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ARTICLE

# *Phytophthora boodjera* sp. nov., a damping-off pathogen in production nurseries and from urban and natural landscapes, with an update on the status of *P. alticola*

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**Abstract:** A new homothallic *Phytophthora* species, isolated in Western Australia (WA), is described as *Phytophthora boodjera* sp. nov. It produces persistent, papillate sporangia, oogonia with thick-walled oospores, and paragynous antheridia. Although morphologically similar to *P. arenaria*, phylogenetic analyses of the ITS, cox1, HSP90, β-tubulin and enolase gene regions revealed *P. boodjera* as a new species. In addition, *P. boodjera* has a higher optimal temperature for growth and a faster growth rate. *Phytophthora boodjera* has only recently been found in Western Australia and has mostly been isolated from dead and dying *Eucalyptus* seedlings in nurseries and from urban tree plantings, and occasionally from disturbed natural ecosystems. It is found in association with declining and dying *Agonis flexuosa*, *Banksia media*, *B. grandis, Corymbia calophylla, Eucalyptus* spp,. and *Xanthorrhoea preissii*. The status of *P. alticola* was also reviewed. The loss of all isolates associated with the original description except one; discrepancies in both sequence data and morphology of the remaining isolate with that presented the original description, and inconclusive holotype material places the status of this species in doubt.

**Key words:**
*Eucalyptus*
multi-gene phylogeny
*Oomycota*
*Phytophthora arenaria*

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## INTRODUCTION

Numerous *Phytophthora* species have been associated with damping-off and seedling diseases in plant production nurseries worldwide (Hardy & Sivasithamparam 1988, Davison *et al*. 2006, Warfield *et al*. 2008, Moralejo *et al*. 2009, Goss *et al.* 2011, Lilja *et al*. 2011, Leonberger *et al*. 2013, Pérez-Sierra & Jung 2013, Prospero *et al*. 2013, Schoebel *et al*. 2014). *Phytophthora* species are dispersed *via* the roots of infected plants, soil from potted plants, growth media and water, and in some cases by aerial transmission. Transfer of plants and plant products by human activity and through globalisation in trading is now generally accepted as the main method of introduction of exotic pathogens and pests. The most high-risk pathway for the movement of *Phytophthora* is “plants for plantings” (Brasier 2008, Liebhold *et al*. 2012, Scott *et al*. 2013). Plants infected at production nurseries can potentially distribute *Phytophthora* species to parks and reserves, amenity plantings, plantations, rehabilitation and biodiversity plantings, wildflower farms, retail nurseries, and gardens. Many *Phytophthora* species, such as *P. nicotianae*, *P. plurivora* (often reported as *P. citricola*), *P. cactorum* and *P. citrophthora,* tend to be the most commonly recovered from nurseries worldwide, strongly supporting their dissemination through the nursery trade. Because of the level of attention that has been given to this important topic, it is now rare for a new species to be detected in nurseries (Moralejo *et al*. 2009). Nevertheless the number of reports of *Phytophthora* species damaging to nursery trees, forests and natural ecosystems is increasing and this has significant implications for international plant biosecurity and plant health practice (Kroon *et al.* 2012).

The most significant new detection of the past 20 years is *Phytophthora ramorum* (Grünwald *et al*. 2012, Parke & Grünwald 2012). *Phytophthora ramorum* was first detected infecting *Viburnum* and *Rhododendron* in plant nurseries in Germany and The Netherlands in 1993 (Werres *et al*. 2001), and has subsequently been found in various nurseries all over Europe and North America. It has been recognized as an alien aggressive species in natural areas of the west coast of the USA where it causes sudden oak death, and in Cornwall in the UK (Rizzo *et al*. 2002, Brasier *et al*. 2004). Spread through the international nursery trade, *P. ramorum* poses a serious risk to plant biosecurity worldwide (Brasier 2008, Parke & Lucas 2008, Parke & Grünwald 2012).

In recent years, many new *Phytophthora* species have been described from natural ecosystems in Western Australia (WA) (Burgess *et al.* 2009, Scott *et al.* 2009, Rea *et al*. 2010, Jung *et al.* 2011a, b, Rea *et al*. 2011, Aghighi *et al*. 2012, Burgess *et al.* 2012, Crous *et al.* 2012, Hüberli *et al*. 2013). In 2011, a new damping-off disease was reported in WA nurseries growing *Eucalyptus* and other species for

restoration of agricultural land. ITS sequence data of the isolates did not match any known species, but were closely related to *P. alticola* and *P. arenaria* and were an exact match for a single WA isolate designated as "*P.* taxon arenaria-like" by Rea *et al*. (2011).

*Phytophthora arenaria* has been isolated primarily from Kwongan vegetation and mainly from *Banksia* species on the northern sandplains in south-west WA (Rea *et al.* 2011). *Phytophthora alticola* was first isolated and described by Maseko *et al*. 2007 from cold-tolerant *Eucalyptus* species (*E. dunnii, E. bajensis,* and *E. macarthurii*) with collar and root rot in South African plantations at an altitude above 1150 m. The new taxon has been isolated in WA from dead and dying *Eucalyptus* seedlings in nurseries and from adult plants in the urban landscape, predominantly from eucalypts, and occasionally from *Banksia* species and *Corymbia calophylla* in natural ecosystems.

Further investigation of isolates thought to be *P. arenaria* in the Vegetation Health Service (VHS) collection of the WA Department of Parks and Wildlife (Burgess *et al*. 2009) and other recent collections from urban surveys (Barber *et al*. 2012) revealed two distinct groups of isolates. The first group were of *P. arenaria,* while the second appeared to be a new species related to *P. alticola* (Maseko *et al*. 2007). In the current study, the *P. alticola*/*P. arenaria* species complex was re-evaluated using a combination of morphology and a multi-gene phylogeny resulting in the recognition of a new species, described here as *P. boodjera* sp. nov., and an investigation into the status of *P. alticola.*

## MATERIALS AND METHODS

### Isolates

The majority of isolates used were obtained from the Vegetation Health Service (VHS) Collection, Department of Parks and Wildlife, Perth, Western Australia. All isolates were baited from soil and root material using *Eucalyptus sieberi* cotyledons. The 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. The ex-type isolates of *P. alticola* were obtained from CBS (CBS-KNAW Fungal Biodiversity Centre, Utrecht). Sequence data from related species were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank) the Phytophthora Database (PD; www.phytophthoradb.org), and q-bank (www.q-bank.eu). When all isolates in the CMW collection (Forestry and Agriculture Biotechnology Institute, University of Pretoria, SA) were evaluated and it was found that all isolates of *P. alticola* except CMW 19425 had perished, that isolate was re-numbered CMW 34279. All isolates used in this study are detailed in Table 1, and the status of all *P. alticola* isolates is given in Table 2.

### DNA isolation, amplification and sequencing

The *Phytophthora* isolates were cultured on half-strength potato dextrose agar (PDA) (Becton Dickinson, Sparks, MD), 19.5 g PDA, 7.5 g agar and 1 L of distilled water) at 20 °C for 2 wk. Mycelium was collected by scraping from the agar surface with a sterile blade and placing 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 following the method of Andjic *et al*. (2007). In all cases, the PCR reaction mixtures were as described previously (Andjic *et al*. 2007) but using the PCR conditions described in the original papers (cited below). 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 mitochondrial gene cox1 was amplified with primers FM77 and FM 84 (Martin & Tooley 2003). Heat shock protein 90 (HSP) was amplified with HSP90-F int and HSP90-R1 primers (Blair *et al*. 2008). β-tubulin (BT) was amplified with primers BTF1A and BTR1, and enolase (ENO) was amplified with primers Enl Fy and Enl R1 according to Kroon *et al*. (2004).

All gene regions were sequenced in both directions with the primers used in amplification. The clean-up products and sequencing were accomplished as described previously (Sakalidis *et al.* 2011). All sequences derived in this study were added to GenBank, and the accession numbers are provided in Table 1.

### Phylogenetic analysis

The data set consisted of sequences of *Phyophthora boodjera* sp. nov., *P. alticola* and *P. arenaria* isolates used in this study, and other closely related species in ITS clade 4 (Table 1) which were compiled and manually edited in Geneious v. R7 (http://www.geneious.com/) and Bayesian analysis conducted using a MrBayes (Ronquist *et al*. 2012) plugin within Geneious after determining the most appropriate substitution model with jModelTest-2.1.4 (Darriba *et al*. 2012). Alignment files and trees can be viewed on TreeBASE (http://www.treebase.org/).

### Culture characteristics

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, malt extract agar (MEA), carrot agar (CA; 0.1 L filtered carrot juice, 17 g agar and 0.9 L distilled water) and half-strength PDA (all from BBL, Becton Dickinson, Sparks, MD). Colony morphology was described according to Erwin & Ribeiro (1996). For temperature growth studies, all isolates were subcultured 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. Plates were observed daily to ensure that the colonies did not reach the edge of the Petri dish; the radial growth rate was measured after 4–7 d, along two lines crossing the middle of the inoculum plug at right angles, and the mean growth rates (mm per day) were assessed. After 7 d, plates with no colony growth at 35 °C and 37.5 °C were returned to 20 °C for 7 d to check the isolate viability.

### Morphology

Sporangia were produced by flooding 15 x 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 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 100 g of pine (*Pinus radiata*) bark potting mixture in 1 L distilled water and incubating this on an orbital shaker for 24 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 400x in a BX51 Olympus microscope.

Gametangia were produced by all isolates on V8A in the dark at 20 °C after 7 d. After 14 d, dimensions and characteristic features of 50 randomly-selected mature oogonia, oospores and antheridia were measured at 400x. 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).

The preserved type materials of *P. alticola* available from the National Mycological Herbarium in Pretoria (PREM 59214, PREM 59215, PREM 59216, PREM 59217) were re-examined. The slides were rehydrated with 85 % lactic acid and observed with a Zeiss Axioskop 2 Plus compound microscope fitted with an Axiocam MRc camera. Dimensions were measured using Axiovision v. 4.8 software.

## RESULTS

### Phylogenetic analysis

CMW 19417 was designated as the type isolate of *Phytophora alticola* by Maseko *et al*. (2007), but no sequence data were provided for this isolate. A subsequent sequence of this same isolate, CBS 121937 available on q-bank, actually corresponds to *P. palmivora* (Fig. 1). CMW 19424 and CMW 19425 were originally designated as paratypes and ITS sequence data were provided for these isolates. All of these isolates were subsequently lost except CMW 19425 (= CBS 121939 = CMW 34279 = P19861). ITS sequence data for isolates presented with the original description, including CMW 19425 (DQ988196), differ by 3 bp from all recent sequences of CMW 34279, CBS 121939 and P19861 (Fig. 1). However, when resequenced CMW 19424 (= CBS 121938) was found to actually be an isolate of *P. frigida* (Fig 1). Based on ITS sequence data, the WA isolates investigated in this study cluster with either isolate CMW 34279 or with *P. arenaria* (Fig. 1).

BT sequences data was also provided in the original description (Maseko *et al*. 2007): all isolates assigned to *P. alticola* were identical, but differ by 2 bp from the new sequence of isolate CMW 34279 and by 4 bp from *P. boodjera* sp. nov. (figure available on request from the authors). The *cox*I sequence of isolate CMW 34279 from three separate databases is identical and clusters separately from isolates assigned to *P. boodjera* sp. nov. (figure available on request from the authors). Isolates of *P. arenaria* cluster together, although intraspecific sequence variation is observed. In the concatenated dataset (Fig. 2), isolate CMW 34279 clusters with isolates of *P. boodjera* sp. nov., although it differs by 8 bp across the five gene regions examined. If the isolate is duplicated it forms a strongly supported cluster on its own (data not shown). Isolates of *P. arenaria* also reside in a strongly supported clade, although intraspecific variation is observed (Fig. 2).

### Status of *Phytophthora alticola*

In 2008, the World Phytophthora Collection (WPC; http://phytophthora.ucr.edu/default.html) was sent four isolates from the CMW collection, two isolates each of *P. alticola* and *P. frigida*. When the WPC sequenced them, they realised the identities were incorrect and informed the CMW collection (Table 2). Isolates of *P. alticola* and *P. frigida* were then checked in the CMW collection and it was discovered that all isolates of *P. alticola* had perished or were incorrectly identified, except for CMW19425 which was cleaned and renumbered CMW34279. This isolate was then sent to WPC where it was given the code P19861. Also in 2007, three isolates were sent to CBS; of these, the ex-holotype isolate CBS 121937 (= CMW 19417) is actually *P. palmivora* (the sequence associated with this isolate is available from q-bank), the ex-paratype isolate CBS 121938 (= CMW 19424) was not re-sequenced but is now determined as of *P. frigida*, leaving the same single isolate CBS 121939 (= CMW 34279) (Table 2).

At the start of this project, it was known that the ex-holotype isolate of *P. alticola* had perished, as indeed had all other isolates except an ex-paratype isolate CMW 19425 (= CMW 35429, = CBS 121939, = P19861). The ITS sequence of this isolate from all collections is identical, although there are a few bp different from the ITS sequence of the same isolate in the original description (Fig. 1). The sequence in the original description is short and the differences are at the end of the sequence and could have been erroneously labelled. Controversially, sequence data of other isolates in various collections designated as *P. alticola* match different species (Table 2).

It was originally considered that epitypification would be possible with the intention to designate CMW 34279 as the epitype. However, morphological examination of this isolate revealed that it differed from the original description: the sporangia are not caducous and chlamydospores are not produced (Table 3). Subsequent examination of the holotype and paratypes from PREM were inconclusive (Table 3). Each of the PREM types consisted of a semi-dried agar disc kept at 4 °C and a microscopic slide. The agar disks were all contaminated with bacteria and a dark hyphomycete, most of the mycelia had lysed, but a few aborted oospores were observed in PREM 59216 (= CMW 19424) and PREM 59217 (= CMW 19425). Some reproductive structures were present on the slides. Sporangia and chlamydospores were present for PREM 59214 (= CMW 19416) and PREM 59215 (= CMW 19417). The sporangia were predominantly ovoid, caducous and papillate, and produced in close sympodia (Table 3, Fig. 3). The dimensions of these sporangia match the original description of *P. alticola* (Maseko *et al*. 2007). However, in the original description the sporangia were described as borne on terminal or branched sporangiophores, while the slide associated with the holotype had sporangia borne

**Table 1.** Identity, date and location of isolation, host information and GenBank accession numbers (where available) for *Phytophthora* spp. considered in this study.

| Species | Location | Isolation date | Host association | Isolate number[2] | GenBank Accession No. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | ITS | BT | HSP | ENO | cox1 |
| *P. alticola*[1] | Midillovo, KwaZulu-Natal | 2000-2004[6] | *Eucalyptus badjensis* | CMW 19417 | | | | | |
| ex-holotype | (KZN), South Africa | | | CBS 121937 | q-bank[5] | | | | q-bank[5] |
| *P. alticola*[1] | Midillovo, KZN, South Africa | 2000-2004 | *E. macarthurii* | CMW 19424 | DQ988197 | DQ988236 | | | |
| ex-paratype | | | | CBS 121938 | | | | | |
| *P. alticola*[1] | Paulpetersburg, KZN, | 2000-2004 | *E. dunnii* | CMW 19425 | DQ988196 | DQ988235 | | | |
| ex-paratype | South Africa | | | CBS 121939 | q-bank[5] | | | | q-bank[5] |
| | | | | CMW 34279[4] | HQ013214 | KJ372275 | KJ396703 | KJ396731 | KJ396686 |
| *P. alticola*[1] | unknown | | Unknown | P 16052 | GU259141 | | | | HQ261245 |
| *P. boodjera* | Mt Claremont, Perth, WA | 05/2011 | *Agonis flexuosa* | PAB 11.56[4] | KC748460 | KJ372280 | KJ396708 | KJ396736 | KJ396687 |
| | Dalkeith, Perth, WA | 05/2011 | *Eucalyptus marginata* | PAB 11.67[4] | KC748461 | KJ372276 | KJ396704 | KJ396732 | KJ396682 |
| | Ravensthorpe, WA | 08/2006 | *Banksia media* | VHS 16282[3] | EU301117 | KJ372281 | KJ396709 | KJ396737 | HQ013198 |
| | Kensington, Perth, WA | 02/2012 | *Eucalyptus* sp. | VHS 26631[4] | KJ372240 | KJ372277 | KJ396705 | KJ396733 | KJ396683 |
| ex-holotype | Tincurrin, WA | 03/2012 | Soil dump | VHS 26806[4] | KJ372244 | KJ372283 | KJ396710 | KJ396738 | KJ396688 |
| | | | | CBS 138637 | | | | | |
| | Tincurrin, WA | 04/2012 | *Eucalyptus* sp. | VHS 27016[4] | KJ372245 | | | | |
| | Tincurrin, WA | 04/2012 | *Eucalyptus* sp. | VHS 27017[4] | KJ372246 | KJ372284 | KJ396711 | KJ396739 | KJ396689 |
| | Tincurrin, WA | 04/2012 | *Eucalyptus* sp. | VHS 27018[4] | KJ372247 | KJ372285 | KJ396712 | KJ396740 | KJ396690 |
| | Tincurrin, WA | 04/2012 | *Eucalyptus* sp. | VHS 27020[4] | KJ372248 | KJ372286 | KJ396713 | KJ396741 | KJ396691 |
| | Tincurrin, WA | 04/2012 | *Eucalyptus* sp. | VHS 27021[4] | KJ372249 | KJ372287 | KJ396714 | KJ396742 | KJ396692 |
| | Tincurrin, WA | 04/2012 | *Eucalyptus* sp. | VHS 27022[4] | KJ372250 | KJ372288 | KJ396715 | KJ396743 | KJ396693 |
| | Tincurrin, WA | 04/2012 | *E. polybractea* | VHS 27171[4] | KJ372241 | KJ372278 | KJ396706 | KJ396734 | KJ396684 |
| | Stirling, Perth, WA | 11/2012 | *Xanthorrhoea preissii* | VHS 27382[4] | KJ372242 | KJ372279 | KJ396707 | KJ396735 | KJ396685 |
| | Gingin, WA | 11/2012 | *B. grandis* | VHS 28352 | | | | | |
| | Northam, WA | 09/2013 | *Corymbia calophylla* | TP 13.39 | | | | | |
| *P. arenaria* | Kalbarri, WA | 06/1986 | Kwongan heathland | DDS 1221[4] | EU593266 | KJ372297 | KJ396724 | KJ396752 | HQ013201 |
| | Eneabba, WA | 02/2009 | *E. drummondii* | CBS 125800[4] | HQ013205 | KJ372296 | KJ396723 | KJ396751 | HQ013215 |
| ex-holotype | Eneabba, WA | 02/2009 | *E. drummondii* | CBS 127950[4] | HQ013219 | KJ372289 | KJ396716 | KJ396744 | HQ013203 |
| | Lancelin, WA | 11/2001 | *B. menziesii* | VHS 9861[4] | EU301118 | KJ372290 | KJ396717 | KJ396745 | HQ013202 |
| | | | | IMI 389662 | | | | | |
| | Bunbury, WA | 02/2002 | *B. littoralis* | VHS 10154 | EU301114 | KJ372298 | KJ396725 | KJ396753 | KJ396697 |
| | | | | IMI 389663 | | | | | |
| | Badgingarra, WA | 04/2006 | *B. attenuata* | VHS 15453[4] | EU301115 | KJ372291 | KJ396718 | KJ396746 | HQ013199 |

**Table 1.** (Continued).

| Species | Location | Isolation date | Host association | Isolate number[2] | GenBank Accession No. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | ITS | BT | HSP | ENO | cox1 |
| | Badgingarra, WA | 04/2006 | *B. attenuata* | VHS 15489[4] | HQ013216 | KJ372292 | KJ396719 | KJ396747 | HQ013200 |
| | Eneabba, WA | 06/2008 | *B. attenuata* | VHS 19931[4] | HQ013217 | KJ372293 | KJ396720 | KJ396748 | KJ396694 |
| | Eneabba, WA | 11/2008 | *B. attenuata* | VHS 20537[4] | KJ372253 | KJ372299 | KJ396727 | KJ396754 | KJ396698 |
| | Ellenbrook, Perth, WA | 09/2011 | *Banksia* sp. | VHS 25370[4] | KJ372254 | KJ372300 | KJ396726 | KJ396755 | KJ396699 |
| | Dongara, WA | 11/2012 | *Banksia* sp. | VHS 28145 | KJ372251 | KJ372294 | KJ396721 | KJ396749 | KJ396695 |
| | Muchea, WA | 12/2012 | *X. preissii* | VHS 28269 | KJ372252 | KJ372295 | KJ396722 | KJ396750 | KJ396696 |
| *P. frigida* | South Africa | | *Eucalyptus* sp. | P 16059 | GU259147 | | | | HQ261313 |
| *P. palmivora* | United States | | | P 0113 | GU259121 | EU080465 | EU080468 | EU080467 | HQ261383 |
| *P. heveae* | United States | | | P 10167 | GU259516 | EU080796 | EU080799 | EU080798 | |
| *P. quercetorum* | United States | | | MD 9.2 | | EU080901 | EU080904 | EU080903 | |
| *P. castaneae* | Japan | | | P 10187 | FJ801304 | EUO80803 | EU080806 | EU080805 | HQ261348 |
| *P. megakarya* | Sao Tome and Principe | | | P 8516 | PD[5] | EU079970 | EU079973 | EU079972 | HQ261356 |
| *P. nicotianeae* | Australia | | *Nicotiana tabacum* | 332 | | | | | AY129169 |
| *P. cactorum* | United States | | *Malus sylvestris* | NY 568 | | | | | AY129174 |
| *P. plurivora* | Germany | | *Quercus robur* | CBS 124087 | | | | | FJ237510 |

[1]See Table 2 for explanation on the status of these isolates.

[2]Abbreviations of isolates in culture collections (where known): CBS = Centraalbureau voor Schimmelcultures, The Netherlands; IMI = CABI Bioscience (formerly International Mycological Institute), UK; VHS = Vegetation Health Service Collection, Department of Parks and Wildlife, Perth, Australia; DDS = earlier prefix of VHS Collection; PAB = Paul Barber, in Murdoch University (MU) Culture Collection; TP = Trudy Paap, in Murdoch University (MU) Culture Collection; CMW = culture collection of Forestry and Agriculture Biotechnology Institute, University of Pretoria, South Africa; P = isolate codes from World Phytophthora Collection, University of California, Riverside.

[3]Designated as *Phytophthora* taxon arenaria-like by Rea *et al.* (2011).

[4]Isolates used in the morphological study.

[5]Sequence available on Phytophthora database (http://www.phytophthoradb.org/) or q-bank (http://www.q-bank.eu/).

[6]No specific dates provided by Maseko *et al.* (2004), just date range under 'sampling and isolation'.

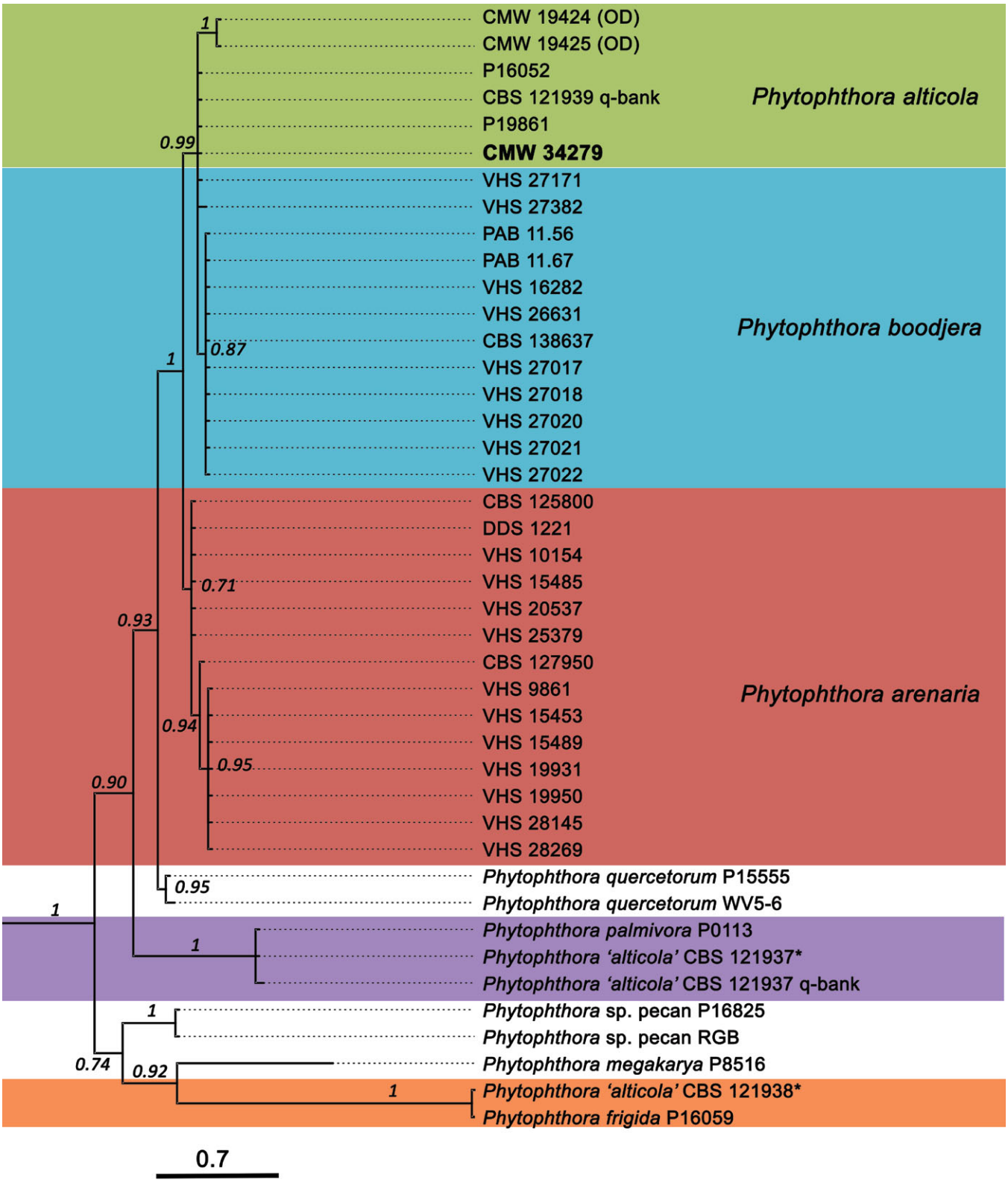


**Fig. 1.** Bayesian inference tree based on ITS sequence data generated in MrBayes using the GTR +G substitution model showing relationship between *P. alticola* nom. dub. (green), *P. boodjera* sp. nov. (blue) and *P. arenaria* (red). Isolates designated as *P. alticola* in CBS correspond to *P. palmivora* (purple) and *P. frigida* (orange). The posterior probability is shown at the nodes. *Phytophthora castaneae* and *P. heaveae* were used as outgroup taxa. Asterisks indicate the re-sequenced isolates CBS 121937 and CBS 121938. CBS 121939 was resequenced, but not included as it was identical to the sequence on q-bank for this isolate.

in close sympodia. These sporangia and their branching patterns resemble more those produced by *P. palmivora* rather than those of living isolate CMW 34279 (Table 3, Fig. 3). Oospores only were present in paratypes PREM 59216 (= CMW 19424) and PREM 59217 (= CMW 19425). The dimensions of these aplerotic oospores match the

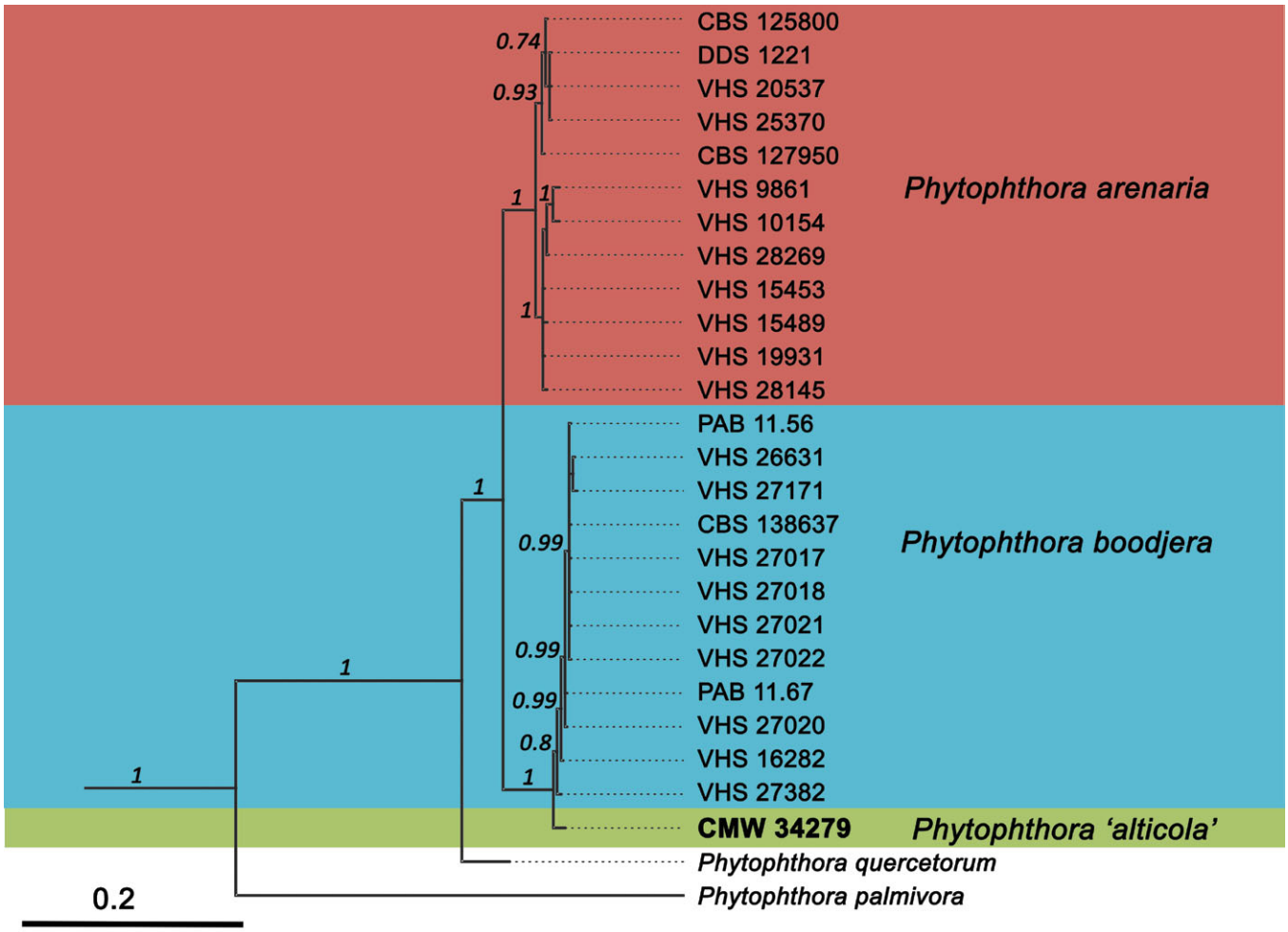


**Fig. 2.** Bayesian inference tree based on concatenated sequence data from ITS, β-tubulin, HSP90, enolase and *cox*I gene regions generated in MrBayes using the GTR +G substitution model showing relationship between *P. alticola* nom. dub. (green), *P. boodjera* sp. nov. (blue) and *P. arenaria* (red). The posterior probability is shown at the nodes. *Phytophthora castaneae* and *P. heaveae* were used as outgroup taxa.

original description and those of living isolate CMW 34279, however antheridia of the types are amphigynous, while those of CMW 34279 are paragynous (Table 3, Figs 3–4). Both *P. frigida* and *P. alticola* were described as having aplerotic oospores with amphigynous antheridia (Table 3), therefore the slides associated with the paratypes are inconclusive.

In the original description (Maseko *et al*. 2007), no sequence data were provided for PREM 59214 (= CMW 19416) and PREM 59215 (= CMW 19417). When the ex-holotype isolate was submitted to CBS and sequenced for q-bank (CBS 121937) it was found to be an isolate of *P. palmivora* (Fig. 1, Table 2). Caducous, papillate sporangia and chlamydospores matching *P. palmivora* were observed in PREM 59214 (= CMW 19416) and PREM 59215 (= CMW 19417) (Fig. 3). When the ex-paratype isolate CMW 19424 was submitted to CBS it was found to be *P. frigida,* as were several isolates labelled as *P. alticola* that were sent to WPC (Fig. 1, Table 2). *Phytophthora frigida* also has aplerotic oogonia with amphigynous antheridia, as observed for PREM 59216 (= CMW 19424) and PREM 59217 (= CMW 19425). Thus, we believe that while in the original description of *P. alticola* the sequence data provided was identical for all isolates, the actual morphological description is based on a set of isolates from more than one species; these are most probably *P. palmivora, P. frigida,* and a species represented by isolate CMW 34279. As there are no other living isolates linked to the original description available for examination and as no more isolates have been recovered in South Africa, despite extensive sampling, it is not possible to amend the description of *P. alticola* or to designate PREM 59217 (= CMW 19425, = CMW 35429) as an epitype. At this point in time the application of the name *P. alticola* is in doubt and will remain so until more isolates from similar hosts or locations can be made and this taxon will be referred to hereafter as *P. alticola nom. dub.*

Compared with the description of *P. alticola nom. dub.*, CMW 34279 has a higher optimum temperature for growth, faster growth rate, persistent sporangia, no chlamydospores and paragynous antheridia, and is very similar in morphology to isolates from Australia described here as *P. boodjera.*

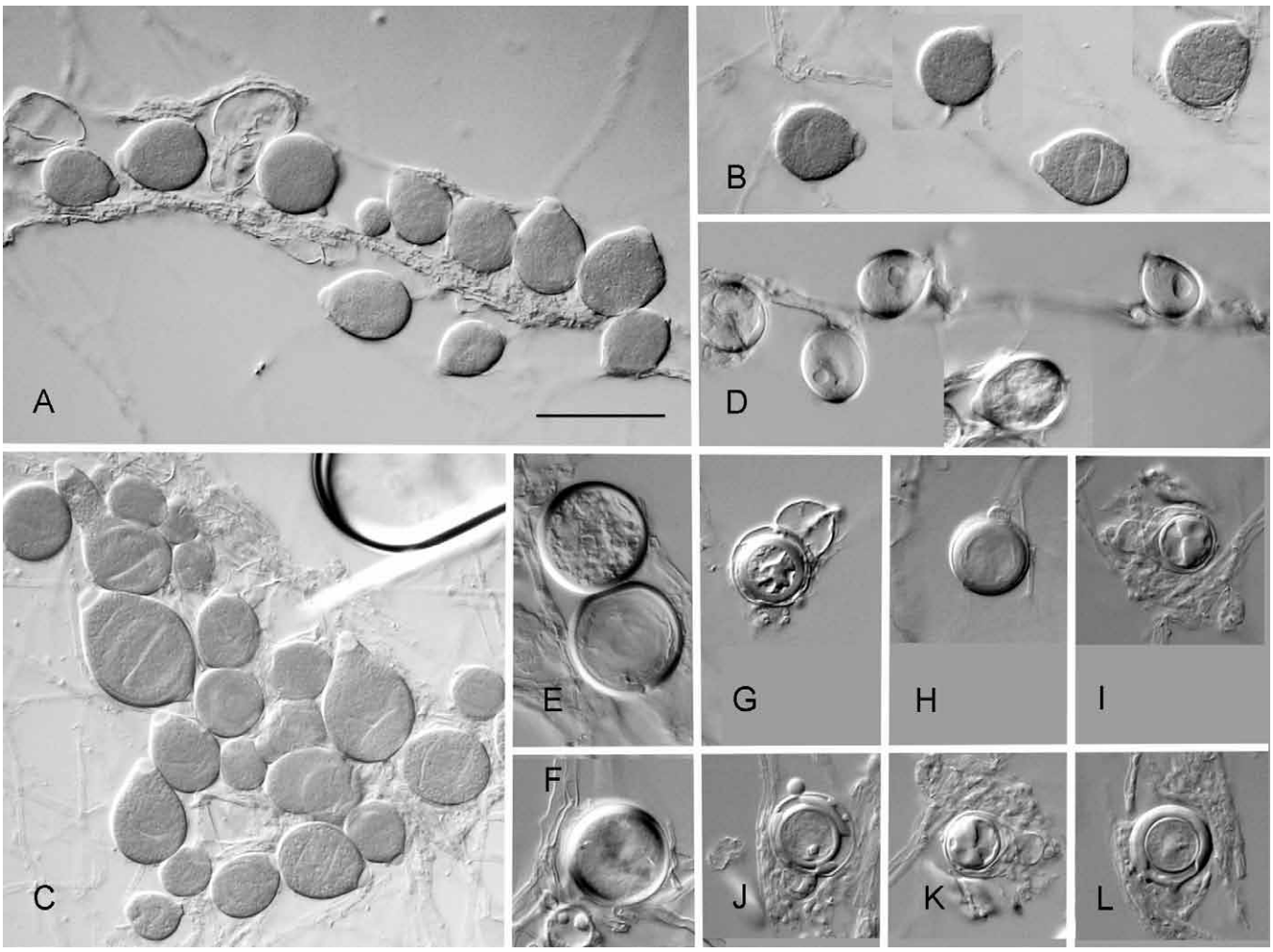


**Fig. 3.** Rehydrated slides of *P. alticola* nom. dub. (type specimens). Sporangia of paratype PREM 59214 = CMW 19416: (a) close sympodia with papillate, ovoid sporangia, (b) papillate, ovoid caducous sporangia with short pedicels, (c) papillate ovoid sporangia. Sporangia and chlamydospores of holotype PREM 59215 = CMW 19417: (d) Papillate, ovoid sporangia, (e–f) chlamydospores. Oospores of paratype PREM 59216 = CMW 19424: (g–h) aplerotic oospores with amphigynous antheridia. Oospores of paratype PREM 59217 = CMW 19425: (i–l) aplerotic oospores with amphigynous antheridia. Bar = 50 µm.

## TAXONOMY

**Phytophthora boodjera** A.V. Simamora & T.I. Burgess, **sp. nov.**
MycoBank MB809223
(Figs 4–5)

*Etymology*: the species name is derived from the Noongar (local Aboriginal) name for earth, ground, or sand plain.

*Type*: **Australia**: *Western Australia*: Tincurrin, from nursery soil dump, Mar. 2012, collected by the Vegetation Health Service of the *Department of Parks and Wildlife* (MURU 470–holotype; cultures ex-type CBS 138637 = VHS 26806). ITS, ß-tubulin, HSP90, enolase and *cox*I sequence GenBank KJ372244, KJ372283, KJ396710, KJ396738 and KJ396688 respectively).

*Diagnosis*: *P. boodjera* is phylogenetically closely related to *P. alticola nom. dub*. but differs in having persistent sporangia, paragynous antheridia and no chlamydospores. *P. boodjera* is morphologically similar to *P. arenaria* but differs in having a higher lethal temperature and larger sporangia and oogonia.

*Description (type)*: Papillate, persistent predominantly ovoid sporangia (52 %) but also limoniform (45 %) and distorted shapes (3 %). Sporangia averaged 34.7 ±1.16 x 27 ± 0.78 µm and ranged 15.2–62.3 x 14.6–42.5 µm. Homothallic; aplerotic oogonia averaged 28.9 ± 2.13 µm, ranging from 24.3–34 µm. Oospores averaging 26.3 ± 1.42 µm diam, range 20.9–29.4 µm. Growth rate at optimum of 25 °C was 11.2 mm/d. Colonies were appressed with no pattern and had regular smooth margins on CA, V8A, MEA and PDA.

*Description (species)*: *Sporangia* papillate, persistent, abundantly produced in soil extract water on simple sporangiophores frequently with globose swellings close to the sporangial base (Fig. 4f). Although predominantly ovoid (64 %, Fig. 4a–g), various sporangial shapes were observed including limoniform (20 %, Fig. 4d right, 4h), peanut-shaped (10 %) and distorted shapes (6 %, Fig. 4i, j). Bipapillate (Fig. 4i) sporangia were also occasionally observed.

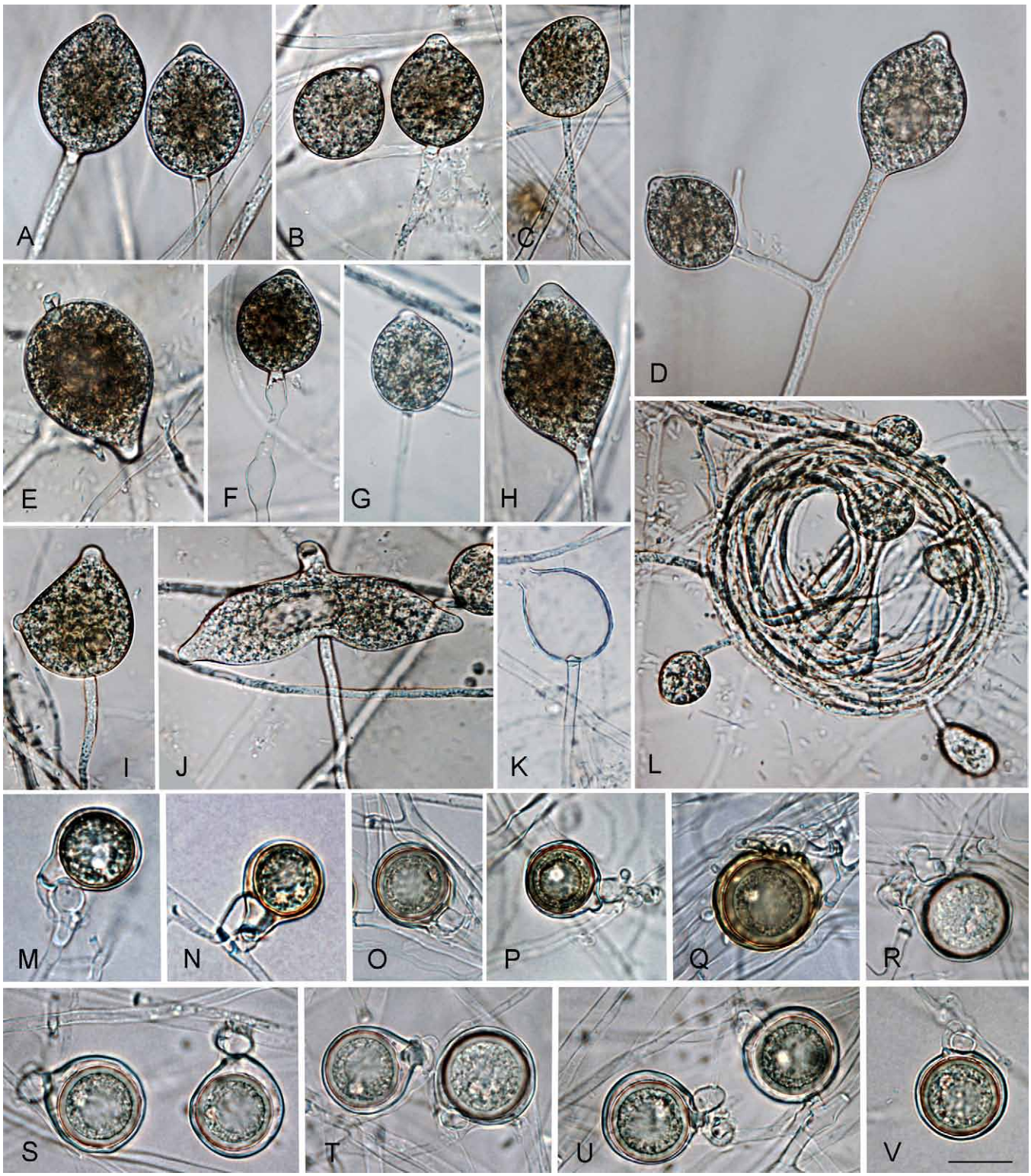


**Fig. 4.** (a–k) Papillate sporangia of *Phytophora boodjera* formed on V8A flooded with soil extract. Ovoid to broadly ovoid (a, b, e, f, g), limoniform (d right, h) bipapillate (i) distorted and bipapillate (j) often with laterally attached sporangiophore (c, k). Branching sporangiophores were rarely observed (d), occasional constriction of sporangiophore near base of sporangia (e), or bulbous sporangiophore (f). Hyphal coils rarely observed (l). Oogonia of *P. alticola nom. dub.* isolate CMW34279 with tapering bases, wavy margins and turning golden brown at maturity, with aplerotic oospores and paragynous antheridia (m–q). Aplerotic oospores of *P. boodjera* with paragynous antheridia (r–v). Scale bar = 20 µm.

Sporangiophores often laterally attached to sporangia (Fig. 4c, k), and sometimes constricted (Fig. 4e); branched sporangiophores rare (Fig. 4d). Sporangia from 12 isolates averaged 39.2 ± 4.4 x 29.7 ± 3.4 µm (range 32.5–44.5 x 24.5–33.5 µm), exit pores narrow, 6 ± 1 µm, length:breadth ratio 1.27 ± 0.16 (Table 3). Chlamydospores absent.

Homothallic, readily producing oogonia (and sporangia) in single culture on CA and V8A. Oospores matured within 14

**Fig. 5.** Colony morphology of (top to bottom) isolate CMW 34279, *Phytophthora boodjera* (VHS 27171, CBS 138637), and *P. arenaria* (CBS 127950, VHS 25370) after 7 d growth at 20 °C on different media: CA, V8A, MEA and half strength PDA (left to right).

to 21 d. Oogonia averaged 29.4 ± 2.3 µm diam with isolate means ranging from 24.6 to 33.4 µm (Table 3). Oospores aplerotic in all isolates, containing ooplasts when semi-mature to mature (Fig. 4s–v). Oospores averaged 25.5 ± 1.9 µm diam with isolate means ranging from 21.3 to 29.5 µm (Table 3). Oospore walls thick (2.5 ± 0.33 µm) (Fig. 4s–v),

**Table 2.** Status of *Phytophthora alticola* isolates submitted to different culture collections.

| Isolate | Sequence[1] | Notes on status of isolate |
|---|---|---|
| CMW 19416<br>PREM 59214-paratype | no sequence (OD) | Lost in CMW collection. Only papillate, caducous sporangia and chlamydospores observed from preserved slide associated with PREM 59214 |
| CMW 19417<br>PREM 59215-holotype<br>CBS 121937 | no sequence (OD)<br>ITS, CO, YPT1, TEF (q-bank) | Lost in CMW collection. Supposed corresponding isolate in CBS is actually *P. palmivora* and all sequence data on q-bank associated with this isolate is *P. palmivora*. Only papillate, caducous sporangia and chlamydospores observed from preserved slide associated with PREM 59215 |
| CMW 19419<br>PD 01642 | ITS and BT (OD) | Lost in CMW collection |
| CMW 19421<br>PD 01641 | ITS and BT (OD) | Lost in CMW collection |
| CMW 19422<br>PD 01640 | ITS and BT (OD) | Lost in CMW collection |
| CMW 19423<br>PD 01639 | ITS and BT (OD) | Lost in CMW collection |
| CMW 19424<br>PREM 59216-paratype<br>CBS 121938<br>PD 01638 | ITS and BT (OD) | Lost in CMW collection. Sequence on q-bank of ITS and BT is from the original description. The ITS of isolate re-sequenced in this study corresponds to *P. frigida*. Only aplerotic oospores and amphigynous antheridia observed from preserved slide associated with PREM59216 |
| CMW 19425<br>PREM 59217-paratype<br>CBS 121939<br>PD 01637 | ITS and BT (OD)<br>ITS, CO, YPT1, TEF (q-bank) | Living in CMW collection and renamed CMW 35429. ITS and BT of re-sequenced isolate differ from original description by 3 and 2 bp respectively. ITS and CO sequence on q-bank is identical to sequence of isolate CMW 35429 obtained in the current study. Only aplerotic oospores and amphigynous antheridia observed from preserved slide associated with PREM 59217 |
| CMW 35429<br>P16948 | ITS, cox1, ENO, HSP, BT<br>ITS (GA) | Was sent to WPC as CMW 35429 as a replacement for *P. alticola* and named WPC 16948. ITS sequence supplied by Gloria Abad is identical to that obtained in the current study for isolate CMW 35429 |
| PD 01914<br>P16053 | cox2 and cox1 (PD) | Was sent to WPC as *P. alticola* isolate CMW 19424 but when sequenced it was identified as being an isolate of *P. frigida* |
| PD 02043<br>P16051 | cox2 and cox1 (PD) | Was sent to WPC as *P. frigida* isolate CMW 19433 and when sequenced it was identified as being an isolate of *P. frigida* |
| PD 02044<br>P16054 | cox2 and cox1 (PD) | Was sent to WPC as *P. alticola* isolate CMW 19425 but when sequenced it was identified as being an isolate of *P. frigida* |
| PD 02775<br>P16052 | cox1 (PD) | Was sent to WPC as *P. frigida* isolate CMW 20311 but when sequenced it was identified as being an isolate of *P. alticola* and thus cannot be linked to any isolate from CMW collection |
| VHS 26631<br>P19861 | ITS, cox1, ENO, HSP, BT | List in WPC as a neotype for *P. alticola,* but this is not recommended as the isolate is from a different host and a different country from the original description. In current study this is considered an isolate of *P. boodjera.* |

[1]OD = original description (Maseko *et al.* 2007), WPC = World *Phytophthora* Collection (http://phytophthora.ucr.edu/), GA = supplied by Gloria Abad, PD = *Phytophthora* database http://www.phytophthoradb.org/, q-bank = http://www.q-bank.eu/.

oospore wall index 0.47 ± 0.05 µm (Table 3). Antheridia paragynous (Fig. 4r–v), averaging 10.4 ± 1.9 x 8.3 ± 1.5 µm. Hyphal swellings catenulate, some with radiating hyphae, formed rarely in non-sterile soil extract water.

*Cultures*: All isolates produced colonies that were appressed with no distinctive growth pattern and regular smooth margins on CA, V8A, MEA, and PDA (Fig. 5). Growth on MEA was sparser than on the other media. Optimum temperature for the growth on V8A 25–30 °C, where the average growth rate was 9.18 ± 0.56 mm/d (Fig. 6). The maximum temperature for growth was 35 °C (Table 3). Although no growth occurred at 37.5 °C, this temperature was not lethal since isolates resumed growth when subsequently incubated at 20 °C.

*Additional specimens examined*: **Australia:** Western Australia: Mt Claremont, Perth, from roots of dying *Agonis flexuosa*, May 2011, *Paul Barber* (PAB 11.56, private collection); Dalkeith, from roots of dying *Eucalyptus marginata,* May 2011 *Paul Barber* (PAB 11.67, private collection); Northam, from *Corymbia calophylla*, Sept. 2013,*Trudy Paap* (TP13.39, private collection). Ravensthorpe, from *Banksia media*, Aug. 2006, (VHS 16282); Kensington, Perth, WA, from *Eucalyptus* sp., Feb. 2012, (VHS 26631); Tincurrin, from *Eucalyptus* spp., Apr. 2012, (VHS 27016, VHS 27017, VHS 27018, VHS 27020, VHS 27021, VHS 27022); Tincurrin, from roots of *E. polybractea*, Apr. 2012, (VHS 27171); Stirling,

**Table 3.** Comparison of morphological characters and dimensions, and temperature-growth relations of *Phytophthora palmivora, P. frigida, P. alticola* (from original description, holotype and paratype material and living isolate CMW 19425 = CMW 34279)*, P. boodjera,* and *P. arenaria.*

| Species and sources of data | *P. palmivora* (Erwin & Ribero, 1995) | *P. frigida* (Maseko 2007) | *P. alticola* (holotype[1]) PREM 59215 | *P. alticola* (paratype[2]) PREM 59217 | *P. alticola* (Maseko 2007) | CMW 34279[3] (this study) | *P. boodjera* (this study) | *P. arenaria* (Rea 2011) | *P. arenaria* (this study) |
|---|---|---|---|---|---|---|---|---|---|
| **No of isolates** | | 10 | | | 10 | 1 | 12 | 10 | 9 |
| **Sporangia** (mm) | | | | | | | | | |
| LxB mean | 45.3 x 29.8 | 33 x 37 | 31.1 ± 5.0 x 30.9± 4.5 | | 36 x 28 | 38.9 ± 5.4 x 28.6 ± 4.3 | 39.2 ± 4.4 x 29.7 ± 3.4 | 31.8 ± 4.6 x 23.7 ± 3.5 | 23.9 ± 3.1 x 19.8 ± 3.4 |
| Range | 40–60 x 25–35 | 24–40 x 20–33 | 27.7–45.7 x 23.0–29.4 | | 30–45 x 20–35 | 20.4–60.7 x 19.0–38.9 | 15.2–64.5 x 13.9–42.5 | 20.2–53.0 x 12.5–35.0 | 12.7–38.5 x 9.9–30.7 |
| Range of isolates means | | | | | na | na | 32.6–44.6 x 24.7–33.3 | 28.9–34.8 x 21.4–28.3 | 19.5–24.9 x 16.0–23.1 |
| L/B ratio | 1.2–1.8 | 1.22 | 1.21 ± 0.12 | | 1.4 (<1.6) | 1.35 ± 0.03 | 1.27 ± 0.16 | 1.40 ± 0.17 | 1.22 ± 0.20 |
| Range of isolates means | | | | | na | na | 1.19–1.35 | 1.2–1.5 | 1.08–1.65 |
| Sporangial characteristics | Papillate | Papillate, rarely bipapillate | Papillate | | Papillate, rarely bipapillate | Papillate, rarely bipapillate or bilobed | Papillate, rarely bipapillate or bilobed | Papillate, rarely bi/tripapillate or bilobed | Papillate, rarely bipapillate or bilobed |
| Persistence | caducous | caducous | semi-caducous | | caducous | persistent | persistent | persistent | persistent |
| Sporangiophores | Lax or close sympodia | simple | Lax or close sympodia | | simple or branched sympodia | simple or branched sympodia often with bulbous base, very often laterally attached | simple or branched sympodia often with bulbous base, very often laterally attached | simple or branched sympodia often with bulbous base | simple or branched sympodia often with bulbous base, very often laterally attached |
| Sporangia shape | ellipsoid, ovoid spherical | ovoid, sometimes obpyriform | Usually ovoid to broad ovoid | | usually ovoid or ellipsoid, sometimes obpyriform or peanut-shaped | ovoid 66 %, limoniform 14 %, peanut-shaped 8 %, obpyriform 6 %, distorted 6 % | ovoid 64 %, limoniform 20 %, peanut-shaped 10 %, distorted 6 % | usually ovoid, also obpyriform or distorted | ovoid 40 %, subglobose 20 %, globose 14 %, obpyriform 12 %, distorted 4 % |
| Proliferation | absent | absent | absent | | absent | absent | absent | absent | absent |
| **Exit pores** (mm) | | | | | | | | | |
| Width | | 5–6 | | | 6 | 6.21 ± 0.53 | 6.09 ± 1.02 | 6.00 ± 1.00 | 5.50 ± 0.95 |
| Width range | | 5–10 | | | 4–8 | 5.00–7.10 | 4.85–8.89 | 3.40–8.90 | 3.88–7.10 |
| **Chlamydospores** (mm) | 32–42 | 24–26 | 42.6 ± 5.8 | | Some isolates 28 (20–35) | absent | absent | absent | absent |
| **Hyphal swellings** | | Spherical | | | Irregular | Catenulate, some with radiating hyphae | Catenulate, some with radiating hyphae | Catenulate, globose to sub-globose, some with radiating hyphae | Catenulate, globose to sub-globose, some with radiating hyphae |
| Mean diameter (mm) | | | | | na | 14.7 | 15.2 | na | 12.8 |
| **Breeding system** | Heterothallic | Heterothallic | | Homothallic | Homothallic | Homothallic | Homothallic | Homothallic | Homothallic |
| **Oogonia** (mm) | | | | | | | | | |
| Mean diameter | | 38 | | 26.2 ± 2.3 | 284 | 27.3 ± 1.9 | 29.4 ± 2.3 | 25.3 ± 2.2 | 26.6 ± 1.6 |
| Diameter range | 22–34.8 | 24–48 | | 24–37 | 20–35 | 22.03–31.07 | 24.3–33.9 | 19.6–34.3 | 20.5–29.6 |
| Range of isolates means | | | | | na | na | 24.6–33.4 | 24.3–28.1 | 23.6–28.8 |

**Table 3.** (Continued).

| Species and sources of data | *P. palmivora* (Erwin & Ribero, 1995) | *P. frigida* (Maseko 2007) | *P. alticola* (holotype[1]) PREM 59215 | *P. alticola* (paratype[2]) PREM 59217 | *P. alticola* (Maseko 2007) | CMW 34279[3] (this study) | *P. boodjera* (this study) | *P. arenaria* (Rea 2011) | *P. arenaria* (this study) |
|---|---|---|---|---|---|---|---|---|---|
| **Oospores** (mm) | | | | | | | | | |
| Mean diameter | 22.8 ± 0.1 | 33 | | 26.2 ± 2.1 | 30 (28.3 x 30.5) | 24.9 ± 2.1 | 25.5 ± 1.9 | 22.3 ± 1.8 | 23.8 ± 1.6 |
| Diameter range | 22.8 | 25–42 | | 21–31 | 24–36 | 20.3–29.5 | 20.92–29.3 | 16.0–28.3 | 17.8–28.6 |
| Range of isolates means | | | | | na | na | 21.3–29.5 | 21.4–23.9 | 21.5–25.9 |
| Wall thickness | | | | | na | 2.51 ± 0.4 | 2.47 ± 0.33 | 2.30 ± 0.34 | 2.57 ± 0.22 |
| Oospore wall index | | na | | 0.57± 0.01 | na | 0.54 ± 0.05 | 0.47 ± 0.05 | 0.50 ± 0.05 | 0.53 ± 0.06 |
| Oogonial characteristics | Aplerotic | Aplerotic | | Aplerotic | Markedly aplerotic, oospores with thick inner walls | Aplerotic oospores Mature oogonia with a slightly wavy surface and golden-brown discoloration often with tapering base | Aplerotic oospores Mature oogonia with a slightly wavy surface and golden-brown discoloration | Aplerotic oospores Mature oogonia with a slightly wavy surface and golden-brown discoloration | Aplerotic oospores Mature oogonia with a slightly wavy surface and golden-brown discoloration |
| **Antheridia** | Amphigynous | Amphigynous | | Amphigynous | Mainly amphigynous | Paragynous, often with finger-like projections | Paragynous | Paragynous, often with finger like projections | Paragynous |
| LxB mean (mm) | | | | | na | 10.6 ± 2.3 x | 10.4 ± 1.9 x | 11.2 ± 1.7 x | 10.0 ± 2.1 x |
| | | | | | | 8.3 ± 1.4 | 8.3± 1.5 | 8.4 ± 1.3 | 7.5 ± 1.3 |
| LxB range (mm) | | | | | na | Na | 8.2–10.9 x | 7.9–16.4 x | 6.4–13.8 x |
| | | | | | | | 7.3–10.6 | 6.0–10.5 | 5.6–12.8 |
| **Growth Characteristics** | | | | | | | | | |
| Max temp (°C) | 34 | 30 to <35 | | | 30 to <35 | 35 | 35 | 32.5 | 35 |
| Opt temp (°C) | 27.5–30 | 25 | | | 25 | 20–25 | 25–30 | 30 | 25 |
| Min temp (°C) | 11 | >5<10 | | | >10<15 | >10<15 | >10<15 | >10<15 | 15 |
| Lethal temp (°C) | | | | | na | >37.5 | >37.5 | na | <37.5 |
| Growth rate at optimum (mm/day) | | *ca.* 7.5 (CA), | | | *ca.* 4.5 (CA), | 8.20 (V8A) | 9.18 (V8A) | 5.9–7.4 (CA) | 8.65 (V8A) |
| | | *ca.* 8 (V8A) | | | *ca.* 7 (V8A) | | | | |
| Growth rate at 20°C (mm/day) | | 5 (V8A), | | | 4.5 (V8A), | 7.75 (V8A) | 6.12 (V8A) | 3.8–5.2 (CA) | 5.96 (V8A) |
| | | 3.0 (CA) | | | 3.0 (CA) | | | | |

**Table 3.** (Continued).

| Species and sources of data | *P. palmivora* (Erwin & Ribero, 1995) | *P. frigida* (Maseko 2007) | *P. alticola* (holotype[1]) PREM 59215 | *P. alticola* (paratype[2]) PREM 59217 | *P. alticola* (Maseko 2007) | CMW 34279[3] (this study) | *P. boodjera* (this study) | *P. arenaria* (Rea 2011) | *P. arenaria* (this study) |
|---|---|---|---|---|---|---|---|---|---|
| **Colony morphology** | On CA, stellate, defined edge, aerial mycelium in centre | Stellate-petaloid on V8, CA, PDA and MEA, moderately fluffy | | | Uniform and fluffy on MEA and V8A, stellate with limited aerial mycelium on CA and PDA | Appressed and cottony with no distinctive growth pattern and regular smooth margins on CA, V8A and PDA; sparse, slow growth on MEA | Appressed with no distinctive growth pattern and regular smooth margins on CA, V8A, MEA and PDA | Radiate to faintly radiate with very limited aerial mycelium and regular smooth margins on CA, V8A, MEA and PDA | Appressed with no distinctive growth pattern and regular smooth margins on CA, V8A and PDA, sometimes slightly petaloid on V8A; sparse on MEA |

[1]Morphological features of paratype PREM 59214 = CMW 19416 same as holotype PREM 59215 = CMW 19417, caducous, papillate sporangia in close sympodia and chlamydospores present. No oospores observed.

[2]Morphological features of paratype PREM 59216 = CMW 19424 same as paratype PREM 59217 = CMW 19425 = CMW 35479; amphigynous, aplerotic oospores turning brown on maturity. No sporangia or chlamydospores observed.

[3]Isolate CMW 19425 = CMW 35429 = CBS 121939 = WPC 16948 is the only isolate still surviving from the original description of *P. alticola* (Maseko *et al*. 2007) and it is linked to PREM 59217. Note: when all isolates were lost in the CMW collection, the remaining isolate CMW 19425 was renamed CMW 35429 and it is this isolate that was sent to the World *Phytophthora* Collection and given the code P 16948.

[4]Measurements from Maseko *et al*. (2007) where oospores were misrepresented to be larger than oogonia.

Perth, from *Xanthorrhoea preissii,* Nov. 2012, (VHS 27382); Gingin, from *Banksia grandis*, Nov. 2012, (VHS 28352); All VHS isolates were collected and are maintained by the Vegetation Health Service of the Department of Parks and Wildlife, Western Australia.

*Notes*: *Phytophthora boodjera* is morphologically very similar to isolate CMW 34279 linked to *P. alticola nom. dub.*; all measurements overlap, although CMW 34279 produces on average smaller sporangia, oogonia and oospores (Table 3). Colony morphologies on malt extract agar also differ (Fig. 5), and *P. boodjera* has a higher optimal temperature for growth and grows faster at higher temperatures (Fig. 6). Isolates of *P. boodjera* differ from CMW 34279 by one fixed single nucleotide polymorphism (SNP) in the ENO gene region, two in HSP and two in BT; three fixed SNPs separate the species in the cox1 gene region.

*Phytophthora boodjera* is closely related to *P. arenaria.* Morphologically, these species are very similar producing abundant thick walled oospores and sporangia of similar shapes and sizes (Table 3). The most marked differences between these species are: (1) 37.5 °C is lethal to *P. arenaria* but not to *P. boodjera*; (2) sporangia as well as oogonia and oospores are smaller in *P. arenaria*; and (3) 34 % of sporangia of *P. arenaria* are globose to subglobose while this shape is rare in *P. boodjera* (Table 3).

## DISCUSSION

*Phytophthora* isolates from plant production nurseries in Western Australia (WA) were identified as closely related to *P. alticola nom. dub.* based on ITS sequence data. These isolates were compared to the single remaining isolate of *P. alticola nom. dub.* from the original description (Maseko *et al*. 2007). Based on morphology and molecular data from four nuclear and one mitochondrial gene region, the isolates from WA were recognized as a new species and described as *P. boodjera*. *Phytophthora boodjera* has emerged as a pathogen in some WA plant production nurseries and is now regularly recovered also from urban environments. However, it has been recovered infrequently (VHS 16282 from Ravensthorpe, VHS 28352 from Gingin, and TP 13.39 from Northam) from natural ecosystems in WA, despite widespread sampling in the region (Burgess *et al*. 2009, Rea *et al*. 2011).

*Phytophthora alticola nom. dub.* was originally described from *Eucalyptus* plantations in South Africa and has never been recovered from sampling within natural ecosystems in that region (Nagel *et al*. 2013, Oh *et al*. 2013). This suggests that *P. alticola* has been introduced into South Africa. Morphological studies of the remaining isolate CMW 34279 revealed three major discrepancies with the original description: firstly, *P. alticola nom. dub.* was described as having caducous sporangia, and secondly, as producing chlamydospores; however, the remaining isolate CMW 34279 has persistent sporangia and produced no chlamydospores. Thirdly, *P. alticola nom. dub.* was described as producing mainly amphigynous and some paragynous antheridia; however, in the remaining isolate CMW 34279, only paragynous

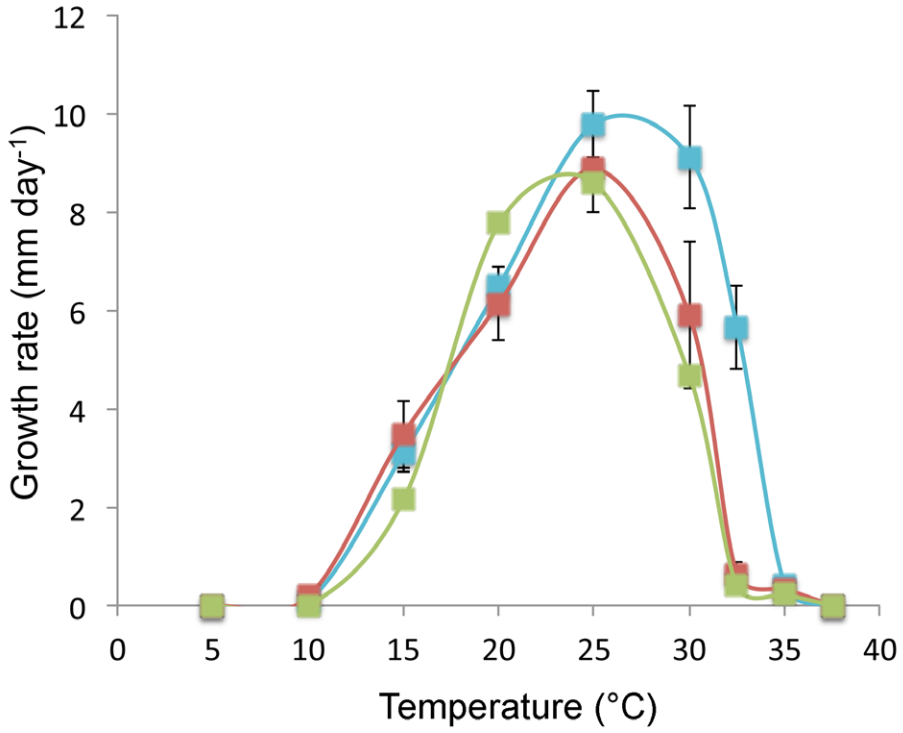


**Fig. 6.** Average growth rate (mm/d ± SE) of *Phytophthora boodjera* (blue), CMW 34279 (green) and *P. arenaria* (red) on V8A across the temperature range from 4–37.5 °C.

antheridia were observed. Although the ex-holotype isolate CMW 19417 has been lost, re-examination of the holotype PREM 59215 revealed sporangia and chlamydospores matching the original description of *P. alticola nom. dub.* except that they were produced in close sympodia rather than simple or branched sympodia (Maseko *et al*. 2007). CMW 19417 was submitted to CBS and the sequence of this isolate reveals that it is *P. palmivora*. The dimensions and characteristics of sporangia and chlamydospores observed in the holotype match those of *P. palmivora.*

Discrepancies in sequence data were found between the original description of *P. alticola nom. dub.* and the remaining ex-paratype isolate CMW 19425 (= CMW 34279). Unfortunately only oospores can be observed on the paratype PREM 59217 (= CMW 19425), but even these differ from the original description in that all antheridia are amphigynous in the holotype material, but they are all paragynous for CMW 34279. Thus, after examining the holotype and paratype material and resequencing isolates submitted to CBS, we have concluded that the original description was based on a mix of species and, as no further isolates similar to CMW 34279 have been recovered in South Africa despite extensive sampling (Oh *et al*. 2013), the status of *P. alticola* is in doubt.

*Phytophthora arenaria* (Rea *et al*. 2011), the species most closely related to *P. boodjera* in Western Australia, has been recovered exclusively from natural Kwongan vegetation on the coastal sand plains of south-west WA, where it was mainly isolated from dead and dying *Banksia* species and from the rhizosphere soil associated with such plants. This species appears to be restricted to the Kwongan vegetation and to be adapted to this ecosystem, suggesting that *P. arenaria* is native to WA. *Phytophthora boodjera* has only recently been found in WA and has mostly been isolated from dead and dying eucalypt seedlings in plant production nurseries and from declining trees (predominantly *Myrtaceae*) in disturbed urban landscapes, and once from *Xanthorrhoea preissii*. It has been isolated from natural ecosystems on only three occasions (from *Banksia media, B. grandis,* and *Corymbia calophylla*) and currently we consider this to be an introduced species.

Recent outbreaks of the damping-off disease of young eucalypt seedlings, caused by *P. boodjera*, have raised new concerns about the risk of *Phytophthora* species in plant production nurseries in WA. The dispersal of *Phytophthora* from nurseries to field plantings in previously non-infested areas may result in serious threats to biodiversity in natural ecosystems in these areas.

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