DOI 10.12905/0380.sydowia69-2017-0161

Phytophthora alticola; emended description based on new collections and a neotype

Tanay Bose[1,*], Treena I. Burgess[2], Jolanda Roux[3], Michael J. Wingfield[1]

[1] Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

[2] Centre for Phytophthora Science and Management, School of Veterinary and Life Sciences, Murdoch University, Murdoch, 6150 Australia

[3] Department of Plant and Soil Sciences, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

* e-mail: tanaybose15@gmail.com

Bose T., Burgess, T.I., Roux J., Wingfield M.J. (2017) *Phytophthora alticola*; emended description based on new collections and a neotype. – Sydowia 69: 161–170.

A *Phytophthora* diversity study in native forests and plantations of exotic *Acacia mearnsii* and *Eucalyptus grandis* yielded several isolates of an apparently new species in *Phytophthora* Clade 4. Multi-gene phylogeny placed these isolates in a single monophyletic clade together with sequence of an isolate CBS 121939 linked to the description of *Phytophthora alticola*. The morphological features of these new isolates were substantially different to those presented in the original description of *P. alticola,* but identical to an ex-paratype isolate (CBS 121939) of that species. The original description of *P. alticola* was likely based on mixed cultures and has been previously recognised as *nomen dubium*. In order to clarify this confusion, we have designated a neotype and emended *P. alticola* based on these new collections.

Keywords: *Eucalyptus grandis*, *Acacia mearnsii*, *Phytophthora arenaria*, *Phytophthora boodjera,* South Africa.

Intensively managed plantations of *Pinus*, *Eucalyptus*, and *Acacia* species provide the foundation for an important forestry industry in South Africa. *Phytophthora* diseases have been reported from these plantations as well as from natural forests in the country (Nagel et al. 2013). *Phytophthora* species causing root and collar-rot of various *Eucalyptus* and *Pinus* species in South Africa include *P. alticola, P. boehmeriae, P. cinnamomi, P. frigida* and *P. nicotianae* (Linde et al. 1994, Maseko et al. 2007). The most common *Phytophthora* disease of *Acacia mearnsii* is known as 'black butt' caused by *P. nicotianae* (Roux et al. 2012, Zeijlemaker 1971, Zeijlemaker & Margot 1970). Other *Phytophthora* species that are associated with *A. mearnsii* in South Africa include *P. boehmeriae* and *P. meadii* (Roux & Wingfield 1997). In addition, *P. cinnamomi* causes root-rot of several native plant species including those in the Ericaceae, Proteaceae and Bruniaceae (Von Broembsen 1984; Von BroembsenKruger 1985).

As part of ongoing projects to identify *Phytophthora* species associated with trees in South Africa, soil was collected from plantations of non-native *Acacia mearnsii* and *Eucalyptus grandis*, as well as from native forests in the vicinity of these plantations. Seven isolates of a Clade 4 *Phytophthora* species were recovered from both the native forest environment and planatations in Commondale, Melmoth and Vryheid. The aim of this study was to identify these isolates utilising morphological and molecular data.

Material and methods

Collection of soil samples and baiting

A total of 144 soil samples were collected from plantations of *E. grandis*, *A. mearnsii* and natural forests from four locations in Mpumalanga and KwaZulu-Natal provinces: Commondale, Howick, Melmoth and Vryheid. For each tree, the rhizosphere soil along with fine roots was collected after removing the leaf debris from the base of the tree and about 4 cm of the top soil.

All the soil samples were baited using leaves of *Rhododendron indicum*, *Hedera helix*, *Duranta repens*, *Hibiscus rosa-sinensis*, white rose petals and cotyledonous leaves of *Eucalyptus sieberi* within 2 days of collection. The baits were monitored regularly for 10 days for any signs of infection in the form of lesions. Lesions from the infected baits were

subsequently isolated on to NARPH medium (Masago et al. 1977) followed by establishment of pure cultures. Pure cultures were maintained on 10 % clarified V8-Agar (10 ml clarified V8 juice, Campbell Soup Company USA ; 15gm Difco™ Agar, Becton, Dickinson and Company, Sparks, USA) as well as half-strength Potato Dextrose Agar (PDA; Becton, Dickinson and Company, Sparks, USA, 19.5 g PDA, 7.5 g of agar and 1l of distilled water). Where the initial batings failed to show signs of infection after two weeks, the same soil was re-baited after drying at room temperature.

DNA isolation, amplification and sequencing

For each of the seven isolates requiring identification, mycelium was harvested from single 10-day-old cultures growing on PDA and genomic DNA was extracted using ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research, USA) following the manufacturer's protocol. Complete ITS (ITS1-5.8S-ITS2), β-tubulin, hsp90 and coxI gene regions were amplified using the respective primers (Tab. 1).

Individual PCR reactions were carried out using 5 µl of 5× GoTaq Flexi Buffer (Promega, MI), 2.5 µl of 25 mM $MgCl_2$ (Promega, MI) , 1.5 µl of 0.1 mM dNTPs (Promega, MI) , 1 µl BSA (Amresco, OH) , 1U GoTaq Hot Start Polymerase (Promega, MI), primers 0.5 µl each (Tab. 1) and the final volume was made up to 25 µl with PCR grade water. The amplifications were carried out with initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 sec, annealing temperature for 30 sec (Tab. 1), 72 °C for 1 min and final elongation at 72 °C for 5 min. Amplicons were sequenced at the DNA Sequencing Facility of the University of Pretoria. Geneious R8 (Kearse et al. 2012) was used to assemble the sequence data. GenBank accession numbers for sequences for the *Phytophthora* isolates considered in this study are provided in Tab. 2.

Phylogenetic analyses

Phylogenetic analyses of the sequence data were performed using both maximum likelihood (ML) and Bayesian (BA) approaches using RAxML-8 (Stamatakis 2014) and Mr. Bayes-3.2.6 (Huelsenbeck & Ronquist 2001) respectively. The datasets comprised of sequences of the unknown *Phytophthora* species, which were compared with those from the study of Simamora et al. (2015) and additional sequences of *Phytophthora* Clade 4 species retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), *Phytophthora* Database (http://

Tab. 1. Annealing temperature and time for primer pairs used for amplification of genomic DNA in the present study.

Gene	Primers		Annealing Temperature (°C)	Time (sec)	References
	Forward	Reverse			
ITS	ITS6	ITS4	55	30	Cooke et al. (2000), White et al. (1990)
β-tubulin	Btub_F1A	Btub_R1	60	30	Blair et al. (2008), Kroon et al. (2004)
cox1	FM84	FM83	56	60	Martin & Tooley (2003)
hsp90	HSP90_F1	HSP90_R2	62	30	Blair et al. (2008)

Tab. 2. GenBank accession numbers for *Phytophthora* isolates sequenced in the current study.

Taxon	Isolates		Gene regions			
			B-tubulin	coxI	hsp90	ITS
P. alticola	CBS141718	CMW48711	KX247592	KX247585	KX247578	KX247599
	CBS141719	CMW48712	KX247593	KX247586	KX247579	KX247600
	CBS141720	CMW48713	KX247594	KX247587	KX247580	KX247601
	CBS141721	CMW48714	KX247595	KX247588	KX247581	KX247602
	CBS141722	CMW48715	KX247596	KX247589	KX247582	KX247603
	CBS141723	CMW48716	KX247597	KX247590	KX247583	KX247604
	CBS141724	CMW48717	KX247598	KX247591	KX247584	KX247605
	CBS121939	CMW34279	KJ372275	KJ396686	KJ396703	HQ013214
P. quercetorum	CBS121119	–	KX759519	KX759520	KX759521	KX759518

www.phytophthoradb.org/) and qBank (http://www.q-bank.eu/). The datasets were aligned using MAFFT-7 (Katoh et al. 2002) and manually edited using Geneious R8 (Kearse et al. 2012). Individual gene region, *i.e.* ITS, β-tubulin, coxI, and hsp90 were analysed following the ML approach using a General Time Reversible model with gamma distribution (GTR GAMMA) as the substitution model selected through jModelTest-2.1.4 (Darriba et al. 2012). The concatenated dataset was analysed using both ML and BA approaches and also using GTR GAMMA model. The sequence alignments and trees were deposited in TreeBASE (Study ID# 20049).

Culture characteristics

Culture characteristics of the unknown *Phytophthora* species were determined on four growth media; 10 % Carrot Agar (CA; 10 ml fresh carrot juice; 15 g Difco™ agar and 1 l of deionized water), 2 % Malt Extract Agar (MEA; 20 g malt extract, Biolab, Merck, Midrand, South Africa; 15 g Difco™ agar and 1 l of deionized water), V8-Agar, and PDA. Circular inoculum plugs (5 mm diam.) were taken from the margins of 6-day-old cultures growing on V8A at 20 ºC in dark. Hyphal and colony morphology was defined using 7-day-old cultures grown at 20 °C in the dark. Colony morphology was described following the recommendations of Erwin & Ribeiro (1996).

All isolates of seven unknown *Phytophthora* species were sub-cultured onto V8A and CA plates and incubated for 24 h at 20 °C. Thereafter, five replicate plates per isolate were incubated at 4, 10, 15, 20, 25, 30, and 35 ºC (± 0.2 ºC). Colony diameters were measured daily from the fourth to seventh day. For viability assessment, plates incubated at 4, 10 and 35 °C were returned to 20 °C for 48 h to check for resumption of growth.

Morphology of reproductive structures

Agar blocks (1 × 1 cm) were cut from the actively growing edges of 6-day-old cultures of the unknown *Phytophthora* sp. on V8A and transferred to sterile petri dishes to produce sporangia. Sterile water was added to each of the Petri dishes containing 4–5 agar blocks and these were incubated under sunlight for a period of 6 hours at 20–25 °C. The water was replaced twice after 4 and 6 h. After the last water change, 1 ml of 10 % unsterilized soil extract was added and the plates were incubated overnight in dark. After 24–72 h, measurements were made for 100 randomly selected mature sporangia under 400x magnification using ZEISS Axioskop 50 and photographed using an AxioCam ICc5 camera.

Gametangia were produced by all the isolates on V8A agar incubated in the dark at 20 °C after 6–9 days. After 21 days, dimensions of 50 randomly selected mature oogonia, oospores and antheridia 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 analyses

Individual gene trees for the *Phytophthora* Clade 4 species had similar topologies (Fig. 1a) and were congruent with the tree generated using the concatenated dataset (Fig. 1b). All the isolates of the unknown *Phytophthora* species formed a monophyletic clade together with an isolate CBS 121939 (=CMW19425), which is the only available culture linked to the description of *P. alticola* (Fig. 1a,b). The species most closely related to the unknown isolates were *P. boodjera* and *P. arenaria* (Fig.1a,b).

Culture characteristics

Isolates of the unknown *Phytophthora* species produced appressed, cottony colonies with smooth and entire margins with no distinctive growth pattern on all four media (CA, V8A, PDA and MEA) after 7 days of incubation at 20 ºC in dark. The colonies produced on CA were dense and increasingly less so on V8A, PDA and MEA (Fig. 2).

The optimum temperature for growth on both V8A and CA for all isolates of the unknown *Phytophthora* species was 25 ºC (Fig. 2). The maximum and minimum temperatures for growth of the isolates were 15 ºC (Fig. 3, 1.05 ± 0.14 mm/day on V8A; 1.88 ± 0.31 mm/day on CA) and 30 ºC (Fig. 3, 2.92 ± 0.29 mm/day on V8A; 4.08 ± 0.29 mm/day on CA). The mean growth rates for the isolates were 3.50 ± 0.67 mm/day (V8A) and 7.42 ± 0.51 mm/day (CA) at 25 ºC (Fig. 3). All the isolates grew much more slowly at 20 ºC, 2.33 ± 0.49mm/day (V8A) and 3.75 ± 0.45 mm/day (Fig. 3, CA). In the viability assessment for the plates incubated at 4, 10 and 35 °C, growth was observed only on the plates incubated at 10 ºC.

Morphological comparisons

Isolates of the unknown *Phytophthora* sp. were morphologically identical to isolate CBS 121939 linked to the description of *P. alticola* (Tab. 3). The measurements for all reproductive structures of the

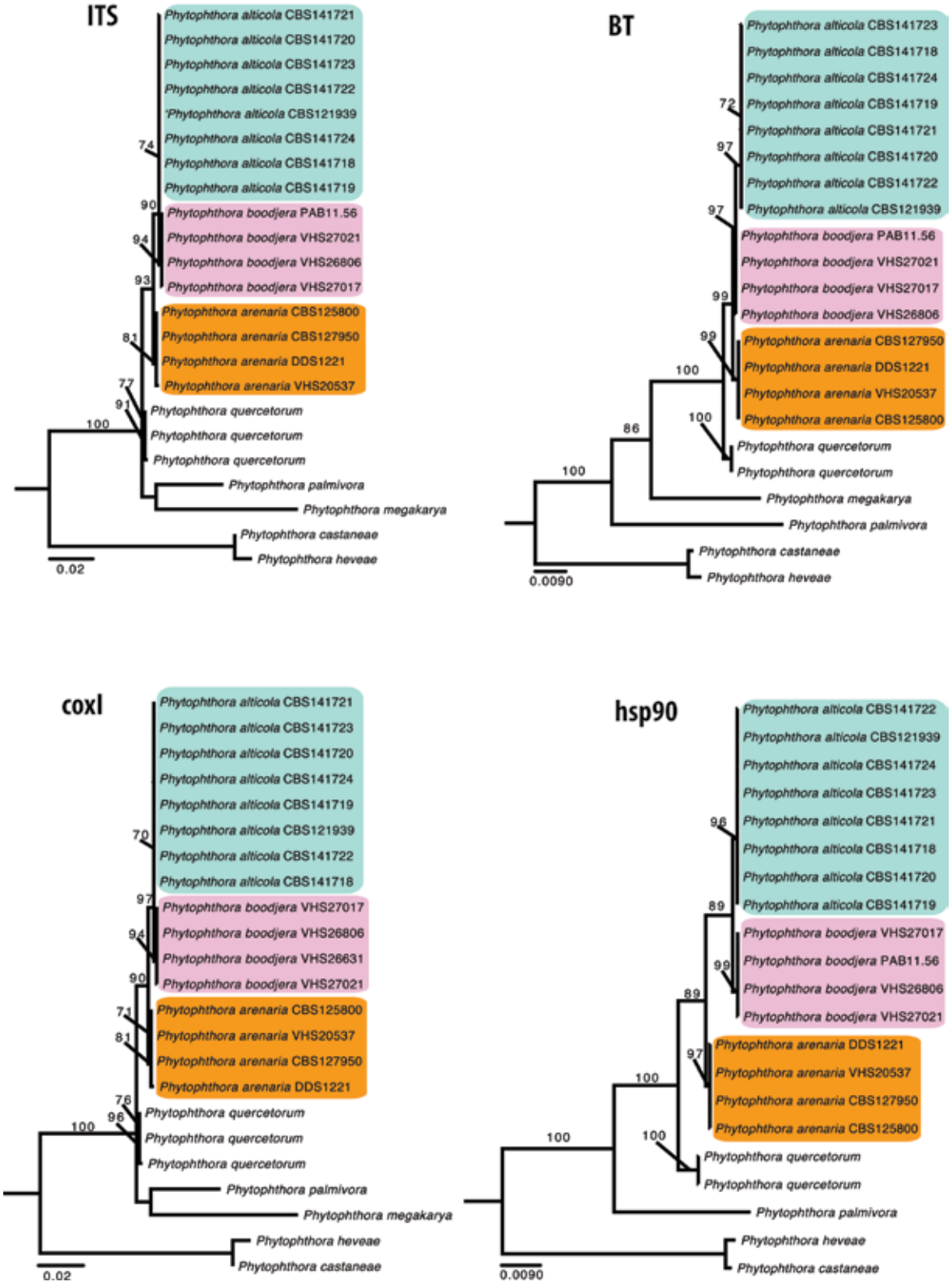


Fig. 1a. Maximum likelihood phylogenies of individual genes ITS, β-tubulin, heat shock protein 90 and cytochrome oxidase I (clockwise) for Clade 4 *Phytophthora* species. In all the trees CBS121939, an isolate of *P. alticola nom. dub.* nests within *P. alticola* isolates recovered from this study. Numbers on the nodes represent bootstrap percentages.

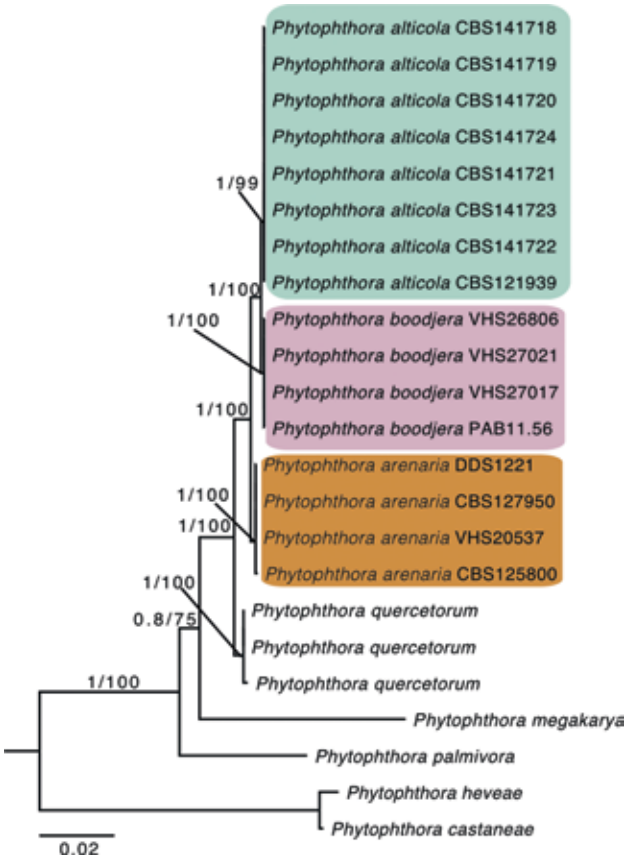


Fig. 1b. Maximum likelihood tree for Clade 4 *Phytophthora* species based on concatenated sequences of the ITS, beta-tubulin, cytochrome oxidase I and heat shock protein 90 gene regions. CBS121939, an isolate of *P. alticola nom. dub.* nests within a clade with *P. alticola* sequences recovered from this study. *Phytophthora boodjera* is a sister taxon to *P. alticola*. Numbers on the nodes represent posterior probabilities/bootstrap percentages.

unknown isolates overlapped with those of *P. boodjera*. However, the unknown isolates had a larger mean sporangial size and were mostly ovoid in contrast to ovoid-limoniform in *P. boodjera* (Tab. 3). The average oogonial and oospore size in the unknown isolates was smaller than those of *P. boodjera* (Tab. 3). The growth rate of the isolates of the unknown species was CA and V8A was 7.4 mm/day (25 °C) and 3.5 mm/day (25 °C) respectively and this is considerably lower than for *P. boodjera* (Table 3, Fig. 3). Maximum growth temperature for the isolates of the unknown *Phytophthora* sp. was 30 °C, which is 5 °C lower than for *P. boodjera* (Tab. 3, Fig. 3). The optimal growth temperature for the unknown *Phytophthora* sp. was 25 °C, while this ranged between 25–30 °C for *P. boodjera*. Lethal temperature for the unknown species was 35 °C, which is lower than that for *P. boodjera*. Based on DNA sequence data, the isolates representing the uknown *Phytophthora* sp. differed from *P. boodjera* by a single nucleotide polymorphism (SNP) in ITS, 2 in hsp90 and β-tubulin and 3 in the cox1 gene region.

Discussion

The isolates of a *Phytophthora* sp. collected in South African plantations and native forest are identical to the ex paratype culture CBS121939 of *P. alticola*. This species is known to have a confused identity which is in detail explained and discussed by Simamora et al. (2015) when describing *P. boodjera*, a close relative of *P. alticola*. In the original description of *Phytophthora alticola* Maseko et al. (2007) mixed three taxa and included three differ-

Tab. 3. Comparison of morphological characters and dimensions, and temperature-growth relations of *P. alticola*, *P. boodjera*, and *P. arenaria*. Main differences between original and new description are highlighted by shading.

Species and source of data	*P. alticola* (present study)	*P. 'alticola'* (CBS 121939) (Simamora et al. 2015)	*P. alticola* (Maseko et al. 2007)	*P. boodjera* (Simamora et al. 2015)	*P. arenaria* (Rea et al. 2011)
No. of isolates	7	1	10	12	10
Sporangia (µm)					
L×B mean	37.6 ± 3.2 × 28.8 ± 4.5	38.9 ± 5.4 × 28.6 ± 4.3	36 × 28	39.2 ± 4.4 × 29.7 ± 3.4	31.8 ± 4.6 × 23.7 ± 3.5
Range	20.8–45.3 × 18.4–33.7	20.4–60.7 × 19.0–38.9	30–45 × 20–35	15.2–64.5 × 13.9–42.5	20.2–53.0 × 12.5– 35.0
Range of isolates means	37.9 ± 4.1 × 27.2 ± 4.5	na	na	32.6–44.6 × 24.7–33.3	28.9–34.8 × 21.4–28.3
L/B ratio	1.28 ± 0.05	1.35 ± 0.03	1.22	1.27 ± 0.16	1.40 ± 0.17
Range of isolates means	1.16–1.33	na	na	1.19–1.35	1.2–1.5

Species and source of data	*P. alticola* (present study)	*P. 'alticola'* (CBS 121939) (Simamora et al. 2015)	*P. alticola* (Maseko et al. 2007)	*P. boodjera* (Simamora et al. 2015)	*P. arenaria* (Rea et al. 2011)
Sporangial characteristics	Papillate, frequently bipapillate, rarely bilobed.	Papillate, rarely bipapillate or bilobed	Papillate, rarely bipapillate	Papillate, rarely bipapillate or bilobed	Papillate, rarely bi/tripapillate or bilobed
Persistence	Persistent	Persistent	Caducous	Persistent	Persistent
Sporangiophores	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	Simple or branched sympodia often with bulbous base, very often laterally attached	Simple or branched sympodia often with bulbous base
Sporangia shape	Ovoid 87%, obpyriform 9%, distorted 4%	Ovoid 66%, limoniform 14%, peanut-shaped 8%, obpyriform 6%, Distorted 6%	Usually ovoid or ellipsoid, sometimes obpyriform or peanut-shaped	Ovoid 64%, Limoniform 20%, peanut-shaped 10%, distorted 6%	Usually ovoid, also obpyriform or distorted
Proliferation	Absent	Absent	Absent	Absent	Absent
Exit pores (µm)					
Width	6.53 ± 1.27	6.21 ± 0.53	6	6.09 ± 1.02	6.00 ± 1.00
Width range	6.07–8.7	5.00–7.10	4–8	4.85–8.89	3.40–8.90
Breeding system	Homothallic	Homothallic	Homothallic	Homothallic	Homothallic
Oogonia (µm)					
Mean diameter	27.6 ± 1.7	27.3 ± 1.9	28.4	29.4 ± 2.3	25.3 ± 2.2
Diameter range	22.4–30.3	22.0–31.0	20–35	24.3–33.9	19.6–34.3
Range of isolates means	20.4–32.3	Na	Na	24.6–33.4	24.3–28.1
Chlamydospores	absent	absent	Some isolates 28 (20–35)	absent	absent
Oospore (µm)					
Mean diameter	24.7 ± 1.9	24.9 ± 2.1	30 (28.3 x 30.5)	25.5 ± 1.9	22.3 ± 1.8–
Diameter range	19.1–29.2	20.3–29.5	24–36	20.92–29.3	16.0–28.3
Range of isolate mean	23.03 ± 2.47	na	na	21.3–29.5	21.4–23.9
Wall thickness	2.48 ± 0.14	2.51 ± 0.4	na	2.47 ± 0.33	2.30 ± 0.34
Oospore wall index	0.51 ± 0.07	0.54 ± 0.05	na	0.47 ± 0.05	0.50 ± 0.05
Oogonial characteristics	Aplerotic oospores, mature oogonia with a slightly wavy surface and golden-brown in colour	Aplerotic oospores, mature oogonia with a slightly wavy surface and golden-brown in colour	Markedly aplerotic, oospores with thick inner walls	Aplerotic oospores, mature oogonia with a slightly wavy surface and golden-brown in colour	Aplerotic oospores, mature oogonia with a slightly wavy surface and golden-brown in colour
Antheridia (µm)					
Position	Paragynous, often with finger-like projections	Paragynous, often with finger-like projections	Mainly amphigynous	Paragynous	Paragynous, often with finger-like projections

Species and source of data	*P. alticola* (present study)	*P. 'alticola'* (CBS 121939) (Simamora et al. 2015)	*P. alticola* (Maseko et al. 2007)	*P. boodjera* (Simamora et al. 2015)	*P. arenaria* (Rea et al. 2011)
LxB mean	10.2 ± 1.2 x 8.2 ± 1.7	10.6 ± 2.3 × 8.3 ± 1.4	na	10.4 ± 1.9 × 8.3 ± 1.5	11.2 ± 1.7 × 8.4 ± 1.3
LxB range	6.2–12.8 × 5.7–10.7	na	na	7.9–16.4 x 6.0–10.5	7.9–16.4 x 6.0–10.5
Growth characteristics					
Max temp (ºC)	30	35	30–<35	35	32.5
Opt temp (ºC)	25	20–25	25	25–30	30
Min temp (ºC)	>10<15	>10<15	>10<15	>10<15	>10<15
Lethal temp (ºC)	35	>37.5	na	>37.5	na
Growth rate at optimum (mm/day)	3.50 (V8A) 7.42 (CA)	8.20 (V8A)	7 (V8A) 4.5 (CA)	9.18 (V8A)	5.9–7.4 (V8A)
Growth rate at 20ºC (mm/day)	2.33 (V8A) 3.75 (CA)	7.75 (V8A)	4.5 (V8A) 3.0 (CA)	6.12 (V8A)	3.8–5.2 (V8A)
Colony morphology	Appressed and cottony with no distinctive growth pattern and regular smooth margins on CA, V8A and PDA; sparse, slow growth on MEA	Appressed and cottony with no distinctive growth pattern and regular smooth margins on CA, V8A and PDA; sparse, slow growth on MEA	Uniform and fluffy on MEA and V8A, stellate with limited aerial mycelium on CA and PDA	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

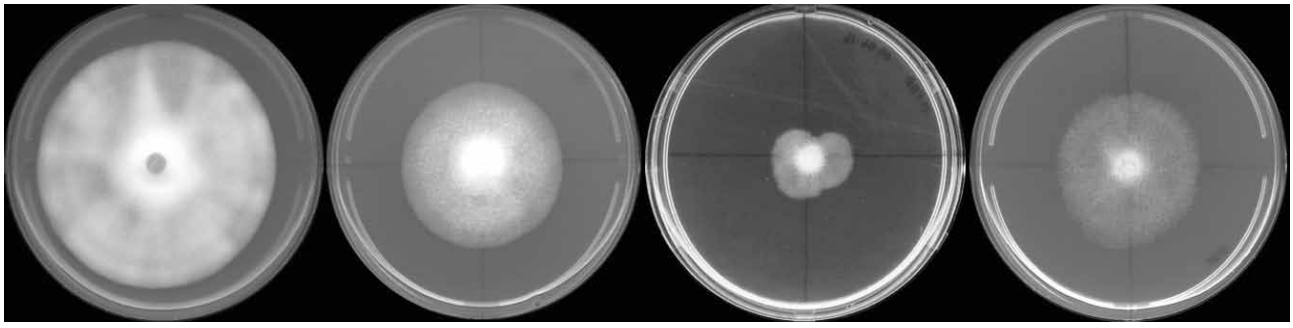


Fig. 2. Colony morphology of *Phytophthora alticola* (CBS141718) after 7 days growth at 20 °C on different media: CA, V8A, PDA and MEA (left to right).

ent sets of morphometric data for *P. alticola* which was obviously caused by the fact that the description was based on the examination of a set of isolates belonging to three different species. The extype isolate of *P. alticola* CBS 121937 (= CMW 19417) submitted to CBS and the World *Phytophthora* Genetic Resource Collection (WPC) turned out to be *P. palmivora*. Another paratype isolate CBS 121938 (= CMW 19424) proved to be *P. frigida*. Only one existing isolate linked to the original description of *P. alticola*, CBS 121939, turned out to be a very close relative to *P. arenaria* and *P. boodjera* from Clade 4 (Hawksworth, pers . comm.). Simamora et al. (2015) concluded that *P. alticola* should be treated as a *nomen dubium*. The sequences available for isolate CBS 121939 differed to those presented in the original description of *P. alticola* by Maseko et al. (2007). Morphological examination of

isolate CBS 121939 also showed that it differed markedly from the original description of Maseko et al. (2007). Specifically, the sporangia were not caducous, chlamydospores were absent and the antheridia were paragynous. Simamora et al. (2015) also examined the dried holotype and paratype specimens of *P. alticola*, these provided inconclusive results and appeared to represent several different species of *Phytophthora* as mentioned above. This included the paratype PREM59217 supposedly linked to isolate CBS 121939. Only oospores were preserved and these had amphigynous antheridia. However, subsequent morphological re-examination of CBS 121939 by Simamora et al. (2015) showed that this isolate produced paragynous antheridia with finger-like projections.

Due to the erronous identifications of the type specimens and the fact that the ex-paratype isolate CBS 121939 does not fit the description of *P. alticola* presented by Maseko et al. (2007) we decided to stabilize the identity of *P. alticola* by proposing a neotype for *P. alticola*. The original holotype (PREM 59215= CMW19417=CBS121937) and paratype (PREM 59214= CMW 19416, PREM59216 = CMW19424=CBS121938, and PREM59217=CMW 19425 = CBS121939) material for this species does not represent *P. alticola*. The neotype presented here as well as other isolates considered in the present study should be used.

Emended description

Phytophthora alticola Maseko, Cout. & M.J. Wingf., Mycological Research 111 (11): 1332 (2007) ***emend.*** – Figs. 4–17

MycoBank No.: MB511177

Neotype (here designated). – SOUTH AFRICA, KwaZulu-Natal, Commondale, from *Eucalyptus grandis* soil sample (S27 14.384 E31 00.904), November 2014, leg. T. Bose neotype PREM61767, ex-neotype culture CBS141718= CMW48711.

Description. – Sporangia papillate, persistent, abundantly produced in non-sterile soil extract water (Figs. 4–7), predominantly ovoid (87 %, Figs. 4–7); other sporangial shapes include obpyriform (9 %,) and distorted shapes (4 %, Fig. 6). Bipapillate (Fig. 6) sporangia were also frequently observed. Sporangiophore simple (Figs. 4,6–8) or rarely branched usually with bulbous base (Fig. 5), attachment usually basal (Fig, 4), often lateral (Figs. 5, 7). Sporangia from seven isolates 19.57–49.19 × 17.39–36.72 (37.89 ± 4.14 × 27.19 ± 4.52) µm, exit pores narrow 5.62–7.75 (6.53 ± 1.27) µm, length: breadth ratio 1.26 ± 0.04. Chlamydospores absent. Homothallic, readily producing oogonia in single culture on V8A. Oogonia matured within 10 to 14 d. Oogonia 20.43–32.33 (27.99 ± 2.48, Figs. 8–13) µm diam., with simple (Fig. 9–13) or bulbous stalk 12.33–18.56 × 6.1–11.23 µm (15.69 ± 2.47 × 7.55 ± 1.84, Fig. 8) µm. Occasionally oogonia peanut-shaped (Fig. 11) or distorted, in chain of 2–3 or branched (Fig. 13). Oospores aplerotic, with slightly wavy surface (Fig. 10), on maturation golden brown. Oospores 19.1–29.23 (23.03 ± 2.47) µm diam.. Oospore walls thick 2.0–2.58 (2.48 ± 0.14, Figs. 10–13) µm, oospore wall index 0.51 ± 0.07 µm. Antheridia paragynous (Figs. 14–15), 6.23–12.78 × 5.66–10.67 (9.92 ± 1.56 x 8.15 ± 1.97) µm. Hyphae coenocytic, septate on maturation, highly branched, often forming coils (Figs. 16–17).

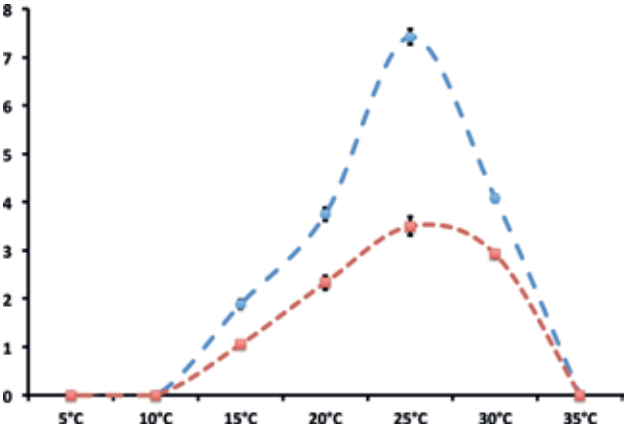


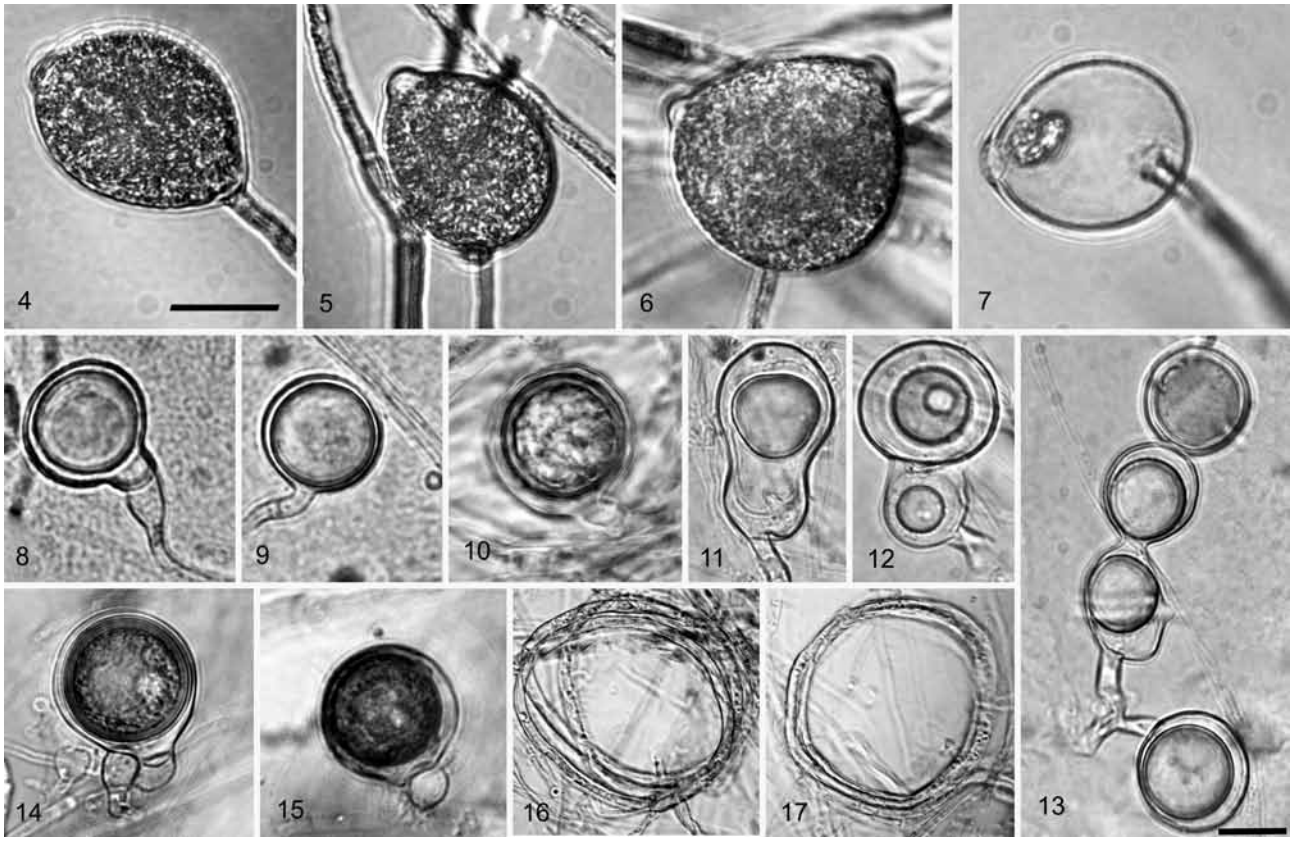
Fig. 3. Mean radial growth rates of *Phytophthora alticola* (seven isolates) on CA (blue line) V8A (red line) at different temperatures. Bars on plot points indicate standard errors.

Distribution. – South Africa, KwaZulu-Natal Province

Substrate. – Isolated by baiting from rhizospheric soil collected from *E. grandis* and *A. mearnsii* plantations, and native forests.

Additional specimens examined. – SOUTH AFRICA. KwaZulu-Natal, Melmoth, from natural forest soil sample (S28 39.919 E31 25.597), November 2015, *leg.* T. Bose, CBS141719= CMW48712; (S28 40.505 E31 25.135), November 2015, *leg.* T. Bose, CBS141720 = CMW48713; Melmoth, from *E. grandis* soil sample (S28 39.793 E31 25.662), March 2016, *leg.* T. Bose, CBS141721 = CMW48714; (S28 39.721 E31 25.604), March 2016, *leg.* T. Bose, CBS141722 = CMW48715. Commondale, from *A. mearnsii* soil sample (S14.527 E31 00.144), March 2016, *leg.* T. Bose, CBS141723 = CMW48716. Vryheid, from *E. grandis* soil sample (S27 39.100 E30 42.928), March 2016, *leg.* T. Bose, CBS141724= CMW48717.

Notes. – *Phytophthora alticola* is both morphologically and phylogenetically closely related to *P. boodjera* and *P. are-*



Figs. 4–17. Micrographs of *Phytophthora alticola*. **4**–**7** Papillate sporangia, ovoid (4, 5, 7), bipapillate (6) often with laterally attached sporangiophore (5), occasional swollen sporangiophore attachment (5); **8**–**13** Oogonia with bulbous (8) and slender (9) stalk, wavy margins and turning golden brown at maturity (10), occasionally oogonia were peanut-shaped (11), or distorted, in chain of 2–3 or branched (12, 13), with aplerotic oospores. **14**–**15** paragynous antheridia. **16**–**17** Hyphal coils. Scale bar = 20 µm (4–12, 14–17), 20 µm (13).

naria, species recovered frequently from nurseries and natural ecosystems in Western Australia. The dimensions of the reproductive structures for *P. alticola* either overlap with those of *P. boodjera* or in some cases they are close to both *P. boodjera* and *P. arenaria*. Phylogenetically *P. alticola* is a sister taxon to *P. boodjera* and its next closest relative is *P. arenaria*. Although these three species have few differences in the gene regions included here for comparison, they resided in distinct monophyletic clades in the phylogenetic analyses, consistent with the analyses performed by Simamora et al. (2015).

Phytophthora alticola was first isolated as a pathogen of cold-tolerant *Eucalyptus* species from KwaZulu-Natal (Maseko et al. 2007). This taxon was not subsequently isolated in surveys conducted in South Africa (Nagel et al. 2013, Oh et al. 2013). It is therefore interesting that *P. alticola* emerged as a relatively common species in the present study. This species was isolated from undisturbed natural forests as well as from *E. grandis* and *A. mearnsii* plantations and has not, to date, been recovered elsewhere in the world. The results suggests *P. alticola* is probably native to South Africa.

Acknowledgements

We thank Dr. Stephen Taerum, Dr. Alistair McTaggart, Mr. Jan Nagel, Me. Mandy Messal, Mr. Conrad Trollip, Mr. Joey Hulbert and the foresters from NCT Forestry Co-operative Limited and TWK Agri for their assistance with surveys and access to field sites. Mrs. Diane White from the Centre for *Phytophthora* Science and Management, Murdoch University provided sequences of *Phytophthora quercetorum* (CBS121119). We acknowledge funding from the University of Pretoria, members of the Tree Protection Cooperative Programme (TPCP), and the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB), South Africa.

References

Blair J.E., Coffey M.D., Park S.-Y., Geiser D.M., Kang S. (2008). A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genetics and Biology* **45**: 266–277.

Cooke D., Drenth A., Duncan J., Wagels G., Brasier C. (2000). A molecular phylogeny of *Phytophthora* and related Oomycetes. *Fungal Genetics and Biology* **30**: 17–32.

Darriba D., Taboada G. L., Doallo R., Posada D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* **9**: 772–772.

Dick M. (1990). Keys to *Pythium*. Reading, UK: University of Reading.

Erwin D.C., Ribeiro O.K. (1996). *Phytophthora* diseases worldwide. St. Paul, Minnesota: American Phytopathological Society.

Huelsenbeck J.P., Ronquist F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Katoh K., Misawa K., Kuma K.I., Miyata T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Kroon L., Bakker F., Van Den Bosch G., Bonants P., Flier W. (2004). Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* **41**: 766–782.

Linde C., Kemp G., Wingfield M. (1994) *Pythium* and *Phytophthora* species associated with eucalypts and pines in South Africa. *European Journal of Forest Pathology* **24**: 345–356.

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

Masago H., Yoshikawa M., Fukada M., Nakanishi N. (1977) Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soils and plants. *Phytopathology* **67**: 425–428.

Maseko B., Burgess T.I., Coutinho T.A., Wingfield M.J. (2007) Two new *Phytophthora* species from South African *Eucalyptus* plantations. *Mycological Research* **111**: 1321–1338.

Nagel J.H., Gryzenhout M., Slippers B., Wingfield M.J. (2013) The occurrence and impact of *Phytophthora* on the African continent. In: Lamour K. (ed.) *Phytophthora*: a global perspective. United Kingdom: CABI International. pp. 204–214.

Oh E., Gryzenhout M., Wingfield B.D., Wingfield M.J., Burgess T.I. (2013) Surveys of soil and water reveal a goldmine of *Phytophthora* diversity in South African natural ecosystems. *IMA Fungus* **4**: 123–131.

Rea A., Burgess T., Hardy G.S.J., Stukely M., Jung T. (2011) Two novel and potentially endemic species of *Phytophthora* associated with episodic dieback of Kwongan vegetation in the south west of Western Australia. *Plant Pathology* **60**: 1055–1068.

Roux J., Hurley B., Wingfield M., Bredenkamp B., Upfold S. (2012) Diseases and pests of eucalypts, pine and wattle. *South African Forestry Handbook*, pp. 303–335.

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

Simamora A.V., Stukely M.J., Hardy G.E.S., Burgess T.I. (2015) *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*. *IMA Fungus* **6**: 319–335.

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

Von Broembsen S. (1984).Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. *Phytophylactica* **16**: 221–225.

Von Broembsen S., Kruger F. (1985) *Phytophthora cinnamomi* associated with mortality of native vegetation in South Africa. *Plant Disease* **69**: 715–717.

White T.J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications **18**: 315–322.

Zeijlemaker F.C.J. (1971) Black-butt disease of black wattle caused by *Phytophthora nicotianae* var. *parasitica*. *Phytopathology* **61**: 144–145.

Zeijlemaker F.C.J., Margot P. (1970) Black-butt disease of black wattle. Report Wattle Research Institute, University of Natal **1971**: 49–50.

(Manuscript accepted 2 May 2017; Corresponding Editor: I. Krisai-Greilhuber)