata* (soil)	GQ847754	GQ847764
P. elongata	VHS 13615 (WAC 13038)[B]	Dwellingup, Western Australia	26.xi.04	*E. marginata* (soil)	EU121961	GQ847765
P. elongata	VHS 15078	Dwellingup, Western Australia	29.xii.05	*E. marginata* (soil)	GQ847755	GQ847766
P. elongata	VHS 13559	Dwellingup, Western Australia	16.xi.04	*E. marginata* (soil)	EU301121	n.a.
P. elongata	VHS 13663	Dwellingup, Western Australia	30.xi.04	*E. marginata* (soil)	EU301123	n.a.
P. elongata	VHS 16103	Pemberton, Western Australia	04.viii.06	*Banksia grandis* (soil)	EU301125	GQ847767
P. taxon 'elongata-like'	VHS 15032[B]	Dwellingup, Western Australia	19.xii.05	*E. marginata* (soil)	GQ847756	GQ847768

[A]Abbreviations of isolates and culture collections: CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; CMW = Culture Collection of the Forestry and Agriculture Biotechnology Institute, University of Pretoria, South Africa; MU = Murdoch University, Murdoch, Western Australia; VHS = Vegetation Health Service of the Department of Environment and Conservation, Perth, Western Australia; VPRI = Primary Industries, Knoxfield, Australia; WAC = Department of Agriculture and Food, Perth, Western Australia.

[B]Isolates used in pathogenicity trials.

[C]n.a. = not available.

Dryandra squarrosa (2008) east of Muchea, north of Perth (M. Stukely, unpubl. data). Its known distribution in WA is restricted to the laterite soils in the hills of the northern and southern jarrah forest and it is not found on the sandy coastal plains. In Victoria, however, it has been found on sandy soils and loams (W. Dunstan, pers. comm.). Based on its unique combination of morphological and physiological characters and ITS DNA and *cox*1 sequences this taxon is described here as a new species to which the name *Phytophthora elongata* sp. nov. is assigned.

Materials and methods

Isolation methods

Samples of soil and root material were baited with *Eucalyptus sieberi* cotyledons (Marks and Kassaby 1974), which were plated after 5 or 10 days onto P10VPH (Tsao and Guy 1977) or NARPH (Hüberli *et al*. 2000) selective agar, from which pure cultures were then isolated. Roots (0.5–15 mm diam.) and collars of dying 1–2-year-old *E. marginata* plants from rehabilitated mine pits were surface sterilised in 70% ethanol and rinsed four times in distilled water before being cut into short lengths (1.0–1.5 cm) and plated onto selective agar as above.

Preparation of Phytophthora *cultures*

The isolates used in this study are detailed in Table 1. All isolates were initially grown on cornmeal agar (CMA; BBL, Becton Dickinson Co., Sparks, MD, USA) plates before being subcultured twice onto *Phytophthora*-selective NARPH agar plates (Hüberli *et al*. 2000). To ensure all isolates used in the pathogenicity trial were equally vigorous, each isolate was passaged through an apple fruit (var. 'Granny Smith') and reisolated after 6 days using NARPH. Isolates were then maintained on V8 agar (V8A) plates (Erwin and Ribeiro 1996).

DNA isolation, amplification and sequencing

The *Phytophthora* isolates were grown on half-strength potato dextrose agar (PDA) (Becton Dickinson Co.,19.5 g PDA, 7.5 g of Difco agar and 1 L of distilled water) at 20°C for 2 weeks. 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). The region spanning the internal transcribed spacer (ITS) 1–5.8S-ITS2 region of the rDNA was amplified using the primers ITS-6 (5′ GAA GGT GAA GTC GTA ACA AGG 3′) (Cooke *et al*. 2000) and ITS-4 (5′ TCC TCC GCT TAT TGA TAT GC 3′) (White *et al*. 1990). The PCR reaction mixture, PCR conditions, clean up of products and sequencing were as described previously (Andjic *et al*. 2007).

The mitochondrial *cox*1 gene was amplified with the primers FM82 (5′ TTG GCA ATT AGG TTT TCA AGA TCC 3′) and FM83 (5′ CTC CAA TAA AAA ATA ACC AAA AAT G 3′) (Martin and Tooley 2003). The PCR reaction mixture was as described previously (Andjic *et al*. 2007). The PCR conditions were as described previously (Martin and Tooley 2003). PCR products were cleaned using the Qiagen PCR clean up kit (Qiagen, Germantown, MD, US) according to the manufacturer's instructions. The *cox*1 region of templates was sequenced in both directions using the primers FM84 (5′ TTT AAT TTT TAG TGC TTT TGC 3′), FM85 (5′ AAC TTG ACT AAT AAT ACC AAA 3′), FM50 (5′ GTT TAC TGT TGG TTT AGA TG 3′) and FM83 (Martin and Tooley 2003).

Phylogenetic analysis

The ITS rDNA and *cox*1 gene were sequenced for the isolates of *P. elongata* used in this study. Species closely related to *P. elongata* in ITS clade2 (Cooke *et al*. 2000) and representative species from other clades within the genus were either sequenced or obtained from GenBank. All sequences derived in this study were deposited in GenBank and accession numbers are shown in Table 1.

Sequence data for the ITS region were initially assembled using Sequence Navigator version 1.01 (Perkin Elmer, Waltham, MA, US) and aligned in Clustal X (Thompson *et al*. 1997). Manual adjustments were made visually by inserting gaps where necessary in BioEdit version 5.06 (Hall 2001). There were no gaps in the *cox*1 alignment. The first 540 bp of the *cox*1 dataset were excluded to allow for alignment with the other sequences available on GenBank.

Parsimony analysis was performed in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2003) and Bayesian analysis with MrBayes version 3.1 (Ronquist and Heuelsenbeck 2003) as described previously (Jung and Burgess 2009). Alignment files and trees can be viewed on TreeBASE (SN4638).

Colony morphology, growth rates and cardinal temperatures

Colony morphology was described from 7-day-old cultures grown at 20°C in the dark on carrot agar (CA), V8A, malt extract agar (MEA) and half-strength PDA (recipes in Jung and Burgess 2009). CA was prepared as described by Ribeiro (1978). Colony morphologies were described according to Brasier and Griffin (1979), Erwin and Ribeiro (1996) and Jung *et al*. (2003). Radial growth rate was recorded 4–6 days following the onset of linear growth along two lines intersecting the centre of the inoculum at right angles (Jung *et al*. 1999). For temperature-growth studies, all isolates were subcultured onto CA plates and incubated for 24 h at 20°C to initiate growth. Three replicates for each isolate were then transferred to incubators set at 4, 10, 15, 20, 25, 30 and 32.5°C, and radial colony growth was measured as above after 4–6 days.

Morphology of sporangia and gametangia

Sporangia and gametangia were produced on CA and V8A and measurements were made as described by Jung *et al*. (1999). Sporangia were obtained by flooding 5 × 5-mm-agar squares taken from the growing margins of 3–5-day-old colonies with enough deionised water to ensure that the discs were submerged and adding 5 mL of non-sterile soil extract in 90-mm Petri dishes. Petri dishes were incubated in the dark at 20°C for 18–36 h. The non-sterile soil extract was made by mixing 100 g of soil with 1 L of deionised water and shaking vigorously every 20 min for 2 h. Soil extract water was left to settle overnight after which the water was filtered through

cheesecloth followed by Whatman no. 1 paper. Dimensions and characteristic features of >50 mature sporangia chosen at random were determined at ×400 magnification (BH-Olympus, Tokyo, Japan) for each isolate. Dimensions and characteristic features of >50 mature oogonia, oospores and antheridia chosen at random were measured for each isolate at ×400 magnification at the surface of ~15-mm cut from the centre of 14–22-day-old V8A cultures grown in the dark at 20°C. For each isolate 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).

Pathogenicity trials

Pathogenicity of *P. elongata* to *E. marginata* (isolates VHS 6362, CBS 125799, VHS 13615 and VHS 15032) and *B. attenuata* (isolates VHS 6362 and CBS 125799) was tested in two separate soil-infestation pot trials. In both trials *P. cinnamomi* isolate MP 9448 was included as a comparison as it is known to kill both hosts in the field.

Pine plugs were used as the source of inoculum (Butcher *et al.* 1984). Plugs ~1.0 cm in diameter × 1.5 cm in length were cut from young debarked branches of *Pinus radiata*. The pine plugs were then washed, placed in 2-L flasks and sterilised by autoclaving three times over 3 days at 121°C for 20 min. Flasks were then stored at room temperature in the dark until required. When the *Phytophthora* isolates had grown almost to the edge of the V8A plates they were added to the flasks containing the sterilised pine plugs. Half a plate of each *Phytophthora* culture was added to separate flasks by cutting the agar into ~1.0 × 1.0-cm squares. Flasks were incubated at 20°C for 7 weeks before inoculation of plants. The flasks were shaken weekly to facilitate even colonisation of the pine plugs. Colonisation of the pine plugs by each isolate was confirmed following surface sterilisation of two plugs from each flask, which were subsequently cut longitudinally in half and plated onto NARPH agar plates. *Phytophthora* grew from all plugs. For both trials, 200-mL free-draining polyurethane pots were filled with potting mix to ~3 cm below the container rim. The potting mix consisted of steam pasteurised composted pine bark, coarse river sand and coco peat mix (2 : 2 : 1) into which was incorporated 60 g Dolomite, 60 g $CaCO_3$, and 40 g Osmocote (Scotts Australia Pty Ltd, Baulkham Hills, NSW) (formulated for Australian native plants) per 40 L of mix. Seedlings of *E. marginata* and *B. attenuata* were purchased from Oakford Tree Farms, WA. For the duration of the trials (175 days for *E. marginata* and 185 days for *B. attenuata*) plants were assessed daily for the development of aboveground disease symptoms. Mortality of seedlings was recorded, and immediately after death reisolations were made from necrotic roots and the root collar using NARPH.

E. marginata trial: four 6-month-old *E. marginata* seedlings still in 'peat pots' were planted in each pot in the first week of August 2007. Plants were distributed equidistantly around the centre of each pot. A total of 240 plants were potted up in 60 pots. Plants were watered daily to container capacity by hand and maintained in an evaporatively cooled glasshouse in which the temperature did not exceed 30°C. To minimise root disturbance and wounding at the time of inoculation, a sterile 15-mL tube was placed in the centre of each pot at planting; this was removed at the time of inoculation to provide a cavity in which to deposit the pine plug inoculum. The plants were grown for 4 months before inoculation.

At inoculation, pots containing *E. marginata* plants were inoculated with pine plugs colonised with either one of three isolates of *P. elongata*, *P.* taxon 'elongata-like' isolate VHS 15032, and *P. cinnamomi* isolate MP 9448 (positive control), or non-colonised pine plugs (negative control). Forty plants (10 pots) were allocated to each isolate/control, and each pot was inoculated with four colonised pine plugs or control plugs placed in the central cavity. Pots were organised in completely randomised blocks on three benches. Immediately after inoculation the pots were individually flooded in buckets for 16–20 h and thereafter on a fortnightly basis for 3 months and then once in each of the following 2 months. Between flooding events plants were watered daily to container capacity by hand.

B. attenuata trial: four *B. attenuata* seedlings were planted in each pot in June 2008. A total of 80 plants were potted up in 20 pots. Ten pots were allocated to each of the two *P. elongata* isolates tested, *P. cinnamomi* or non-colonised pine plugs as a negative control. Pots were organised in completely randomised blocks. Plants were watered daily by hand and maintained as described above.

Pots containing *B. attenuata* were inoculated in early October 2008 as described for *E. marginata* except that the pots were not flooded initially. Pots were first flooded as described above in mid December 2008, and then in January, February and March 2009 to stimulate the production of sporangia and pathogen spread and infection via zoospores.

Results

Phylogenetic analysis

The ITS dataset consisted of 911 characters of which 313 were parsimony informative. The dataset contained significant ($P < 0.001$, g1 = –0.32) phylogenetic signal. Heuristic searches resulted in 12 most parsimonious trees of 703 steps (CI = 0.66, RI = 0.92) (TreeBASE SN4638, Fig. 2). The topology of the Bayesian tree was very similar (TreeBASE SN4638). Sequences of all isolates of *P. elongata* are identical (9 isolates presented here and an additional 17 isolates from the VHS collection, M. Stukely, unpubl. data) and reside in a strongly supported terminal clade, separate from the closest species *P. bisheria*, *P. frigida* and *P. multivesiculata* by 34, 52 and 45 bp, respectively. Of these species only *P. multivesiculata* was included in the phylogenetic analysis of Cooke *et al.* (2000). In their analysis, *P. multivesiculata* was separate from other species but was placed in the ITS clade 2. Now with the inclusion of the additional species, clade 2 is divided into two subclades; clade 2a containing *P. citricola*, *P. multivora*, *P. plurivora*, *P. citrophthora*, *P. colocasiae* and *P. capsici* and clade 2b containing *P. bisheria*, *P. frigida*, *P. elongata* and *P. multivesiculata* (Fig. 2).

The *cox*1 dataset consisted of 742 characters of which 96 were parsimony informative. The dataset contained significant ($P < 0.001$, g1 = –0.62) phylogenetic signal. Heuristic searches resulted in two most parsimonious trees of 242 steps (CI = 0.54, RI = 0.76) (TreeBASE SN4638, Fig. 3). The topology of the

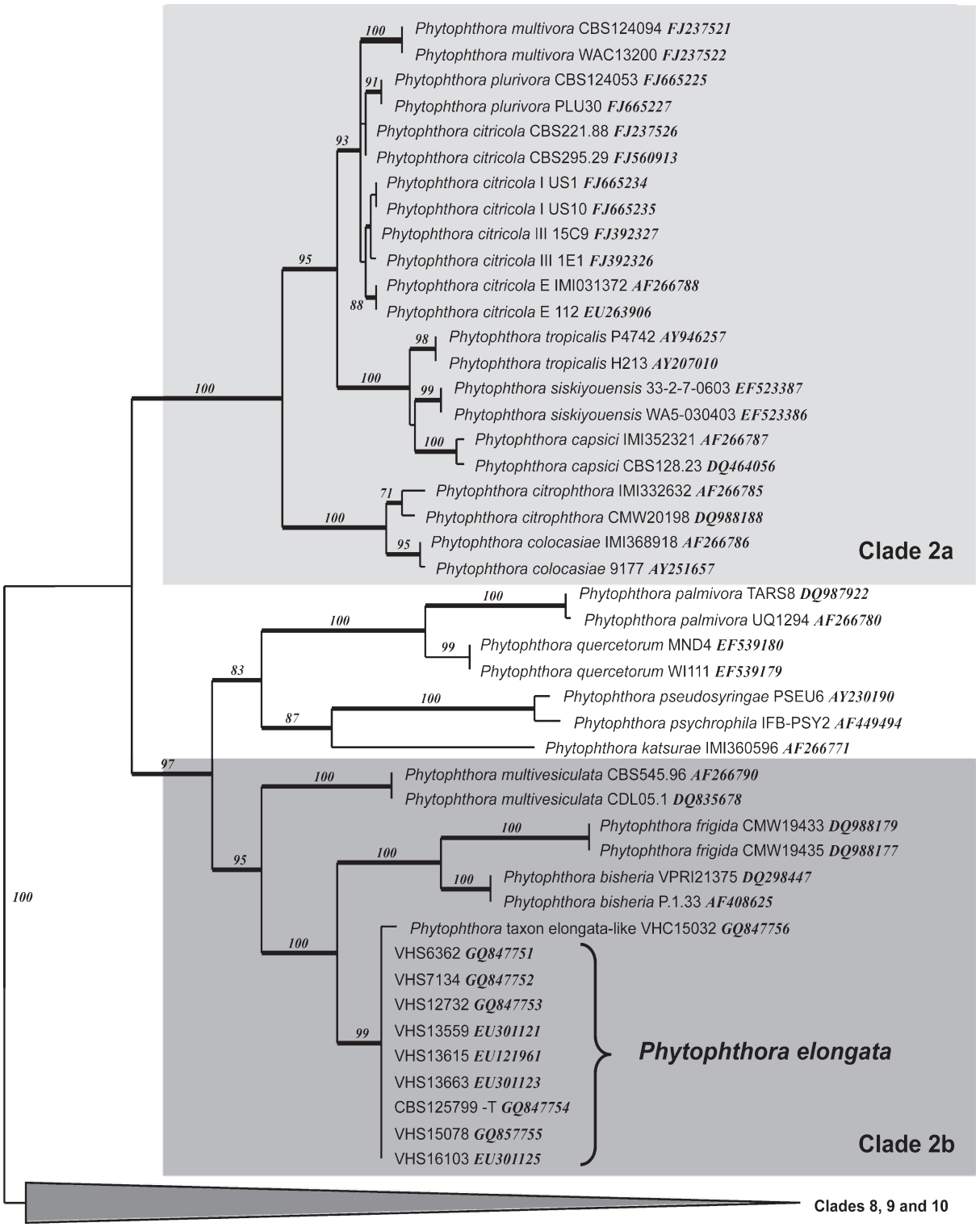

Fig. 2. One of 12 most parsimonious trees of 703 steps based on analysis of rDNA internal transcribed spacer (ITS) sequence data showing phylogenetic relationships between *Phytophthora elongata* and related species from ITS clades 2a, 2b and 4. Numbers in italics represent bootstrap support for the nodes. Thickened branches indicate a posterior probability based on Bayesian analysis of greater than 0.80.

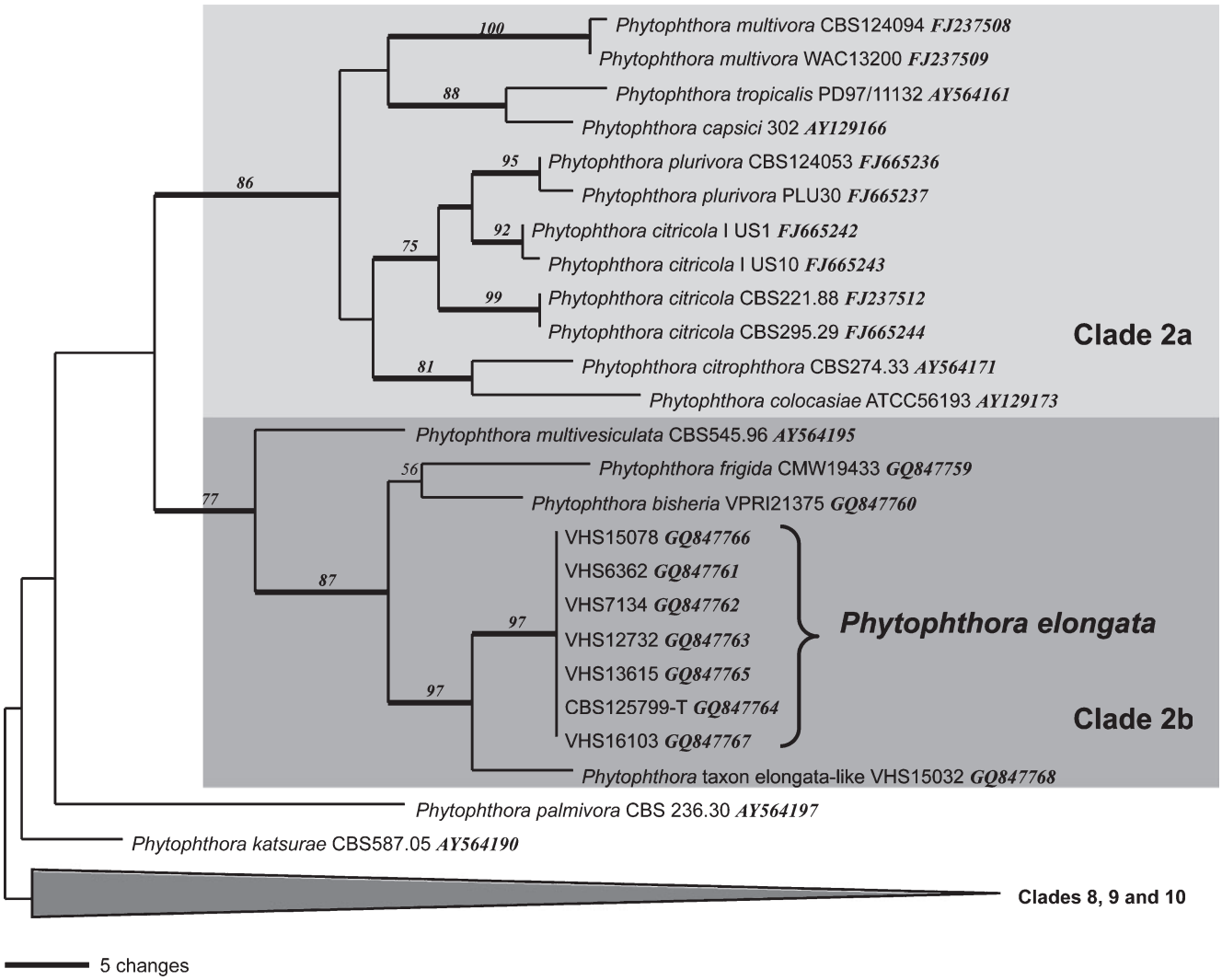

Fig. 3. One of two most parsimonious trees of 242 steps based on analysis of mitochondrial gene *cox*I sequence, showing phylogenetic relationships between *Phytophthora elongata* and related species from internal transcribed spacer clade 2. Numbers in italics represent bootstrap support for the nodes. Thickened branches indicate a posterior probability based on Bayesian analysis of greater than 0.80.

Bayesian tree was very similar (TreeBASE SN4638). Species from ITS clade 2 grouped together with strong support. Sequences of all isolates of *P. elongata* were identical and resided in a strongly supported terminal clade separate from their closest relatives (ITS clade 2b) *P. bisheria*, *P. frigida* and *P. multivesiculata* by 18, 22 and 27 bp, respectively (Fig. 3).

For both ITS and *cox*1, the sequence for isolate VHS 15032 was different from *P. elongata* by 3 and 6 bp, respectively, consequently this single isolate has been designated as *P.* taxon elongata-like.

Colony morphology, growth rates and cardinal temperatures

All *P. elongata* isolates produced colonies with a petaloid morphology on MEA and PDA. On V8A and CA colonies of the ex-type isolate CBS 125799 were faintly petaloid to radiate while isolates VHS 7134 and VHS 15078 produced petaloid colonies. Isolate VHS 15032 (*P.* taxon elongata-like**)** produced cottony colonies which were uniform on MEA and PDA and faintly stellate on V8A and CA. Both taxa formed aerial mycelium on all four agar media assessed (Fig. 4). Colonies of *P. frigida* were stellate on CA, V8A and MEA and faintly stellate on PDA. Aerial mycelium was formed on CA, V8A and PDA. *P. bisheria* produced uniform colonies on all four media with dome-shaped aerial mycelium at the inoculum plug on V8A and PDA (Fig. 4).

P. elongata had a mean radial growth rate on CA of 6.29 ± 0.21 mm d^{-1} at its optimum temperature of ~25°C. The maximum temperature for growth was 32.5°C (Fig. 5). Although growth at this temperature ceased after 2 days, 32.5°C was not lethal as isolates resumed growth when subsequently incubated at 20°C. Isolate VHS 15032 (*P.* taxon elongata-like**)** had cardinal temperatures identical to those of *P. elongata* but grew slightly

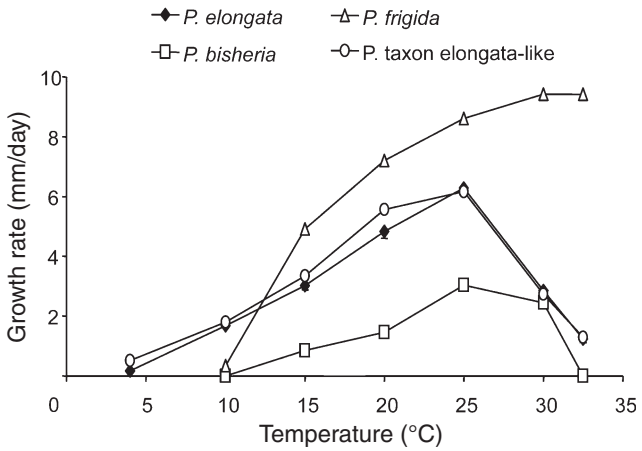

Fig. 4. Radial growth rates of *Phytophthora elongata* (means and standard errors calculated from three isolates), *P.* taxon elongata-like (VHS 15032), *P. bisheria* (VPRI 21375) and *P. frigida* (CMW 19435) on carrot agar at different temperatures.

faster (Fig. 5, Table 2). Compared with *P. elongata*, *P. frigida* had higher growth rates at optimum on CA and at 20°C on all media while *P. bisheria* was much slower growing at all temperatures and on all media (Fig. 5, Table 3).

TAXONOMY

Phytophthora elongata sp. nov. **A. Rea, M. Stukely & T. Jung, sp. nov.** Figs 6, 7.

MycoBank no.: MB 515142.

Etymology: name refers to the occurrence of elongated sporangia.

Systema sexus homothallica; oogonia globosa vel rare subglobosa ad irregularia, interdum cum basim attenuata vel petiolo longo, 31.3 ± 2.7 µm. Oosporae apleroticae vel pleroticae, 27.3 ± 3 µm, paries 2.2 ± 0.2 µm. Antheridia paragynosa, saepe cum appendicibus brevibus. Sporangia abundantia in cultura liquida, persistentia, terminalia, semi-papillata, ovoidea, obpyriformia, limoniformia vel distorta, saepe cum obturamento conspicuo basale, apex saepe elongatus, interdum praeacutus vel arcuatus, 45.0 ± 4.6 × 28.0 ± 3.1 µm (53.5 ± 8.5 × 23.4 ± 3.6 µm sporangiis elongatis), ratio longitudo ad altitudinem 1.6 ± 0.1 µm (2.3 ± 0.5 sporangiis elongatis). Germinatio directa sporangiorum saepe observatae iam intra 24 h. Sporangiophora simplicia aut ramosa sympodiis laxis irregularibus; interdum cum inflationes irregulares. Chlamydosporae non-observatae. Temperaturae crescentiae in agaro 'V8A', optima ~25°C et maxima ~32.5°C. Coloniae in agaro 'V8A' modice lente crescentes (4.2 $mm.d^{-1}$ ad 20°C), tenue radiatae cum mycelio aerio restricto. Regiones 'rDNA ITS', '*cox*I' cum unica sequentia (GenBank GQ847754, GQ847764).

Typus: Western Australia: Dwellingup, isol. ex solo rhizosphaerae arboris, *Eucalyptus marginata*, April 2004, M. Stukely, MURU 453 cultura exsiccata in agaro 'V8A' ('Herbarium of Murdoch University, Western Australia', CBS 125799 (VHS 13482) culturae vivae).

P. elongata is homothallic and readily produced oogonia in single culture on CA and V8A, containing oospores which matured within ~2 weeks. Oogonia from 5 isolates averaged 31.2 ± 3.2 µm (type isolate CBS 125799 31.3 ± 2.7 µm) (Table 2) with isolate means ranging from 30.5 to 33.4 µm. Many oogonia had a tapering base (Fig. 6*c*, *e*, *f*). In all isolates some oogonia had a very long stalk (up to 79.7 µm), which could be either thick or thin and were usually curved (Fig. 6*g–i*). Both plerotic and aplerotic oospores occurred in all isolates (percentage of plerotic oospores of isolates varied between 22 and 48%). They averaged 27.4 ± 3.3 µm diameter with isolate means ranging from 26.7 to 29.7 µm. Oospore walls were moderately thick (2.27 ± 0.36 µm) and often turned golden-brown with age (Fig. 6*b*, *d–f*, *i*). The oospore wall index was 0.42 ± 0.05 µm (Table 2), with isolate means ranging from 0.39 to 0.44 µm. Irregular hyphal swellings were formed by all isolates (Fig. 6*j*, *k*).

Antheridia were exclusively paragynous, usually club-shaped, with some having a short hyphal extension (Fig. 6*c*), and were usually attached either close to the oogonial stalk (Fig. 6*a*, *b*, *e*, *f*) or at an angle of up to 90° (Fig. 6*c*, *h*), ~12 ± 1.6 × 9.5 ± 1.4 µm. Sometimes oogonia with more than one antheridium were observed (Fig. 6*d*).

Semipapillate persistent sporangia were abundantly produced in non-sterile soil extract water in mostly lax sympodia or on simple sporangiophores. Up to 8–10 sporangia per sporangiophore were observed although there were usually fewer. Sporangial proliferation was external and often occurred immediately below the old sporangium (Fig. 7*a*, *b*, *e*, *f*, *h*, *k*). In all isolates the sporangiophore occasionally widened towards the base of the sporangium (Fig. 7*i*). Basal swellings were sometimes observed on sporangiophores. Sporangial apices were usually flat but could occasionally be pointed (Fig. 7*c*). Sporangial shapes varied widely, ranging from ovoid (Fig. 7*a*, *f*) and ovoid-obpyriform (Fig. 7*c*, *d*) to obpyriform (Fig. 7*b*). Sporangia often had special features such as curved or displaced apices (Fig. 7*d*, *h*, *j*), a large vacuole (Fig. 7*g*) or a conspicuous basal plug that protruded into the empty sporangium (Fig. 7*f*; length 2.1–8.3 µm, average 4.5 ± 1.7 µm). Normal-sized sporangia averaged 45.8 ± 6.3 µm in length and 28.4 ± 3.5 µm in width, with exit pores 7 ± 1.1 µm wide. In addition, all isolates of *P. elongata* also produced some markedly elongated sporangia with elongated obpyriform, ampuliform, limoniform or distorted shapes (Fig. 7*g–j*), which averaged 58.2 ± 12.7 µm in length and 24.5 ± 4.3 µm in breadth. The l : b ratio for normal-sized sporangia was 1.62 ± 0.15 µm, while for elongated sporangia it was 2.40 ± 0.48 µm. Direct germination of a proportion of sporangia occurred even within the first flush of sporangia after 12–24 h (Fig. 7*k*, *l*).

Apart from a generally lower sporangial l : b ratio, a larger oospore wall index and a lower variability in sporangial shapes, the morphological structures and morphometric data of isolate VHS 15032 (*P.* taxon elongata-like**)** resembled those of *P. elongata* (Table 2).

Notes: In previous studies *P. elongata* is referred to as *Phytophthora* sp. 2 (Burgess *et al*. 2009), *Phytophthora* sp. WA2 (Stukely *et al*. 2007) and *P. citricola* isozyme subgroup SG1 (Bunny 1996; Stukely *et al*. 2007). Despite many morphological similarities and a similar optimum temperature for growth (~25°C), *P. elongata* can easily be separated from

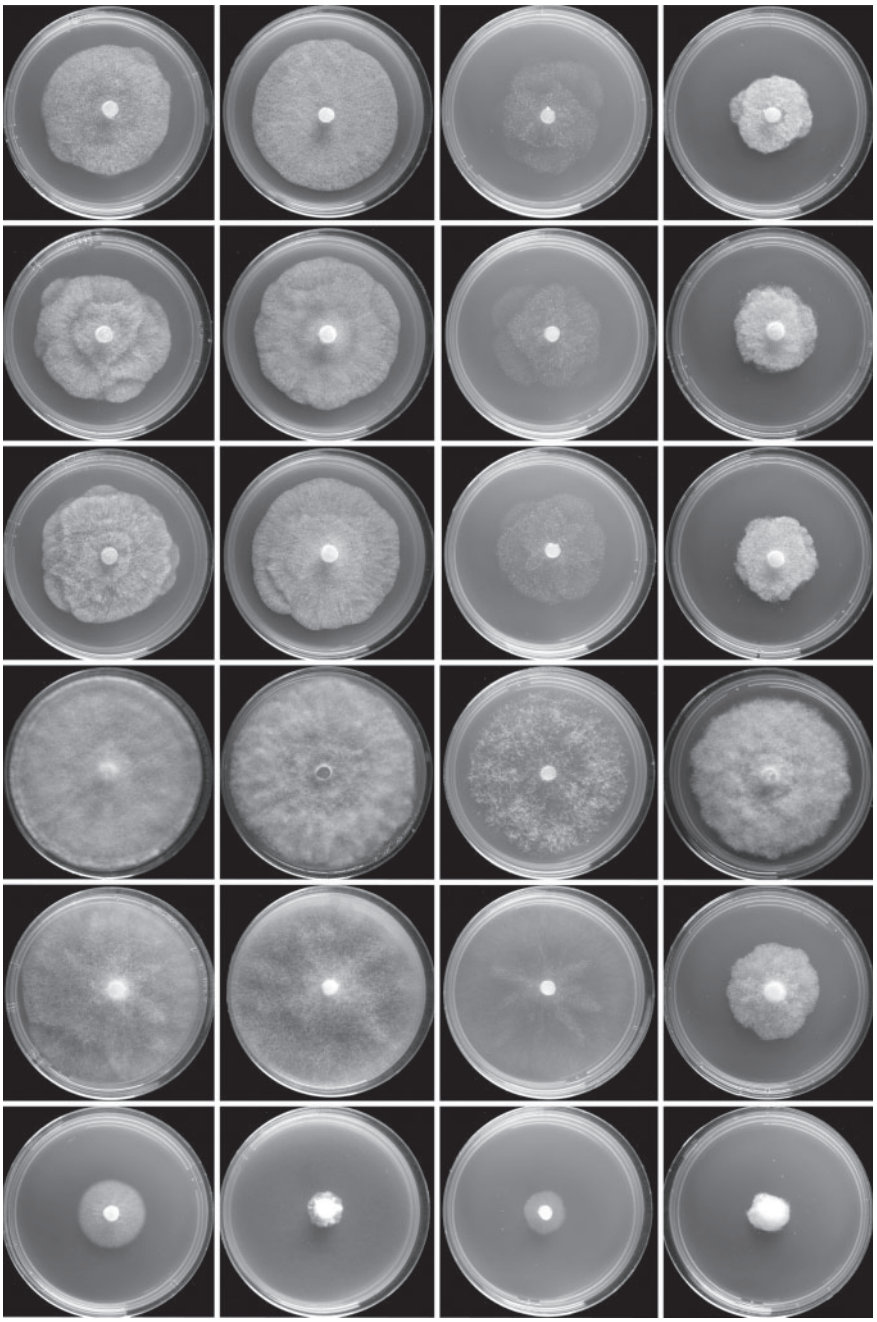

Fig. 5. Colony morphology of isolates CBS 125799 (ex-type), VHS 7134 and VHS 15078 of *Phytophthora elongata*, VHS 15032 of *P.* taxon elongata-like, CMW 19433 of *P. frigida* and VPRI 21375 of *P. bisheria* (from top to bottom) after 7 days' growth at 20°C on carrot agar, V8 agar, malt extract agar and potato dextrose agar (from left to right).

P. bisheria, *P. frigida* and *P. multivesiculata* by its unique combination of morphological characters (Table 3). Like *P. elongata*, *P. bisheria* produces both large distorted sporangia as well as ovoid-obpyriform and obpyriform sporangia, which may have a large vacuole. *P. elongata* can be distinguished from *P. bisheria* by the absence of bipapillate sporangia and globose sporangial shapes, by considerably higher growth rates (Abad *et al*. 2008), the occasional presence of long oogonial stalks in all isolates, and different colony growth patterns. *P. elongata* can be easily distinguished from *P. frigida* by the production of oogonia with paragynous antheridia in single culture, and persistent semipapillate sporangia which on average are much larger, the occurrence of elongated sometimes distorted sporangia which may have a vacuole, the occasional presence of long oogonial stalks in all isolates, the absence of chlamydospores, different colony growth

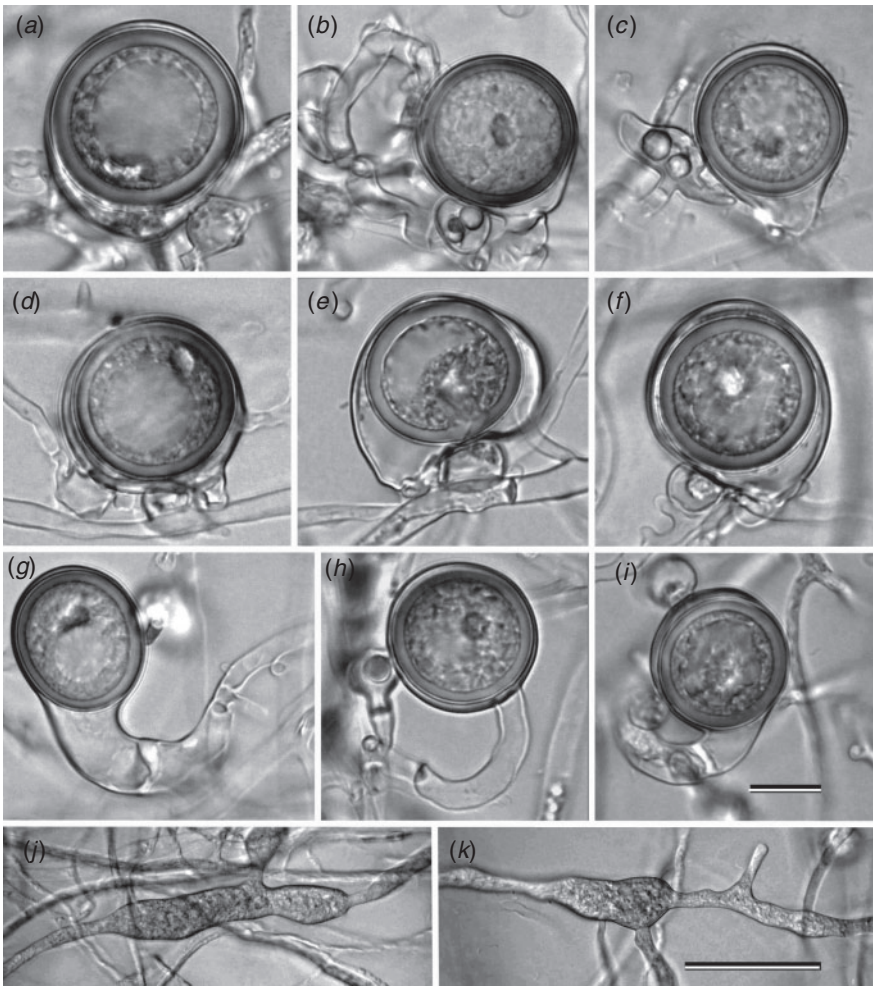

Fig. 6. Morphological structures of *Phytophthora elongata* formed on solid V8 agar. (*a–i*) Mature oogonia with oospores containing ooplasts; (*a*) slightly excentric oogonium with aplerotic oospore and paragynous antheridium; (*b*) oogonium with plerotic, slightly golden-brown oospore and paragynous antheridium; (*c*) oogonium with tapering base, almost plerotic oospore and paragynous antheridium with finger-like hyphal projections; (*d*) oogonium with aplerotic slightly golden-brown oospore and two paragynous antheridia; (*e*) excentric reniform oogonium with tapering base, markedly aplerotic, slightly golden-brown oospore and paragynous antheridium; (*f*) elongated oogonium with tapering base, markedly aplerotic, golden-brown oospore and paragynous antheridium; (*g*) oogonium with plerotic oospore and very long thick oogonial stalk; (*h*) oogonium with plerotic oospore, very long thin oogonial stalk and paragynous antheridium; (*i*) oogonium with plerotic, golden-brown oospore, very long oogonial stalk and paragynous antheridium; scale bar = 25 µm, applies to *a–i*; (*j*, *k*) irregular hyphal swellings. Scale bars = 25 µm, applies to *j*, *k*.

patterns and higher cardinal temperatures (Maseko *et al*. 2007). Similarities between *P. multivesiculata* and *P. elongata* include the production of ovoid and obpyriform semipapillate sporangia which may directly germinate and form another sporangium (Ilieva *et al*. 1998). *P. elongata* can be separated from *P. multivesiculata* by the occurrence of elongated sometimes distorted sporangia which may have a vacuole, the absence of nonpapillate or bipapillate sporangia, the occasional presence of long oogonial stalks in all isolates, the absence of amphigynous antheridia and chlamydospores, and the production of distinct colony growth patterns (Ilieva *et al*. 1998).

Although there are morphological similarities between *P. elongata*, *P. citricola* and *P. multivora*, several features enable a clear discrimination between these species. *P. elongata* does not produce sporangia with more than one papilla, and does not display the range of distorted sporangial shapes typical of *P. citricola* and produced occasionally by *P. multivora* (Scott *et al*. 2009). *P. elongata* produces markedly elongated sporangia with large vacuoles, and long oogonial stalks which are not observed in *P. citricola* and *P. multivora*. Furthermore, oospores of *P. elongata* have significantly thicker walls and a higher oospore wall index

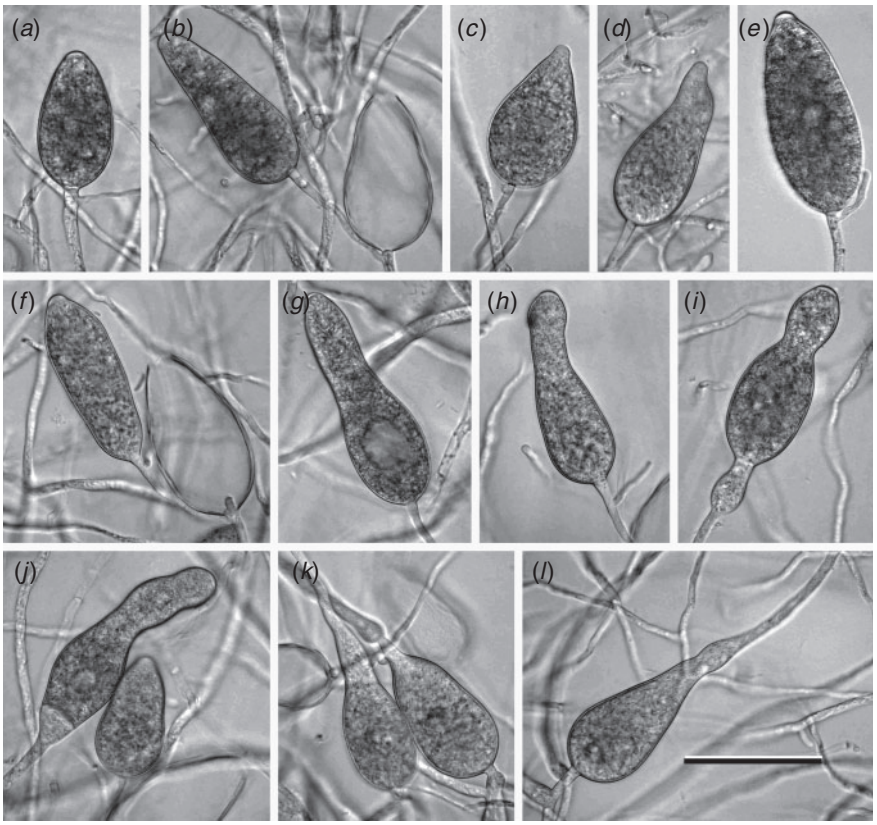

Fig. 7. Semipapillate sporangia of *Phytophthora elongata* on V8 agar flooded with soil extract. (*a*) Ovoid, with external proliferation close to the sporangial base; (*b*) sympodium with an empty obpyriform sporangium and mature elongated-obpyriform sporangium; (*c*) mature ovoid-obpyriform sporangium with a pointed apex; (*d*) elongated-ovoid with a laterally displaced pointed apex; (*e*) limoniform, with external proliferation close to the sporangial base; (*f*) empty ovoid sporangium with a conspicuous basal plug and a mature elongated-obturbinate sporangium; (*g*) elongated-obpyriform with a large vacuole (*h*) elongated-obpyriform with a curved apex; (*i*) ampuliform with a widening of the sporangiophore towards the sporangial base; (*j*) ovoid sporangium and large distorted sporangium with elongated curved apex and a tapering base; (*k*, *l*) mature sporangia germinating directly. Scale bar = 50 µm, applies to *a–l*.

than those of *P. citricola*, possibly indicative of adaptation to seasonally high temperatures and extreme droughts as encountered in the south-west of WA. This has also been discussed for *P. multivora* (Scott *et al.* 2009), which occurs widely within the same ecosystems, and for *P. quercina* in European oak stands (Jung *et al*. 2000). *P. multivora* can also be distinguished from *P. elongata* by its higher oospore wall index, the lack of excentric and elongated oogonia, the absence of long oogonial stalks and hyphal swellings typical of *P. elongata*, and the production of distinct stellate growth patterns on V8A, CMA and MEA (Stukely *et al*. 2007; Scott *et al*. 2009).

Pathogenicity

The three *P. elongata* isolates differed in their aggressiveness to *E. marginata*. While isolate VHS 6362 and *P. cinnamomi* were similar in aggressiveness killing 10 and 7.5% of the seedlings, respectively, isolate CBS 125799 and *P.* taxon elongata-like caused no mortality (Table 4).

In the *B. attenuata* trial the first plant deaths occurred before the initial flooding on day 64. Both *P. elongata* isolates (VHS 6362 and CBS 125799) showed similar aggressiveness killing 17.5 and 20% of the seedlings, respectively (Table 4). Both isolates caused deaths in 5 of 10 pots and did not require a flooding stimulus since first deaths occurred after 34 days. As expected, *P. cinnamomi* killed all *B. attenuata* seedlings with the first and the last plant dying after 20 and 110 days, respectively.

In both trials *P. cinnamomi* and *P. elongata* were recovered from the roots and collars of all inoculated dead plants. In addition, the ability of both *P. elongata* and *P.* taxon elongata-like VHS 15032 to persist in the pine plug inoculum used in the *E. marginata* pathogenicity trial was assessed by surface sterilising recovered plugs and plating them on NARPH agar plates 20 months after inoculation. For each isolate one pot was chosen at random and all plugs, at varying depths, were removed. All isolates were reisolated from all inoculated plugs.

Table 2. Morphological characters and dimensions (μm) and temperature-growth relations of *Phytophthora elongata*, *Phytophthora* taxon 'elongata-like', *P. bisheria*, *P. frigida* and *P. multivesiculata*

	P. elongata	*P.* taxon 'elongata-like'	*P. bisheria*	*P. frigida*	*P. multivesiculata*
No. of isolates studied/ additional sources	5[A]	1	1[B] Abad *et al.* (2008)	1[C] Maseko *et al.* (2007)	Ilieva *et al.* (1998)
Sporangia	Semipapillate	Semipapillate	Semipapillate	Papillate	Semi- or nonpapillate
Caducity	No	No	No	Yes	No
Normal-sized sporangia					
l × b mean	45.8 ± 6.3 × 28.4 ± 3.5	50.5 ± 5.7 × 32.8 ± 3.6	34 × 27 (ovoid) 49 × 30 (obpyriform) (62.3 ± 17.1 × 37.2 ± 4.8)	33 × 27 (37.4 ± 6 × 27.3 ± 4.4)	45 × 33
Range of isolate means	44.9–49.4 × 27.7–30.2	n.a.[D]	n.a.	31–34 × 26–28	n.a.
Total range	26–76 × 19–42	35–67 × 21–42	26–72 × 21–36	24–40 × 20–33	30–60 × 20–41
l : b ratio	1.62 ± 0.15	1.54 ± 0.15	1.26 (ovoid) 1.63 (obpyriform)	1.22 (1.38 ± 0.16)	1.43 (1.1–1.8)
Elongated sporangia					
l × b mean	58.2 ± 12.7 × 24.5 ± 4.3	57.7 ± 7.2 × 25.3 ± 3.7	81 × 29 (79.8 ± 24 × 35.4 ± 4.6)	–	–
Range of isolate means	51.5–78.8 × 23.2–31.2	n.a.	n.a.	–	–
Total range	34–90 × 16–40	37–75 × 18–35	72–90 × 26–32	–	–
l : b ratio	2.40 ± 0.48	2.30 ± 0.26	2.79	–	–
Oogonia/breeding system	Homothallic	Homothallic	Homothallic	Heterothallic	Homothallic
Mean diam.	31.2 ± 3.2	30.7 ± 2.4	35 (35.3 ± 3.8)	33	41
Diam. range	20.9–41.5	25.3–38.5	24–46 (26–43)	25–42	28–50
Range of isolate means	30.5–33.4	n.a.	n.a.	31–34	n.a.
Long oogonial stalks	Yes	No	No	No	No
Oospores	Plerotic and aplerotic	Mainly aplerotic	Mainly aplerotic	Aplerotic	Mainly aplerotic
Plerotic oospores	22–48%	Occasionally	18%	n.a.	Rare
Mean diam.	27.4 ± 3.3	27.13 ± 2.2	28 (31.8 ± 3.3)	28	33
Diam. range	17.9–37.8	22.7–35.2	25–31 (24–39.9)	19–38	24–42
Wall diam.	2.27 ± 0.36	2.61 ± 0.30	5 (2.45 ± 0.59)	n.a.	3–4
Oospore wall index	0.42 ± 0.05	0.47 ± 0.05	(0.39 ± 0.07)	n.a.	n.a.
Antheridia	Paragynous	Paragynous	Paragynous	Amphigynous	Amphigynous (95%)
l × b mean	12.0 ± 1.6 × 9.5 ± 1.4	11.5 ± 1.4 × 9.3 ± 1.2	12.9 ± 1.9 × 8.7 ± 1.3	n.a.	n.a.
l × b range	8.6–18 × 6.2–15.6	9.0–14.9 × 6.8–12.1	8–20 × 5–14	n.a.	n.a.
Chlamydospores	No	No	No	Yes	Yes
Hyphal swellings	Yes	Yes	No	Yes	Yes
Maximum temperature (°C)	32.5	32.5	32.5	30 (32.5)	35
Optimum temperature (°C)	25	25	26	25 (30)	n.a.
Growth rate on carrot agar at optimum (mm/day)	6.29 ± 0.2	6.16	3.03 ± 0.06	4.80 (9.42)	6.8 ± 0.6
Growth rate at 20°C (mm/day)					
Carrot agar	4.83	5.56	(1.22)	4.8 (6.97)	6.8 ± 0.6
V8 agar	4.24	5.88	3.7–4.9 (2.08)	5 (6.08)	7.6 ± 0.35
Malt extract agar	3.59	5.23	(1.25)	4 (5.9)	n.a.
Potato dextrose agar	2.84	5.22	(1.33)	3 (3.13)	5.5 ± 0.31

[A]Three of the five isolates of *P. elongata* were included in the growth tests.
[B]Data in brackets are from measurements of isolate VPRI 21375 in this study.
[C]Data in brackets are from measurements of isolate CMW 19435 in this study.
[D]n.a. = not available.

Table 3. Morphological and physiological characters discriminating *Phytophthora elongata* from *P. bisheria*, *P. frigida*, *P. multivesiculata* and *P. multivora*

	Characters discriminating from *P. elongata*[A]			
	P. bisheria	*P. frigida*	*P. multivesiculata*[B]	*P. multivora*[C]
Sporangia	Occasional production of bipapillate sporangia	Papillate, caducous, on av. markedly smaller, absence of markedly elongated sporangia, production of bipapillate sporangia	Partly nonpapillate, absence of markedly elongated sporangia and of vacuoles, production of bipapillate sporangia, occurrence of internal proliferation	Absence of markedly elongated sporangia and of vacuoles, occasional production of bipapillate sporangia
L : b ratio	–	Significantly lower	Slightly lower	–
Oogonia, oospores, breeding system	Absence of long oogonial stalks	Absence of excentric and elongated oogonia long oogonial stalks, heterothallic	Absence of excentric and elongated oogonia and long oogonial stalks, on av. markedly larger	Absence of excentric and elongated oogonia and long oogonial stalks, on av. smaller with thicker oospore walls and higher oospore wall index, germination of most oospores after 4 weeks at 20°C
Antheridia	–	Amphigynous	Mostly amphigynous	–
Chlamydospores	–	Present	Present	–
Hyphal swellings	–	–	Also formed on solid agar	No hyphal swellings formed
Colony growth patterns different from *P. elongata* on	V8A, CA, MEA, PDA	V8A, CA, MEA	V8A, CA, MEA, PDA (no growth patterns formed)	V8A, CA, CMA, MEA, PDA
Maximum temp.	–	Slightly higher	Higher	Slightly lower
Growth rate at 20°C on				
Carrot agar (CA)	Significantly slower	Significantly faster	Faster	n.a.
V8 agar (V8A)	Significantly slower	Significantly faster	Faster	Slightly slower
Malt extract agar (MEA)	Significantly slower	Significantly faster	n.a.[D]	Faster
Potato dextrose agar (PDA)	Significantly slower	Slightly faster	Faster	Slightly faster

[A]For morphometric and growth-temperature data see Table 3.
[B]Data from Ilieva *et al*. (1998).
[C]Data from Scott *et al*. (2009).
[D]n.a. = not available.

Table 4. Mortality of *Eucalyptus marginata* and *Banksia attenuata* seedlings caused by *Phytophthora cinnamomi*, *P. elongata* and *Phytophthora* taxon 'elongata-like' in two separate soil infestation pot trials

Phytophthora taxa	Isolates	Mortality (%)	
		E. marginata[A]	*B. attenuata*[B]
P. elongata	VHS 6362	10.0	17.5
	CBS 125799	0	20.0
	VHS 13615	2.5	n.t.[C]
Average of isolates		4.2	18.75
P. taxon 'elongata-like'	VHS 15032	0	n.t.
P. cinnamomi	MP 9448	7.5	100.0

[A]Trial finished after 175 days.
[B]Trial finished after 185 days.
[C]n.t. = not tested.

Discussion

P. elongata is a homothallic species mainly isolated from necrotic collars and roots and rhizosphere soil of dead and dying *E. marginata* saplings and trees in both restored mine pits and unmined but pre-logged native forests in WA. Infrequently, it has also been found associated with dead and dying *C. calophylla* (only in restored pits; G. Hardy unpubl. data) and *B. grandis*, as well as with the jarrah forest understorey plants *D. squarrosa*, an *Andersonia* sp., *L. propinquus* and the monocotyledonous *P. xanthina*, *X. preissii* and *X. gracilis* (Burgess *et al*. 2009; M. Stukely unpubl. data). Its known distribution is limited to lateritic soils in the northern and southern jarrah forest of south-west WA, and from sandy loam and clay soils in Victoria (W. Dunstan, pers. comm.). Isolations of *P. elongata* date as far back as the mid 1980s but most recoveries have occurred since 2003, with a frequency spike in the summer of 2004–05 from the Dwellingup area (Stukely *et al*. 2007). As with the recently described *P. multivora* (Scott *et al*. 2009), it was previously identified as *P. citricola* based solely on morphological and physiological characters. Phylogenetic analyses of both the nuclear ITS rDNA and mitochondrial *cox*1 gene in this study show *P. elongata* to be a unique species residing in ITS clade 2 of Cooke *et al*. (2000).

Worldwide, a large number of *Phytophthora* isolates have been classified as *P. citricola* despite displaying morphological variation (Balci and Halmschlager 2003; Jung *et al*. 2005) and several approaches have been used in attempts to unravel the *P. citricola* complex. Five distinct subgroups were recognised in an isozyme analysis of a global collection of 125 isolates of *P. citricola* (Oudemans *et al*. 1994). Studies by Gallegly and Hong (2008) and Kong *et al*. (2003) using single-strand conformation polymorphism fingerprinting techniques divided *P. citricola* into four different subgroups. More recently Jung and Burgess (2009) reevaluated European and North American isolates from within the *P. citricola* complex by sequencing the ITS, *cox*1 and β-tubulin gene regions. The complex could be divided into five clades corresponding to *P. citricola* s. str. (to which the type isolates collected by Sawada in Taiwan and Japan belong), *P. multivora*, *P. citricola* E, *P. plurivora* (= *P. citricola* II) and *P. citricola* I. Though more distantly related *P. elongata* represents another species previously classified as *P. citricola* on morphological criteria that has been elucidated as a distinct species by DNA sequencing.

Morphologically, the species most similar to *P. elongata* are *P. citricola*, *P. multivora* and *P. plurivora*, while phylogenetically *P. bisheria*, *P. frigida*, and *P. multivesiculata* are the most closely related species. *P. elongata* differs from *P. bisheria* by 34 and 18 bp in the ITS region and *cox*1 sequence, respectively. *P. frigida* and *P. multivesiculata* are more distantly related, differing by 52 and 45 bp, respectively, in the ITS region and 22 and 27 bp in the *cox*1 sequence. *P. elongata* can be clearly distinguished from these species by a range of morphological and physiological characters (Tables 2 and 3).

P. multivora displays considerable variation in the *cox*1 sequence suggestive of endemism or an introduction into WA soon after human settlement or a non-clonal introduction on several occasions (Scott *et al*. 2009). In contrast, the *cox*1 sequences of all *P. elongata* isolates tested were identical. Similarly, in the isozyme analysis of *P. citricola* SG1, now known to be *P. elongata*, the 52 assessed isolates were genetically indistinguishable and homozygous across 10 loci (Bunny 1996). This low diversity could be evidence for a recent clonal introduction of *P. elongata* into WA. *P. bisheria*, the species most closely related to *P. elongata*, which was only recorded from nursery stock in North America, Australia and the Netherlands (Abad *et al*. 2008), was recently found in undisturbed native *Chamaecyparis* forests in Taiwan (Brasier *et al.* 2010). Therefore, it is likely that both *P. bisheria* and *P. elongata* are native to forests in south-east Asia and like so many other *Phytophthora* species were spread to other continents via the international nursery trade. *P. elongata* may have been introduced to the jarrah forest with infested nursery stock used for site rehabilitation or with infested gravel used for road building in a similar fashion to *P. cinnamomi* (Shearer and Tippett 1989). *P. frigida* was isolated from exotic eucalypt plantations in South Africa (Maseko *et al*. 2007). The origin of this species is currently unknown.

To date ~150 isolates of *P. elongata* have been identified morphologically within the VHS collection, of which 30 have been sequenced. The ITS sequence of all these isolates is identical except for one isolate (VHS 15032) which differs from *P. elongata* in the ITS sequence by 3 bp, and in the *cox*1 sequence by 6 bp. Its colony morphology is also distinct from *P. elongata*, and it has a slightly higher growth rate at 20°C. Morphologically, this isolate is very similar to *P. elongata*, but it does not display the same range of sporangial shapes with most being ovoid. It also has a lower sporangial l : b ratio and a larger oospore wall index than *P. elongata*. Although it appears from the ITS and *cox*1 data that the WA population of *P. elongata* has been recently introduced, it is interesting that this closely related isolate occurs in the same ecosystem. It is sufficiently distinct that a separate species description may be warranted if more isolates were available. Meanwhile, this taxon is informally designated as *P.* taxon elongata-like.

In the soil infestation tests of this study, *P. elongata* showed similar aggressiveness to *E. marginata* as *P. cinnamomi* and was reisolated from the roots and lower stem of dead plants. In a previous study (Bunny 1996) under the name *P. citricola* SG1, *P. elongata* (isolates 2952 and NX22) was found to be more aggressive than *P. cinnamomi* to *E. marginata* as assessed by lesion length following under-bark inoculation (47–77 versus 42–49 mm after 11 days). *P. elongata* also proved to be pathogenic towards *B. attenuata* in the soil infestation trial of the present study. Like *P. cinnamomi*, *P. elongata* did not require a flooding stimulus to initiate infection and cause death of *B. attenuata* although flooding did substantially increase mortality. Though *B. attenuata* does occur to some extent in the jarrah forest, *P. elongata* has not yet been isolated from this host in the field. *B. attenuata* is a significant structural and floristic component of the Banksia woodland and sandplain/Kwongan floristic communities of the Swan Coastal Plain (Beard 1984; Dodd and Griffin 1989). Thus, *P. elongata* could have a detrimental impact on these plant communities if it were to spread beyond its current range, which at present is restricted to the lateritic soils of the jarrah forest in the Darling Range and further south.

Two new species, *P. elongata* and *P. multivora*, previously incorrectly assigned to *P. citricola* based on morphology alone, have now been shown to be present in natural ecosystems in WA for at least 30 years. However, *P. elongata* appears to be more limited in its distribution and impact than the recently described species *P. multivora*. Of the isolates sequenced so far from the VHS collection, 170 isolates have been found to be *P. multivora* while 30 isolates are *P. elongata* (M. Stukely, unpubl. data). In contrast to *P. multivora*, which has been found associated with many hosts over a wide geographical range, *P. elongata* is predominantly associated with *E. marginata* in the Darling Range south-east of Perth. However, *P. elongata* may have a wide host range as it has also been found in association with plants from the families Proteaceae, Myrtaceae, Epacridaceae, Xanthorrhoeaceae and Iridaceae. More pathogenicity tests on a wider range of native plant species are needed to assess the host range of *P. elongata* and its invasive potential in WA and other parts of Australia.

To date, research in WA has concentrated on impact of *P. cinnamomi* and is just embarking on studies to formally describe the multitude of new *Phytophthora* taxa in WA and understand their contribution to *Phytophthora*-driven declines in the region.

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