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## *Phytophthora acaciivora* sp. nov. associated with dying *Acacia mangium* in Vietnam

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forest health

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*Phytophthora acaciae*

*Phytophthora frigida*

plantation forestry

**Abstract:** *Acacia mangium* plantations account for more than 50 % of the exotic plantations in Vietnam. A new black butt symptom was discovered in 2012, followed by the wilting sign in *Acacia* seedlings in Tuyen Quang Province. Isolations recovered two *Phytophthora* species, the well-known *Acacia* pathogen *P. cinnamomi*, and an unknown species. The new species is described here as *Phytophthora acaciivora* sp. nov. Phylogenetically this species resides in clade 2d and is most closely related to *P. frigida*. *Phytophthora acaciivora* is a heterothallic species, oospores are aplerotic and antheridia are amphigynous. It produces predominantly elongated ovoid, semi papillate, persistent sporangia, no hyphal swellings and no chlamydospores. Optimum temperature for the growth is 25–30 °C and the maximum temperature is over 37.5 °C. Studies are underway to determine the impact of this new species on *Acacia* plantations in Vietnam.

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## INTRODUCTION

*Acacia mangium* is a tree in the family *Fabaceae*, native to Papua, western Irian Jaya and the Maluku islands in Indonesia, Papua New Guinea and north-eastern Queensland in Australia (Hegde *et al.* 2013). The wood from this tree can be used for furniture, cabinets, floors, plywood, firewood, charcoal, and pulpwood. Compared with other species, *A. mangium* is fast-growing, tolerant of nutrient-poor soils and is adapted to a wide range of acidic soils in moist tropical lowlands (Hegde *et al.* 2013). As a consequence of these properties, in the last few decades plantation areas in the humid tropics of Asia have increased dramatically.

*Acacia* spp. were introduced in the 1960s into southern Vietnam and in early 1980s in the northern part of the country (Nambiar *et al.* 2015). By 2014, Vietnam had over 1 M ha of *Acacia* spp., and *A. mangium* accounted for around 600 000 ha (Harwood & Nambiar 2014). In addition, *A. mangium* is playing an increasingly important role in efforts to sustain a commercial supply of tree products, whilst reducing pressure on native forests, and has been planted extensively all over the country. In Vietnam, *A. mangium* has a similar growth rate and wood quality to *Eucalyptus* and *Pinus* species, but there is also a huge market for their wood products, making this the preferred plantation species in the region.

Vietnam is located in the tropics, where temperature and humidity are optimum for the growth of *A. mangium*, but these

conditions create a perfect environment for the development of many diseases. Notably, 48 diseases have been investigated from *A. mangium* plantations and nurseries in five regions in Vietnam (Pham 2016). Among those, heart rot caused by *Ganoderma* spp., wilt caused by *Ceratocystis manginecans* and pink disease caused *Corticium salmonicolor* have a significant impact on yield. In 2013, *Phytophthora cinnamomi* was first detected from soil baiting under declining *A. mangium* plantations in Tuyen Quang province (Pham *et al.* 2013). Subsequently, in the same province, in a nursery survey we found another *Phytophthora* sp. causing root rot and wilting (Fig. 1). The disease level was around 30 % in the nursery. This species is characterised in this study and described as *Phytophthora acaciivora* sp. nov.

## MATERIALS AND METHODS

### Sampling and isolation

Soil samples were collected from *A. mangium* seedling pots showing wilting and root rot symptoms in nurseries. All samples were placed in a sterile zip bags placed in a cool box to protect them from high temperatures and direct sunlight and carried to the laboratory for further examination and isolation.

After washing, diseased roots were surface-sterilized (by dipping diseased roots in 70 % ethanol or 1 % bleach for 30–60 s, washed twice in sterile water) and blotted dry on paper



**Fig. 1.** Symptoms of *Phytophthora* root rot of *Acacia mangium* seedlings in the nursery. Symptoms include: **A–C.** Root and collar root leading to patchy seedling death and **D.** Leaf discoloration.

towels. Then, roots were cut into 5–6 mm pieces and placed onto *Phytophthora* selective medium, NARPH (Hüberli *et al.* 2000). The plates were then placed in the dark at room temperature. Soil baiting was conducted using locally available bait leaves, *Castanopsis* spp., *Castanea* spp., *Lithocarpus* spp., *Acacia mangium* and *Rosa* sp. petals. All isolates were maintained in 90 mm Petri dishes on V8 agar (V8A, 0.1 L filtered V8 juice, 17 g agar, 0.1 g CaCO<sub>3</sub>, 0.9 L distilled water) and on 5 mm V8A discs stored in 20 mL sterile water in McCartney bottles at room temperature.

#### DNA extraction, amplification and sequencing

Representative isolates were cultured on half-strength potato dextrose agar (PDA) (Becton, Dickinson and Company, Sparks, USA, 19.5 g PDA, 7.5 g agar and 1 L of distilled water) at 20 °C for 2 wk. Mycelia were collected by scraping from the agar surface with a sterile blade and placed in a 1.5 mL sterile Eppendorf® tube. It was frozen in liquid nitrogen and crushed to a fine powder, and genomic DNA was extracted using ZR Fungal/Bacterial DNA Miniprep™ (Zymo Research, Irvine, California, USA). For all isolates, 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 contained 12.5 µL GoTaq® Green Master Mix 2× (Promega Corporation, Madison, Wisconsin, USA), 0.5 µL of each primer, 10 µL water and 1.5 µL of DNA. PCR conditions were 2 min at 94 °C, 30 cycles of 30 s at 94 °C, 45 s at 55 °C and 1 min at 72 °C with a final extension of 5 min at 72 °C.

For isolates of new species additional gene regions were amplified and sequenced using the PCR conditions described by the original authors. The mitochondrial gene *cox1* was amplified with FM77 and FM84 and *cox2* was amplified with primers FM35 and FMPhy-10b (Martin & Tooley 2003). NADH dehydrogenase subunit 1 (*nadH1*) was amplified with NADH-F1 and NADH-R1 and β-tubulin was amplified with BTF1A and BTR1 primers (Kroon *et al.* 2004) (Kroon *et al.* 2004). Heat shock protein 90 (*hsp90*) was amplified with HSP90-F int and HSP90-R1 primers (Blair *et al.* 2008).

All gene regions were sequenced in both directions with primers used in amplification. PCR and sequencing products were cleaned using Sephadex® G-50 columns as described previously (Sakalidis *et al.* 2011). All sequences derived in this

study were added to GenBank and accession numbers are provided in Table 1.

#### Phylogenetic analysis

The data set consisted of sequences of the *P. acaciivora* sp. nov. and closely related species in ITS clade 2d (<https://idtools.org/id/phytophthora/>) were either sequenced for this study or taken from GenBank (<http://ncbi.nlm.nih.gov/>) (Table 1). Nuclear and mitochondrial genes were analysed separately. Sequence data were compiled and edited in Geneious Prime® (Biomatters; available from <http://www.geneious.com>). Bayesian analysis with MrBayes v. 3.2.6 (Ronquist *et al.* 2011) and Maximum Likelihood analysis using RAxML v. 8 (Stamatakis 2014) were performed using plugins within the Geneious software. Data has been submitted to TreeBASE (<https://www.treebase.org/>) SN26126.

#### Colony morphology, growth rates, cardinal temperatures

Circular inoculum plugs (5 mm diam) were taken from the margin of 7-d-old cultures on V8A and placed in the centre of 90 mm Petri dishes of the test media. Morphology of hyphal and colony growth patterns were defined from 7-d-old cultures grown at 20 °C in the dark on V8A, 2 % malt extract agar (MEA; BBL, Becton, Dickinson & Co, Sparks MD 21152 USA), carrot agar (CA) (100 mL of filtered carrot juice, 17 g agar and 900 mL of distilled water) and half-strength potato dextrose agar (PDA; BBL, Becton, Dickinson & Co, Sparks MD 21152 USA). Colony morphology was described according to Erwin & Ribeiro (1996). Radial growth rate was measured 5–7 d after the onset of linear growth, along two lines crossing the middle of the inoculum plug at right angles, and the mean growth rates (mm/d) were assessed. For temperature growth studies, all isolates were sub-cultured onto V8A plates and incubated for 24 h at 20 °C for growth stimulation. The plates were then moved to incubators fixed at 4, 10, 15, 20, 25, 30, 32.5, 35 and 37.5 °C. Radial growth rates were determined by the aforementioned method, after 5 d.

#### Morphology of sporangia and gametangia

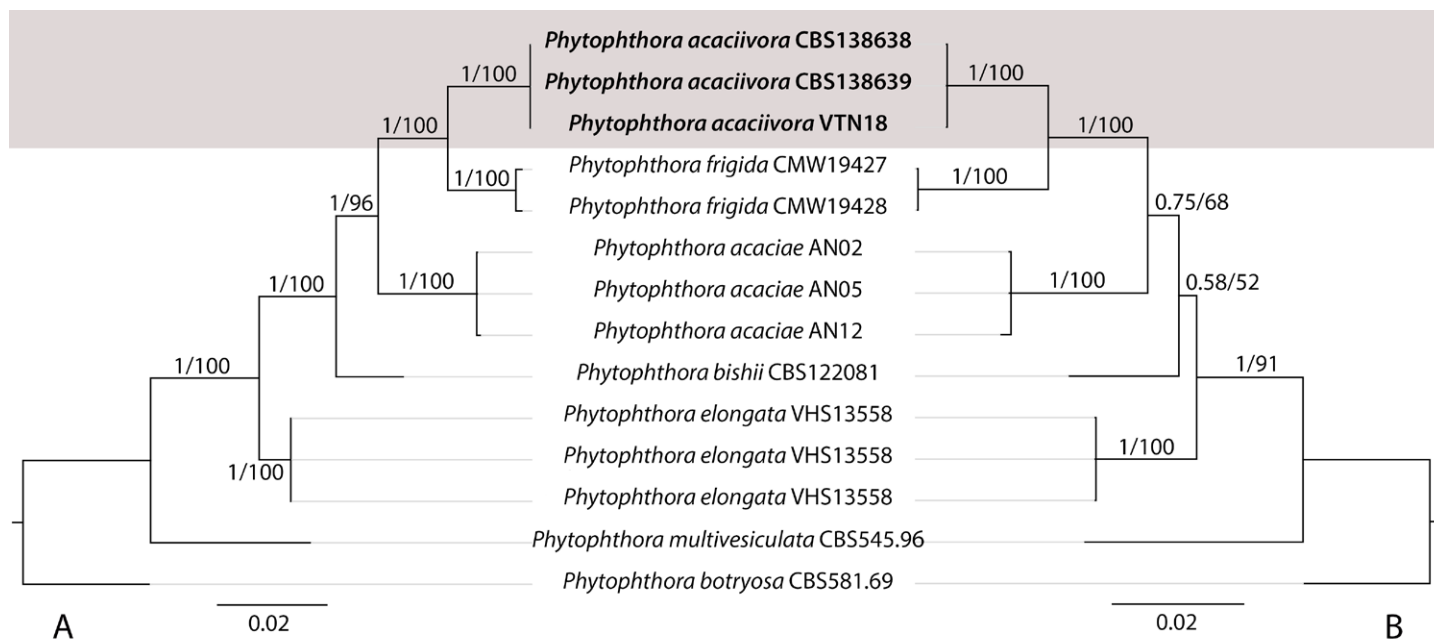
Sporangia were produced by flooding 15 × 15 mm square agar discs, removed from the growing edge of 3–5-d-old colonies on V8A in 90 mm Petri dishes, with sterile water at 18–25 °C

**Table 1.** Identity, host information, collection location, date, and GenBank accession numbers for *Phytophthora* spp. considered in this study.

Identity	Code <sup>1,2</sup>	Host	Year	Location	GenBank Accession Numbers					
					ITS	$\beta$ -tubulin	<i>hsp90</i>	<i>cox1</i>	<i>cox2</i>	<i>nadh1</i>
<i>Phytophthora acaciivora</i>	CBS 138638	<i>Acacia mangium</i>	2014	Vietnam, Tuyen Quang	KX011263	MN991983	KX011238	MN991990	KX011228	KX011286
	CBS 138639 (ET)	<i>A. mangium</i>	2014	Vietnam, Tuyen Quang	KX011264	MN991984	KX011239	MN991991	KX011229	KX011287
	VTN18	<i>Melia azedarach</i>	2014	Vietnam, Tuyen Quang	KX011265	n/d	MN991995	n/d	KX011230	KX011288
<i>P. acaciae</i>	AN02 (ET)	<i>Acacia mearnsii</i>	1999	Brazil, RS, Triunfo	KX396303	KX396338	n/a	KX396267	KX396279	n/a
	AN05	<i>A. mearnsii</i>	1999	Brazil, RS, Triunfo	KX396304	KX396339	n/a	KX396268	KX396280	n/a
	AN12	<i>A. mearnsii</i>	1999	Brazil, RS, Triunfo	KX396305	KX396340	n/a	KX396269	KX396281	n/a
	CBS 122081 (ET) P10117	<i>Fragaria ananassa</i>	1999	USA, North Carolina, Wake	MG783381	MN991985	KX011243	MH136851	GU221940	KX011289
<i>P. elongata</i>	CBS 125799 (ET) VHS13482 P19596	<i>Eucalyptus marginata</i>	2004	Australia, WA, Dwellingup	MG865485	MH493932	MK020301	MH136881	KX011232	KX011290
<i>P. frigida</i>	VHS 13558	<i>E. marginata</i>	2004	Australia, WA, Dwellingup	KX011268	MN991987	MN991997	MH136881	KX011234	KX011292
	VHS 13483	<i>E. marginata</i>	2004	Australia, WA, Dwellingup	KX011267	MN991986	MN991996	MN991992	KX011233	KX011291
	CMW20311 (ET)	<i>E. smithii</i>	2002	South Africa, KZN, Ixopo	MG865496	DQ988221	n/a	MH136892	n/a	n/a
	CMW19427	<i>Eucalyptus smithii</i>	2002	South Africa, KZN, Bloemendal	KX011269	MN991988	KX011241	MN991993	KX011235	KX011293
<i>P. multivesiculata</i>	CMW19428	<i>Acacia decurrens</i>	2002	South Africa, KZN, Seven Oaks	KX011270	MN991989	MN991998	MN991994	KX011236	KX011294
	CBS 545.96 (ET) P10410	<i>Cymbidium</i> sp.	1996	Netherlands, Mijdrecht	MG865544	MH493982	KX011247	MH136937	KX011237	KX011295
<i>P. botryosa</i>	CBS 581.69 (ET) P3425	<i>Hevea brasiliensis</i>	1966	Malaysia, Perlis	MK496516	MH493910	KX250541	MH136855	JN618604	AY563993

<sup>1</sup> ET = ex-type culture.<sup>2</sup> Abbreviations of isolate in culture collections (where known): CBS = Culture collection of the Westerdijk Fungal Biodiversity Institute, the Netherlands; P = isolate codes from World Phytophthora Collection, University of California, Riverside; VHS = Vegetation Health Service, Department of Biosecurity, Conservation and Attractions, Perth, Western Australia; CMW = culture collection of Forestry and Agriculture Biotechnology Institute, University of Pretoria, South Africa.





**Fig. 2.** Bayesian trees produced from concatenated **A.** ITS, *hsp90* and  $\beta$ -tubulin, and **B.** *cox1*, *cox2* and *nadh1* genes regions using GTR + I model showing the phylogenetic position of *Phytophthora acaciivora* and related species. Maximum likelihood was conducted on the same dataset with RAxML and resulted in the same topology. Numbers above the branches reflect support obtained from the analysis of the same dataset (Bayesian posterior probabilities/Bootstrap values estimated by RAxML). *Phytophthora botryosa* was used as an outgroup taxon. The scale bar corresponds to substitutions per nucleotide site.

with their surfaces submerged, in natural daylight. This water was decanted and replaced twice (after 4 and 6 h). In the final change, 1 mL of non-sterile soil extract was also added, and the Petri dishes were incubated overnight. The soil extract was made by suspending 10 g of rhizosphere soil from beneath a *Quercus* sp. in 100 mL distilled water and incubating this on an orbital shaker for 12 h at 20 °C before filtering through Whatman no. 1 paper to remove soil particles. After 18–36 h, dimensions and characteristic features of 50 mature sporangia of each isolate, selected at random, were ascertained at 400 × magnification (BX51 Olympus).

Isolates of *P. acaciivora* failed to produce oospores in single culture and were paired on V8A with isolates of the same species, with A1 and A2 tester strains of *P. cambivora* (MP45, MP73), *P. cinnamomi* (MP75, DCE60) and *P. cryptogea* (MP21, MP22) (tester strains available from Phytophthora Science and Management culture collection, Murdoch University, Perth, Western Australia). Inoculum plugs (5 mm diam) of the isolate to be tested and the tester isolate were placed on opposite sides of a 90 mm Petri dish, 1 cm from the edge. The plates were incubated at 20 °C in darkness and scored for oogonial formation 21 d after the two colonies had met. For each isolate producing oogonia, dimensions and characteristic features of 50 randomly selected mature oogonia, oospores and antheridia chosen at random were measured at 400× magnification. The oospore wall index was calculated as the ratio between the volume of the oospore wall and the volume of the whole oospore (Dick 1990).

## RESULTS

### Phylogenetic analysis

Tree topologies using Bayesian and Maximum Likelihood analysis were identical. Tree topologies of individual genes were congruent and in all cases the new species resided in a highly supported terminal clade most closely related to *P. frigida* (Fig S1). In a phylogeny of concatenated nuclear gene regions (Fig. 2A) and concatenated mitochondrial genes (Fig. 2B), isolates of the new species recovered from Vietnam reside in a strongly supported terminal clade. It is most closely related to *P. frigida* (97.2 % similarity across the gene regions examined), followed by *P. acaciae* and *P. bishii* (95.9 %), *P. elongata* (95.6 %) and *P. multivesiculata* (93.5 %). Together these species reside in *Phytophthora* clade 2d.

## TAXONOMY

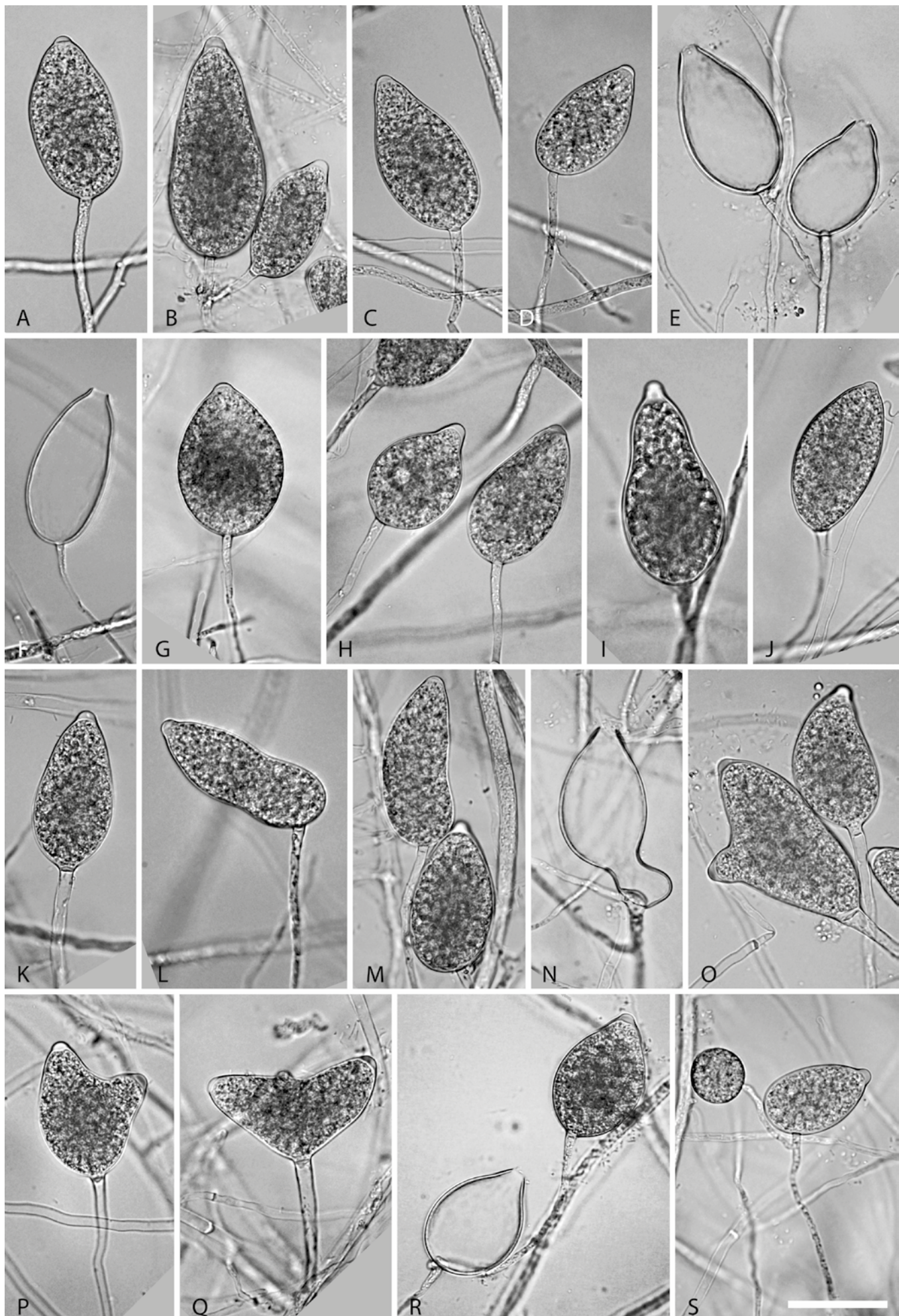
***Phytophthora acaciivora*** T.Q. Pham, T.I. Burgess and Q.N. Dang, *sp. nov.* MycoBank MB834471. Figs 3, 4.

*Etymology:* Named after its host, *Acacia mangium*.

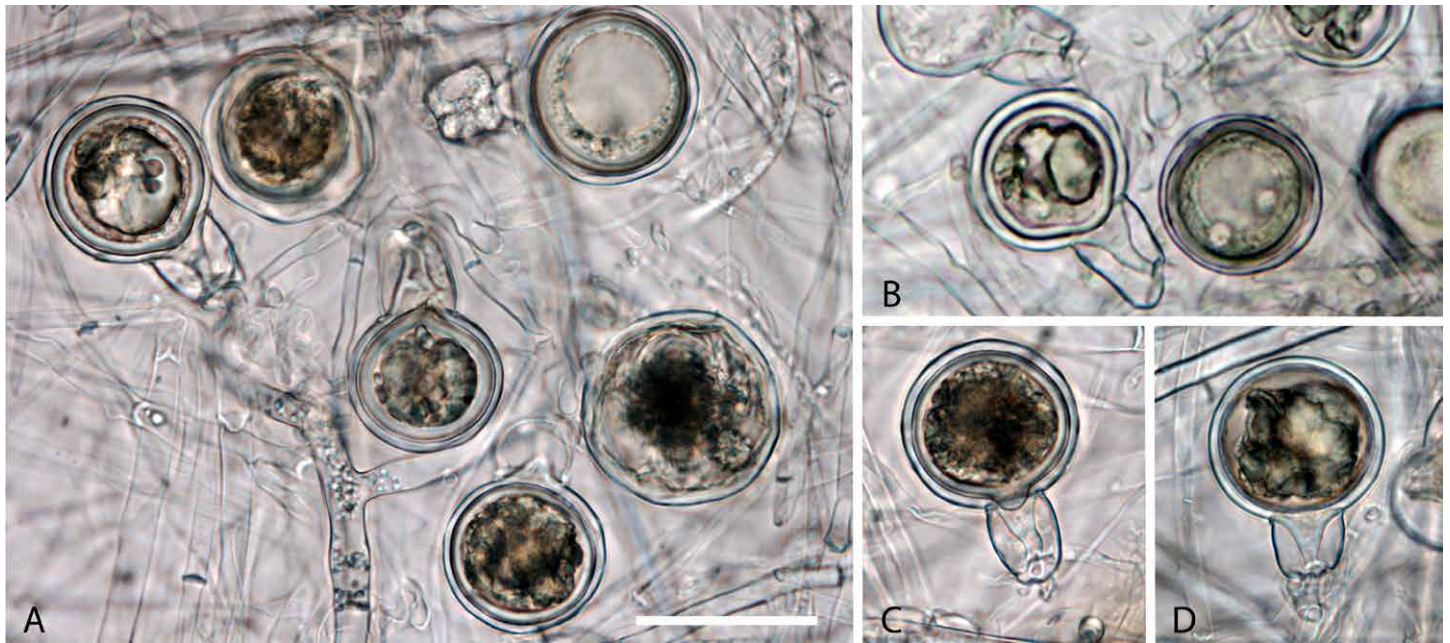
*Typus:* **Vietnam**, Tuyen Quang, from dying *Acacia mangium* seedlings, Mar. 2012, T.Q. Pham (**holotype** MURU 480, cultures ex-type CBS 138638 = AMTQ1; ITS,  $\beta$ -tubulin, *HSP90*, *cox1*, *coxII* and *nadh1* sequences GenBank KX011263, MN991983, KX011238, MN991990, KX011228 and KX011286 respectively).

**Fig. 3.** Papillate to semi-papillate sporangia of *Phytophthora acaciivora* formed on V8 agar flooded with soil extract. Shapes observed included: **A–F, H, M.** Elongated ovoid, **E, G, H, O, R.** Ovoid, **J, K.** Limoniform, **I.** Obpyriform, **L.** Peanut and **M, N.** Distorted. **C–E, H, L–O, S.** Displaced papilla were very common and **O–Q.** Bipapillate sporangia were frequently observed. **R, S.** Proliferation was via external secondary lateral sporangia (r–s). Scale bar = 25  $\mu$ m.









**Fig. 4.** Amphigynous, aplerotic oogonia of *Phytophthora acaciivora* formed after pairing with tester strains. **A–D.** Oogonia had relatively thin walls and large ooplasm on maturity. Most oospores aborted after the formation of oospore walls but few aborted before the formation of walls. A few aborted before the formation of walls. Scale bar = 25  $\mu$ m.

**Description:** Semi papillate, persistent sporangia were abundantly produced in soil extract water on simple sporangiophores. Proliferation was external (Fig. 3B, E, N, R, S). Although predominantly elongated ovoid (60 %, Fig. 3 A–F), various sporangial shapes were observed including ovoid, obpyriform, ellipsoid and limoniform (Fig. 3). Bipapillate sporangia were also occasionally observed (Fig. 3O–Q). *Sporangiophores* were often laterally attached to sporangia resulting in displaced papilla (Fig. 3D, L, S). *Sporangia* from three isolates averaged  $(21\text{--})56.3 \pm 13.4\text{--}(102)$   $\mu$ m in length and  $(15\text{--})32.6 \pm 36.3\text{--}(49)$   $\mu$ m in width, with narrow exit pores of  $5.93 \pm 0.88$   $\mu$ m and a length : breadth ratio of  $1.73 \pm 0.29$  (Table 2). *Chlamydospores* absent. *Hyphal swellings* absent.

*Phytophthora acaciivora* is heterothallic and readily produced oogonia in paired cultures on V8A within 14 to 21 d. All three isolates were of A1 mating type. Oogonia from three isolates averaged  $34.0 \pm 4.1$   $\mu$ m ranging from 24.5 to 46.2  $\mu$ m (Table 2). Oospores were aplerotic in all isolates, but over 90 % aborted, usually after the formation of the oospore walls (Fig. 4). Oospores averaged  $30.7 \pm 3.6$   $\mu$ m diam ranging from 22.6 to 39.2  $\mu$ m (Table 2). Oospore walls were  $1.71 \pm 0.46$   $\mu$ m and the oospore wall index was  $0.30 \pm 0.08$   $\mu$ m (Table 2). Antheridia were amphigynous, cylindrical and two-celled (Fig. 4). Antheridia averaged  $20.5 \pm 3.9 \times 14.8 \pm 2.5$   $\mu$ m.

**Cultures:** All *P. acaciivora* isolates produced colonies that were uniform and cottony with no distinctive growth pattern and regular smooth margins on CA, V8A, MEA and PDA (Fig. 5). Optimum temperature for the growth of *P. acaciivora* on CA was 25–30 °C where the average growth rate was 10 mm/d (range of isolate means) (Fig. 6). The minimum temperature for growth was 10 °C, the maximum temperature for growth was greater than 37.5 °C (Table 2).

**Additional materials examined:** **Vietnam**, Tuyen Quang, from dying *Acacia mangium* seedlings, Mar. 2012, T.Q. Pham, CBS 138639; from canker of dying *Melia azedarach* Mar. 2012, T.Q. Pham, VTN18.

**Notes:** *Phytophthora acaciivora* resides in clade 2d in the complete *Phytophthora* phylogeny, closely related to *P. frigida*, *P. acacia*, *P. elongata*, *P. bichii* and *P. multivesiculata*. Several of these species have distorted and/or elongated sporangia. On average, *P. acaciivora* has much larger sporangia than other species in clade 2d; the average size overlaps with the elongated sporangia type found in *P. elongata* (Table 2). While many morphological features are similar, *P. acaciivora* differs from the most closely related species *P. frigida* in having persistent sporangia, external proliferation and no chlamydospores or hyphal swellings (Table 2). Most species in clade 2d have a temperature optimum of around 25 °C, However *P. acaciivora* and *P. acacia* have a broader temperature maxima of 25–30 °C and *P. acaciivora* still grows at 37.5 °C.

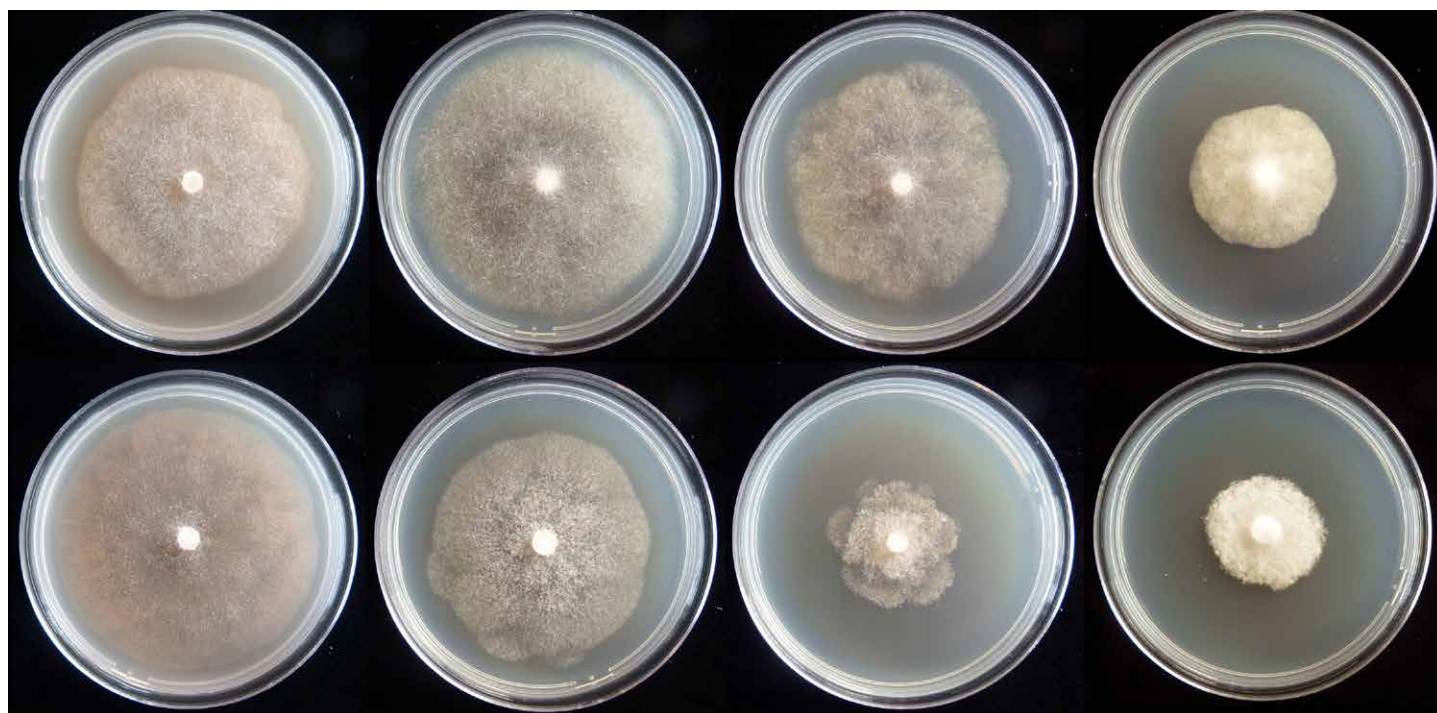
## DISCUSSION

Several *Phytophthora* species have been found associated with various diseases in *Acacia* plantations globally (Table 3) These *Phytophthora* species are mostly unrelated. However the species described here, *Phytophthora acaciivora*, is closely related to *P. acacia*, a species recently described causing gummosis of *Acacia mearnsii* (black wattle) in Brazil (Alves et al. 2019). It is also closely related to *P. elongata* and *P. frigida*, two species described from Australia and South Africa respectively, which had, at the time of their description, been recovered predominately from *Eucalyptus* (Maseko et al. 2007, Rea et al. 2010). One isolate in the original description of *Phytophthora frigida*, was found associated with collar and root rot on *Acacia deccurrens* (Maseko et al. 2007). More recently *P. frigida* has been reported to cause gummosis on *Acacia mearnsii* in Southern Brazil (Alves et al. 2016).

In Vietnam there are several notable diseases of *Acacia* plantations such as root rot, heart rot, pink disease, wilting caused by *Ganoderma* spp., *Erythricium salmonicolor*, *Ceratocystis*

**Table 2.** Comparison of morphological characters and dimensions, and temperature-growth relations of *P. acaciavora*, *P. frigida*, *P. acaciae*, *P. elongata*, *P. bishii* and *P. multivesiculata*. All measurements except growth rate are in  $\mu\text{m}$ .

Character	<i>P. acaciavora</i>	<i>P. frigida</i>	<i>P. acaciae</i>	<i>P. elongata</i>	<i>P. bishii</i>	<i>P. multivesiculata</i>
	This study	Maseko <i>et al.</i> (2007)	Alves <i>et al.</i> (2019)	Rea <i>et al.</i> (2010)	Abad <i>et al.</i> (2008)	Ilieva <i>et al.</i> (1998)
No. of isolates	3	5	25	5	5	7
Hyphal swellings	Absent	Globose	Absent	Ellipsoid (rare)	Coralloid	Globose and catenulate
<b>Sporangia</b>						
Characteristics	Semi-papillate, rarely bipapillate, persistent, displaced apices common	Papillate, persistent, displaced apices common	Papillate, persistent, displaced apices common	Semi-papillate, persistent, displaced apices common	Semi-papillate, rarely bipapillate, persistent	Semi-papillate, rarely bipapillate, persistent
Shapes observed	Elongated ovoid, ovoid, obpyriform	Ovoid, obpyriform	Ellipsoid, ovoid, obpyriform	Ovoid, obpyriform, elongated	Ovoid, obpyriform, elongated	Ovoid, obpyriform
Distorted shapes	Present	Present	Present	Present		
Sporangiophores	Simple or lax sympodia	Lax sympodia	Lax sympodia	Simple or lax sympodia	Simple and often long	Simple, often long and twisted with basal swellings
Exit pores	5.9	3–7	3–12	7	n/a	8–14
Range ( $\mu\text{m}$ )	21–102 $\times$ 15–49	24–40 $\times$ 20–33	28–85 $\times$ 21–50	26–76 $\times$ 19–42	26–72 $\times$ 21–36	30–60 $\times$ 20–41
Length $\times$ breadth mean	56 $\times$ 32.6	33 $\times$ 27	51 $\times$ 31	46 $\times$ 28 58 $\times$ 24 (elongated)	34 $\times$ 27 (ovoid) 49 $\times$ 30 (obpyriform) 89 $\times$ 29 (elongated)	45 $\times$ 33
Length : breadth ratio (mean)	1.73	1.2	1.6	1.6	1.6	1.43
Proliferation	External	Absent	Absent	External	External	Internal and external
<b>Chlamydospores</b>						
	Absent	Present	Present	Absent	Absent	Present
Range ( $\mu\text{m}$ )		20–35	15–55			n/a
Mean diam ( $\mu\text{m}$ )		25	32			n/a
<b>Oogonia</b>						
	Aplerotic	Aplerotic	Aplerotic	Plerotic (30 %) and Aplerotic	Aplerotic	Aplerotic
Range diam ( $\mu\text{m}$ )	24.5–46.2	25–42	20–34	21–42	24–46	28–50
Mean diam ( $\mu\text{m}$ )	34	33	25	31	35	
<b>Oospores</b>						
Range diam ( $\mu\text{m}$ )	22.6–39.2	19–38	17–30	18–38	25–31	24–42
Mean diam ( $\mu\text{m}$ )	30.7	28	22	27	28	33
Oospore Wall index	0.3	0.35	0.54	0.42	0.39	0.45
<b>Antheridia</b>						
	Amphigynous	Amphigynous	Amphigynous	Paragynous	Paragynous	Amphigynous
Sex	Heterothallic	Heterothallic	Heterothallic	Homothallic	Homothallic	Homothallic
<b>Growth characteristics</b>						
Max temp ( $^{\circ}\text{C}$ )	>37.5	30	36	32.5	32.5	35
Opt temp ( $^{\circ}\text{C}$ )	25	25	18–24	25	26	
Min temp ( $^{\circ}\text{C}$ )	10	10	6	4	10	
Growth rate on CA at optimum (mm/d)	10	5.8	12	6.3	3	6.8



**Fig. 5.** Colony morphology of (top to bottom) *P. acaciivora* (CBS 138638) and *P. frigida* (CMW 19427) after 7 d growth at 20 °C on different media: CA, V8A, MEA and half strength PDA (left to right).

**Table 3.** *Phytophthora* species reported causing disease of *Acacia* species.

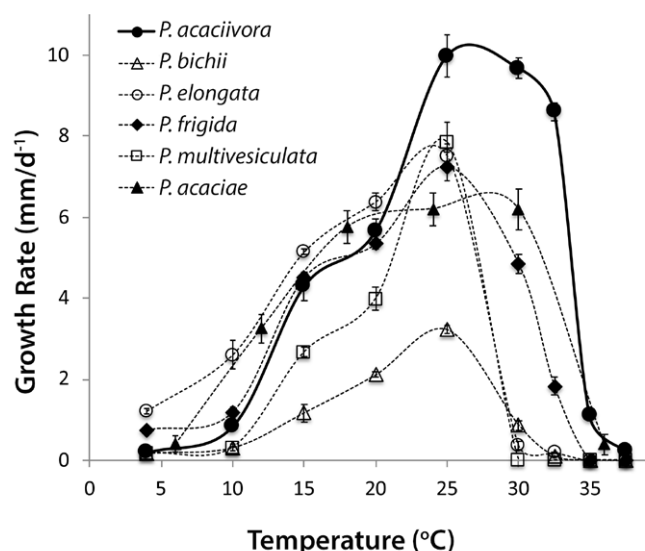
<i>Phytophthora</i> species <sup>1,2</sup>	Clade	<i>Acacia</i> species	Year	Country	Symptoms	Reference
<i>Phytophthora nicotianae</i>	1	<i>Acacia mearnsii</i>	1967	South Africa	Black butt and gummosis	Zeijlemaker (1971)
	1	<i>A. drummondii</i>	1989	Australia		USDA <sup>3</sup>
	1	<i>A. mearnsii</i>	2004	Brazil	Gummosis	Dos Santos <i>et al.</i> (2005)
<i>P. meadii</i>	2a	<i>A. mearnsii</i>	1995	South Africa	Black butt	Roux & Wingfield (1997)
<i>P. acaciae</i>	2d	<i>A. mearnsii</i>	2016	Brazil	Gummosis	Alves <i>et al.</i> (2019)
<i>P. acaciivora</i>	2d	<i>A. mangium</i>	2014	Vietnam	Nursery root rot and wilt	This study
<i>P. frigida</i>	2d	<i>A. deccurrens</i>	2006	South Africa	Black butt	Maseko <i>et al.</i> (2007)
	2d	<i>A. mearnsii</i>	2008	Brazil	Gummosis	Alves <i>et al.</i> (2016)
<i>P. palmivora</i>	4	<i>A. dealbata</i>	1973	Greece		USDA
	4	<i>A. mangium</i>	2000	Indonesia	Black canker	Nair (2000)
	4	<i>A. mangium</i>	2012	Vietnam	Root rot and wilt	Pham <i>et al.</i> (2014)
<i>P. gibbosa</i>	6	<i>A. pycnantha</i>	2009	Australia	Roots	Jung <i>et al.</i> (2011)
<i>P. cinnamomi</i>	7	<i>A. melanoxylon</i>	1981	USA		USDA
	7	<i>A. dealbata</i>	1982	Australia		USDA
	7	<i>A. baileyana</i>	1989	New Zealand		USDA
	7	<i>A. mangium</i>	2012	Vietnam	Root rot and cankers	Dell <i>et al.</i> (2012)
<i>P. niederhauserii</i>	7	<i>A. dealbata</i>	2010	Italy		USDA
<i>P. parvispora</i>	7	<i>A. mangium</i>	2013	Vietnam	Root rot	Pham <i>et al.</i> (2014)
<i>P. cryptogea</i>	8	<i>A. longifolia</i>	1989	Australia		USDA
<i>P. boehmeriae</i>	10	<i>A. mearnsii</i>	1995	South Africa	Gummosis	Roux & Wingfield (1997)
	10	<i>A. mearnsii</i>	2006	Brazil	Gummosis	Dos Santos <i>et al.</i> (2006)

<sup>1</sup> For each *Phytophthora* species from an individual country only the reference for the first report and first host is provided. For example *P. cinnamomi* has been recorded from many *Acacia* species in Australia, but only the first record is provided here.

<sup>2</sup> It is important to note that many of these records were before molecular identification became common and could, upon further investigation, prove to be a different species. However, the purpose of this table is to show records as they have appeared in published literature.

<sup>3</sup> USDA = United States Department of Agriculture disease database.





**Fig. 6.** Average growth rate (mm/d  $\pm$  SE) of *P. acaciivora* compared to other species in clade 2d on V8A across the temperature range from 4–37.5°C. Data for *P. acaciae* from Alves *et al.* (2019).

spp. respectively (Pham *et al.* 2010, Chi *et al.* 2019). With the description of *Phytophthora acaciivora* and the recovery of *P. cinnamomi* from plantations, it is now also important to pay attention to the diseases caused by these species. Even if the disease is not fatal to the tree alone, it may weaken the tree making them less able to defend themselves from other diseases.

The origin of *P. acaciivora* remains unknown. Recently, numerous new *Phytophthora* species have been recognised from Vietnam, many of which are yet to be described (Puglisi *et al.* 2017, Jung *et al.* 2020). However, *P. acaciivora* was not recovered in the extensive study of natural ecosystems conducted by Jung *et al.* (2020). In the current study, *P. acaciivora* was also recovered from *Melia azedarach*, a species with a wide native distribution that includes both Australia and Vietnam. The nursery disease caused by *P. acaciivora* on *Acacia mangium*, an exotic plantation tree species, is of concern in Vietnam. As the origin of the species remains unclear, care should be taken not to introduce this species to other countries where *Acacia* spp. are grown in plantations or to Australia where they are endemic.

**Conflict of interest:** The authors declare that there is no conflict of interest.

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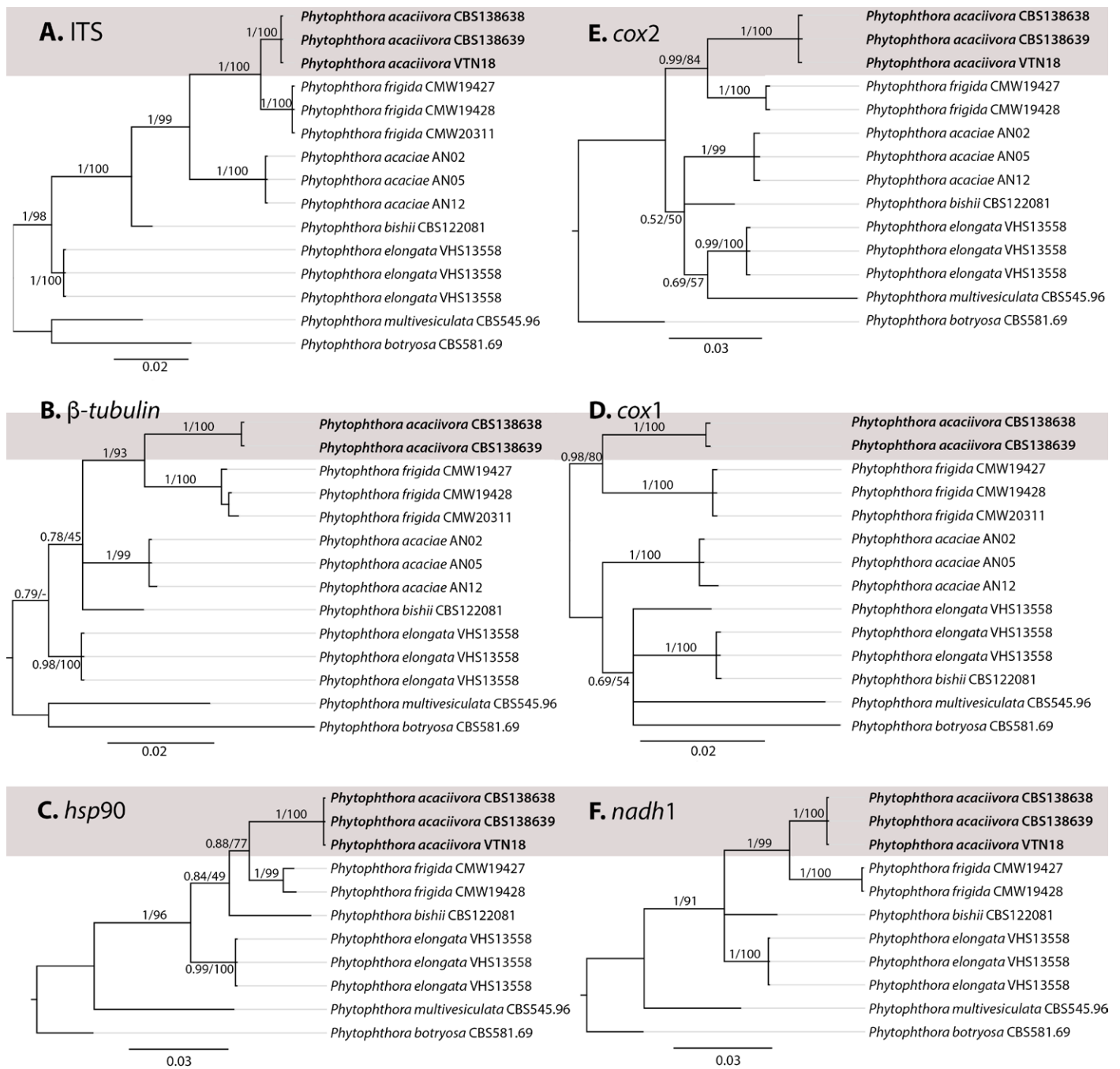
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**Supplementary Material:** <http://fuse-journal.org/>

**Supplementary Fig 1.** Bayesian trees produced from **A.** ITS, **B.**  $\beta$ -tubulin, **C.** *hsp90*, **D.** *cox2*, **E.** *cox1* and **F.** *nadh1* genes regions using GTR + I model showing the phylogenetic position of *Phytophthora acaciivora* and related species. Maximum likelihood was conducted on the same dataset with RAxML and resulted in the same topology. Numbers above the branches reflect support obtained from the analysis of the same dataset (Bayesian posterior probabilities/Bootstrap values estimated by RAxML). *Phytophthora botryosa* was used as an outgroup taxon. The scale bar corresponds to nucleotide substitutions per site.



**Figure S1**



**Supplementary Fig 1.** Bayesian trees produced from **A.** ITS, **B.**  $\beta$ -tubulin, **C.** *hsp90*, **D.** *cox2*, **E.** *cox1* and **F.** *nadh1* genes regions using GTR + I model showing the phylogenetic position of *Phytophthora acaciivora* and related species. Maximum likelihood was conducted on the same dataset with RAxML and resulted in the same topology. Numbers above the branches reflect support obtained from the analysis of the same dataset (Bayesian posterior probabilities/Bootstrap values estimated by RAxML). *Phytophthora botryosa* was used as an outgroup taxon. The scale bar corresponds to nucleotide substitutions per site.