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

T.I. Burgess1*, Q.N. Dang1,2, B.V. Le2, N.Q. Pham1, D. White1, T.Q. Pham2

1Phytophthora Science and Management, Environmental and Conservation Sciences, Murdoch University, 90 South St, 6150, Australia
2Forest Protection Research Centre, Vietnamese Academy of Forest Sciences, 46 Duc Thang Ward, Bac Tu Liem District, Hanoi City, Vietnam

*Corresponding author: tburgess@murdoch.edu.au

Key words: forest health, new taxon, nursery diseases, 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 amphiogenous. 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.

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 pulpwod. 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
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 CaCO₃, 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 MF77 and MF84 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. acaciicora 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 | Code 1, 2 | Host | Year | Location | GenBank Accession Numbers | GenBank Accession Numbers | GenBank Accession Numbers | GenBank Accession Numbers | GenBank Accession Numbers |
|----------|------|-----------|------|------|----------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Phytophthora acaciivora | CBS 138638 | Acacia mangium | 2014 | Vietnam, Tuyen Quang | KX011263 | MN991983 | KX011238 | MN991990 | KX011228 | KX011286 |
| Phytophthora acaciivora | CBS 138639 (ET) | A. mangium | 2014 | Vietnam, Tuyen Quang | KX011264 | MN991984 | KX011239 | MN991991 | KX011229 | KX011287 |
| Phytophthora acaciivora | VTN18 | Melia azedarach | 2014 | Vietnam, Tuyen Quang | KX011265 | n/d | MN991995 | n/d | KX011230 | KX011288 |
| Phaeoacremonium | AN02 (ET) | Acacia mearnsii | 1999 | Brazil, RS, Triunfo | KX396303 | KX396338 | n/a | KX396267 | KX396279 | n/a |
| Phaeoacremonium | AN05 | A. mearnsii | 1999 | Brazil, RS, Triunfo | KX396304 | KX396339 | n/a | KX396268 | KX396280 | n/a |
| Phaeoacremonium | AN12 | A. mearnsii | 1999 | Brazil, RS, Triunfo | KX396305 | KX396340 | n/a | KX396269 | KX396281 | n/a |
| Phytophthora bishii | CBS 122081 (ET) | Fragaria ananassa | 1999 | USA, North Carolina, Wake | MG783381 | MN991985 | KX011243 | MH136851 | GU221940 | KX011289 |
| Phytophthora elongata | CBS 125799 (ET) | Eucalyptus marginata | 2004 | Australia, WA, Dwellingup | MG865485 | MH493932 | MK020301 | MH136881 | KX011232 | KX011290 |
| Phytophthora elongata | VHS 13483 | E. marginata | 2004 | Australia, WA, Dwellingup | KX011268 | MN991987 | MN991997 | MH136881 | KX011234 | KX011292 |
| Phytophthora frigida | VHS 13483 | E. marginata | 2004 | Australia, WA, Dwellingup | KX011267 | MN991986 | MN991996 | MN991992 | KX011233 | KX011291 |
| Phytophthora frigida | CMW20311 (ET) | E. smithii | 2002 | South Africa, KZN, Ixopo | MG865496 | DG988221 | n/a | MH136892 | n/a | n/a |
| Phytophthora frigida | CMW19427 | Eucalyptus smithii | 2002 | South Africa, KZN, Bloemendal | KX011269 | MN991988 | KX011241 | MN991993 | KX011235 | KX011293 |
| Phytophthora frigida | CMW19428 | Acacia decurrens | 2002 | South Africa, KZN, Seven Oaks | KX011270 | MN991989 | MN991998 | MN991994 | KX011236 | KX011294 |
| Phytophthora multivesiculata | CBS 545.96 (ET) | Cymbidium sp. | 1996 | Netherlands, Mijdrecht | MG865544 | MH493982 | KX011247 | MH136937 | KX011237 | KX011295 |
| Phytophthora botryosa | CBS 581.69 (ET) | Hevea brasiliensis | 1966 | Malaysia, Perlis | MK496516 | MH493910 | KX250541 | MH136855 | JN618604 | AY563993 |

1 ET = ex-type culture.
2 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.
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. bichii* (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, β-tubulin, HSP90, coxI, coxII and nadh1 sequences GenBank KX011263, MN991983, KX011238, MN991990, KX011228 and KX011286 respectively).

**Fig. 2.** Bayesian trees produced from concatenated A. ITS, hsp90 and β-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.

**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 μm.
Phytophthora acaciivora sp. nov.

Editor-in-Chief
Prof. dr P.W. Crous,
Westerdijk Fungal Biodiversity Institute,
P.O. Box 85167, 3508 AD Utrecht, The Netherlands.
E-mail: p.crous@westerdijkinstitute.nl
Fig. 4. Amphigynous, aplerotic oogonia of *Phytophthora acaciivora* formed after pairing with tester strains. A–D. Oogonia had relatively thin walls and large ooplasts 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 μm.

**Description:** Semi papillate, persistent sporangia were abundantly produced in soil extract water on simple sporangiophores. Proliferation was external (Fig. 3A–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, I, S). **Sporangia** from three isolates averaged (21–)56.3 (±13.4–102) μm in length and (15–)32.6 (±36.3–49) μm in width, with narrow exit pores of 5.93 ± 0.88 μm and a length : breadth ratio of 1.73 ± 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 ± 4.1 μm ranging from 24.5 to 46.2 μm (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 ± 3.6 μm diam ranging from 22.6 to 39.2 μm (Table 2). Oospore walls were 1.71 ± 0.46 μm and the oospore wall index was 0.30 ± 0.08 μm (Table 2). Antheridia were amphigynous, cylindrical and two-celled (Fig. 4). Antheridia averaged 20.5 ± 3.9 x 14.8 ± 2.5 μm.

**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).

**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 decurrens* (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. acaciivora*, *P. frigida*, *P. acacia*, *P. elongata*, *P. bishii* and *P. multivesiculata*. All measurements except growth rate are in µm.

| Character                      | *P. acaciivora* | *P. frigida* | *P. acacia* | *P. elongata* | *P. bishii* | *P. multivesiculata* |
|--------------------------------|-----------------|--------------|-------------|---------------|-------------|----------------------|
|                                | This study      | Maseko et al. (2007) | Alves et al. (2019) | Rea et al. (2010) | Abad et al. (2008) | Ilieva et al. (1998) |
| No. of isolates                | 3               | 5            | 25          | 5             | 5           | 7                    |
| Hyphal swellings               | Absent          | Globose      | Absent      | Ellipsoid (rare) | Coralloid   | Globose and catenulate |
| Sporangia                      |                 |              |             |               |             |                      |
| Characteristics                | Semi-papillate, rarely bipapillate, 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 oviod, oviod, obpyriform | Ovoid, obpyriform | Ellipsoid, oviod, 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 (µm)                     | 21–102 × 15–49  | 24–40 × 20–33| 28–85 × 21–50| 26–76 × 19–42 | 26–72 × 21–36 | 30–60 × 20–41         |
| Length × breadth mean          | 56 × 32.6       | 33 × 27      | 51 × 31     | 46 × 28       | 58 × 24 (elongated) | 89 × 29 (elongated)    |
| 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 |
| Chlamydospores                 | Absent          | Present      | Present     | Absent        | Absent      | Present              |
| Range (µm)                     | 20–35           | 15–55        | 32          |               | n/a         |                       |
| Mean diam (µm)                 | 25              | 32           |             |               | n/a         |                      |
| Oogonia                        | Aplerotic       | Aplerotic    | Aplerotic   | Plerotic (30 %) and Aplerotic | Aplerotic | Aplerotic |
| Range diam (µm)                | 24.5–46.2       | 25–42        | 20–34       | 21–42         | 24–46       | 28–50                |
| Mean diam (µm)                 | 34              | 33           | 25          | 31            | 35          |                      |
| Oospores                       |                 |              |             |               |             |                      |
| Range diam (µm)                | 22.6–39.2       | 19–38        | 17–30       | 18–38         | 25–31       | 24–42                |
| Mean diam (µm)                 | 30.7            | 28           | 22          | 27            | 28          | 33                   |
| Oospore Wall index             | 0.3             | 0.35         | 0.54        | 0.42          | 0.39        | 0.45                 |
| Antheridia                     | Amphigynous      | Amphigynous  | Amphigynous | Paragynous    | Paragynous  | Amphigynous           |
| Sex                            | Heterothallic   | Heterothallic| Heterothallic| Homothallic  | Homothallic | Homothallic          |
| Growth characteristics         |                 |              |             |               |             |                      |
| Max temp (°C)                  | >37.5           | 30           | 36          | 32.5          | 32.5        | 35                   |
| Opt temp (°C)                  | 25              | 25           | 18–24       | 25            | 26          |                      |
| Min temp (°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).

| Phytophthora species | Clade | Acacia species | Year | Country | Symptoms | Reference          |
|----------------------|-------|----------------|------|---------|----------|--------------------|
| *Phytophthora nicotianae* | 1     | *Acacia mearnsii* | 1967 | South Africa | Black butt and gummosis | Zeijlemaker (1971) |
| 1                    | A. drummondii | 1989 | Australia |         | Gummosis | USDA⁴               |
| 1                    | A. mearnsii | 2004 | Brazil |         | Gummosis | Dos Santos et al. (2005) |
| *P. meadii*          | 2a    | A. mearnsii     | 1995 | South Africa | Black butt | Roux & Wingfield (1997) |
| *P. acaciae*         | 2d    | A. mearnsii     | 2016 | Brazil | Gummosis | Maseko et al. (2019) |
| *P. acaciivora*      | 2d    | A. mearnsii     | 2004 | Brazil | Gummosis | Maseko et al. (2019) |
| *P. frigida*         | 2d    | A. mearnsii     | 2016 | Brazil | Gummosis | Alves et al. (2019) |
| *P. palmivora*       | 4     | A. dealbata     | 1973 | Greece |         | USDA               |
| 4                    | A. mangium | 2000 | Indonesia |         | Black canker | Nair (2000) |
| 4                    | A. mangium | 2012 | Vietnam |         | Root rot and wilt | Pham et al. (2014) |
| *P. gibbosa*         | 6     | A. pycnantha    | 2009 | Australia | Roots | Jung et al. (2011) |
| *P. cinnamomi*       | 7     | A. melanoxyron | 1981 | USA |         | USDA               |
| 7                    | A. dealbata | 1982 | Australia |         |         | USDA               |
| 7                    | A. baileyana | 1989 | New Zealand |         |         | USDA               |
| 7                    | A. mangium | 2012 | Vietnam |         | Root rot and cankers | Dell et al. (2012) |
| *P. niederhauserii*  | 7     | A. dealbata     | 2010 | Italy |         | USDA               |
| *P. parvispora*      | 7     | A. mangium     | 2013 | Vietnam | Root rot | Pham et al. (2014) |
| *P. cryptogea*       | 8     | A. longifolia   | 1989 | Australia |         | USDA               |
| *P. boehmeriae*      | 10    | A. mearnsii     | 1995 | South Africa | Gummosis | Roux & Wingfield (1997) |
| 10                   | A. mearnsii | 2006 | Brazil |         | Gummosis | Dos Santos et al. (2006) |

¹For 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.

²It 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.

³USDA = United States Department of Agriculture disease database.
Phytophthora acaciivora sp. nov.

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

REFERENCES

Abad ZG, Abad JA, Coffey MD, et al. (2008). Phytophthora bshirea sp. nov., a new species from the Rosaceae, raspberry, rose and strawberry in three continents. Mycologia 100: 99–110.

Alves TCA, Tessmann DJ, Ivors KL, et al. (2019). Phytophthora acacea sp. nov., a new species causing gummosis of black wattle in Brazil. Mycologia 111: 445–455.

Alves TCA, Tessmann DJ, Ivors KL, et al. (2016). First report of gummosis caused by Phytophthora frigida on Black Wattle in Brazil. Plant Disease 100: 2336.

Blair JE, Coffey MD, Park S-Y, et al. (2008). A multi-locus phylogeny for Phytophthora utilizing markers derived from complete genome sequences. Fungal Genetics and Biology 45: 266–277.

Chi NM, Thu PQ, Mohammed C (2019). Screening disease resistance of Acacia auriculiformis clones against Ceratocystis manginecans by artificial and natural inoculation methods. Australasian Plant Pathology 48: 617–624.

Cooke DEL, Drenth A, Duncan JM, et al. (2000). A molecular phylogeny of Phytophthora and related Oomycetes. Fungal Genetics and Biology 30: 17–32.

Dell B, Xu D, Thu PQ (2012). Managing threats to the health of tree plantations in Asia. In: AR Bandani (ed), New perspectives in Plant Protection: 63–92. InTech, Rijeka.

Dick MW (1990). Keys to Pythium. University of Reading Press, Reading, UK.

Dos Santos A, Luz E, De Souza J (2006). First report of Phytophthora boehmeriae on black wattle in Brazil. Plant Pathology 55: 813.

Dos Santos A, Luz E, de Souza J (2005). Phytophthora nicotianae: causal agent of gummosis of black wattle in Brazil. Fitopatologia Brasileira 30: 81–84.

Erwin DC, Ribeiro OK (1996). Phytophthora Diseases Worldwide. APS Press, St. Paul, Minnesota.

Harwood CE, Nambiar EKS (2014). Productivity of acacia and eucalypt plantations in Southeast Asia. 2. Trends and variations. International Forestry Review 16: 249–260.

Hegde M, Palanisamy K, Yi JS (2013). Acacia mangium Willd. – A fast growing tree for tropical plantation. Journal of Forest and Environmental Science 29: 1–14.

Hüberli D, Tommerup IC, Hardy GES (2000). False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with Phytophthora cinnamomi. Australasian Plant Pathology 29: 164–169.

Illiew E, Man in’t Veld WA, Veenbaas-Rijks W, et al. (1998). Phytophthora multivesiculata, a new species causing root rot of Cymbidium. European Journal of Plant Pathology 104: 667–684.

Jung T, Scanu B, Brasier CM, et al. (2020). A survey in natural forest ecosystems of Vietnam reveals high diversity of both new and described Phytophthora taxa including P. ramorum. Forests 11: 93.

Jung T, Stukely MJC, Hardy GES, et al. (2011). Multiple new Phytophthora species from ITS clade 6 associated with natural ecosystems in Australia: evolutionary and ecological implications. Persoonia 26: 13–39.

Kroon LPNM, Bakker FT, van den Bosch GBM, et al. (2004). Phylogenetic analysis of Phytophthora species based on mitochondrial and nuclear DNA sequences. Fungal Genetics and Biology 41: 766–782.

Martin FN, Tooley PW (2003). Phylogenetic relationships among Phytophthora species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. Mycologia 95: 269–284.

Maseko B, Coutinho TA, Burgess TI, et al. (2007). Two new species of Phytophthora from South African eucalypt plantations. Mycological Research 111: 1321–1338.

Nair KSS (2000). Insect pests and diseases in Indonesian forest: an assessment of the major threats, research efforts and literature: 101. Center for International Forestry Research, Bogor, Indonesia.

Nambiar EKS, Harwood CE, Kien ND (2015). Acacia plantations in Vietnam: research and knowledge application to secure a sustainable future. Southern Forests 77: 1–10.

Pham TQ (2016). Surveys of Pythiacae causing root rot diseases of Acacia mangium and Acacia hybrid in some provinces of North Vietnam. Vietnamese Academy of Forest Science Journal 1/2016: 4251–4256.

Pham TQ, Dang Q, Burgess TI, et al. (2014). Phytophthora – an emerging threat to plantation forestry in Vietnam. 7th International Union of Research Organisations. 7th International Union of Research
Organisations. IUFRO Working Party 7 Feb. 2009, Phytophthora in Forests and Natural Ecosystems. 9–14 Nov., Esquel, Argentina.

Pham TQ, Dang Q, Dell B (2013). The occurrence of *Phytophthora cinnamomi* caused the root rot disease on *Acacia mangium* in Yen Son, Tuyen Quang Province. *Journal of Plant Protection* 3: 3–5.

Pham TQ, Griffiths M, Pegg GS, et al. (2010). Healthy plantations. *A field guide to pests and pathogens of Acacia, Eucalyptus and Pinus in Vietnam*. The State of Queensland, Department of Employment, Economic Development and Innovation.

Pham TQ, Dang Q, Dell B (2013). The occurrence of *Phytophthora cinnamomi* caused the root rot disease on *Acacia mangium* in Yen Son, Tuyen Quang Province. *Journal of Plant Protection* 3: 3–5.

Pham TQ, Griffiths M, Pegg GS, et al. (2010). Healthy plantations. *A field guide to pests and pathogens of Acacia, Eucalyptus and Pinus in Vietnam*. The State of Queensland, Department of Employment, Economic Development and Innovation.

Puglisi I, De Patrizio A, Schena L, et al. (2017). Two previously unknown *Phytophthora* species associated with brown rot of Pomelo (*Citrus grandis*) fruits in Vietnam. *PloS One* 12: e0172085.

Roux J, Wingfield MJ (1997). Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. *Forest Ecology and Management* 99: 327–336.

Sakalidis ML, Ray JD, Lanoiselet V, et al. (2011). Pathogenic *Botryosphaeriaceae* associated with *Mangifera indica* in the Kimberley Region of Western Australia. *European Journal of Plant Pathology* 130: 379–391.

Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

White TJ, Bruns T, Lee S, et al. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: MA Innes, DH Gelfand, JJ Sninsky, et al. (eds), *PCR Protocols: A Guide to Methods and Applications*: 315–322. Academic Press, San Diego.

Zeijlemaker F (1971). Black-butt disease of black wattle caused by *Phytophthora-nicotianae var parasitica*. *Phytopathology* 61: 144.

Supplementary Material: http://fuse-journal.org/

Supplementary Fig 1. Bayesian trees produced from A. ITS, B. β-tubulin, C. *hsp*90, D. *cox*2, E. *cox*1 and F. *nadh*1 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.