Plant Pathology (2014) **63**, 1095–1109 Doi: 10.1111/ppa.12194

Phytophthora pachypleura sp. nov., a new species causing root rot of *Aucuba japonica* and other ornamentals in the United Kingdom

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Isolates of an unknown *Phytophthora* species from the '*Phytophthora citricola* complex' have been found associated with mortality of *Aucuba japonica* in the UK. Based on morphological characteristics, growth–temperature relationships, sequences of five DNA regions and pathogenicity assays, the proposed novel species is described as *Phytophthora pachypleura*. Being homothallic with paragynous antheridia and semipapillate sporangia, *P. pachypleura* resembles other species in the '*P. citricola* complex' but can be discriminated by its distinctively thick-walled oospores with an oospore wall index of 0·71. In the phylogenetic analysis based on three nuclear (ITS, β-tubulin, *EF-1α*) and two mitochondrial (*cox1*, *nadh1*) DNA regions, *P. pachypleura* formed a distinct clade within the '*P. citricola* complex' with *P. citricola s. str.*, *P. citricola* E and *P. acerina* as its closest relatives. *Phytophthora pachypleura* is more aggressive to *A. japonica* than *P. plurivora* and *P. multivora* and has the potential to affect other ornamental species.

Keywords: pathogenicity, phylogeny, *Phytophthora citricola*, *Phytophthora multivora*, *Phytophthora plurivora*

Introduction

Currently, more than 120 *Phytophthora* species are officially described and another 40–50 *Phytophthora* taxa have been informally designated (Erwin & Ribeiro, 1996; Blair *et al.*, 2008; Brasier, 2009; Jung *et al.*, 2011; Hansen *et al.*, 2012). Species of *Phytophthora* are among the most significant pathogens affecting a broad range of horticultural, forest and ornamental plant species, including annuals, perennials, trees and shrubs.

The profile of *Phytophthora* changed considerably after several epidemic disease outbreaks which have occurred since the 1990s, including dieback of cork and holm oaks in southern Europe (e.g. Jung *et al.*, 2013), oak and beech decline in central and northern Europe (Jung, 2009; Jung *et al.*, 2013), alder mortality across Europe (Brasier & Kirk, 2001; Brasier, 2008; Jung *et al.*, 2013) and most significantly ramorum dieback or sudden oak death, which caused high mortality in native oak and tanoak populations in California and Oregon (Rizzo *et al.*, 2002) and in Japanese larch plantations in the UK (Webber *et al.*, 2010).

Plant trade has been recognized as a major pathway for the introduction and spread of exotic invasive *Phytophthora* species (e.g. Brasier, 2008; Jung, 2009; Jung *et al.*, 2013). During intensive surveys for *Phytophthora* in nurseries, plantations and seminatural ecosystems in Europe, North America and Australia (e.g. Brasier, 2009; Jung *et al.*, 2011, 2013; Hansen *et al.*, 2012) and recent expeditions in remote areas of Asia and South America, many new *Phytophthora* species have been detected and the origins and possible pathways of well-known *Phytophthora* species have been elucidated (Brasier *et al.*, 2010; Vettraino *et al.*, 2011; Huai *et al.*, 2013; Y. Balci, University of Maryland, USA, personal communication).

In addition, molecular tools have developed rapidly since the publication of the first ITS-based phylogeny of *Phytophthora* (Cooke *et al.*, 2000). Several nuclear and mitochondrial regions are now available for species comparison and identification (Martin & Tooley, 2003; Kroon *et al.*, 2004; Blair *et al.*, 2008). This has helped to differentiate cryptic species and unravel complexes of morphologically similar, phylogenetically related, species such as the '*Phytophthora citricola* complex' (Jung & Burgess, 2009; Bezuidenhout *et al.*, 2010; Hong *et al.*, 2011). Detailed phenotypic and phylogenetic analyses resulted in the separation of the morphospecies *P. citricola sensu lato* into *P. citricola sensu stricto* and seven new closely related taxa that together form the '*P. citricola* complex' in ITS Clade 2a, i.e. *P. capensis*, *P. multivora*, *P. pini*, *P. plurivora*, *P. citricola* III, *P. citricola* E and *P.* taxon 'emzansi' (Hong *et al.*, 2009, 2011; Jung & Burgess, 2009; Scott *et al.*, 2009; Bezuidenhout *et al.*, 2010). In addition, two other new species, *P. mengei* and *P. elongata*, were segre-

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Published online 6 March 2014

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gated and belong to the more distantly related subclade 2b (Hong *et al.*, 2009; Rea *et al.*, 2010).

Whilst there are many published reports on the incidence of *Phytophthora* species in the wide environment and on ornamental plants grown in nurseries, gardens in the UK have received little attention when it comes to surveying for species other than *P. ramorum* and *P. kernoviae*. In the UK, gardens are an important ecosystem, covering an area of 432 924 ha and containing around 28·7 million trees (Davies *et al.*, 2009). At the Royal Horticultural Society (RHS) Advisory Service, *Phytophthora* diseases are among the most frequently diagnosed causes of plant death in gardens, with species of the '*P. citricola* complex' being most common followed by *P. cryptogea* and *P. infestans* (RHS, unpublished data).

Aucuba japonica, commonly called spotted laurel, is native to Japan and was introduced to the UK in 1783 (Reader's Digest's, 2001). It is widely cultivated for the ornamental effect of its foliage and red berries and the ability to grow on any soils in deep shade. Since 2001, an unidentified *Phytophthora* species closely related to *P. citricola s. str.* and its relatives in the '*P. citricola* complex' has been found associated with root rot on *A. japonica* and also sporadically on other hosts growing in UK gardens. On *A. japonica*, above-ground symptoms include blackened leaves, branch dieback and eventually plant death. Due to its unique combination of morphological features, nuclear and mitochondrial sequences and pathogenicity to *A. japonica* and several other ornamental hosts, this taxon is described here as a new species, *Phytophthora pachypleura* sp. nov.

Material and methods

Phytophthora isolation and storage

Phytophthora species were isolated from rhizosphere soil, root and stem samples using different baiting techniques (apples, hemp seeds or rhododendron leaves) or by direct plating of necrotic roots or stem onto selective media as follows. Roots were thoroughly washed to eliminate adhering soil particles before being baited or plated. Soil, stem, root and leaf samples of plants with symptoms received at the RHS Advisory Service at Wisley were flooded in 6 × 6 × 8 cm plastic pots overnight. Five or six hemp seeds (Nature's Harvest) previously sterilized at 121°C for 30 min were added to the water and left overnight. Following overnight incubation, the plant and soil samples were baited using green apple fruits ('Granny Smith') as baits (Erwin & Ribeiro, 1996). Apple baits were incubated in the dark at 20°C for 2 weeks. Hemp seeds were removed from the water using sterile tweezers and dried on filter paper (Whatman grade 2). Hemp seeds were then plated on a P5ARP medium (cornmeal agar amended with 5 mg L^{-1} pimaricin, 250 mg L^{-1} ampicillin, 10 mg L^{-1} rifampicin, 100 mg L^{-1} PCNB; Erwin & Ribeiro, 1996) and incubated at 20°C in the dark. Infected apple tissues were removed aseptically with a scalpel from the margin of the necrosis and plated on P5ARP medium. Plates were incubated at 20°C in the dark.

For baiting with rhododendron leaves, 1-year-old or older, freshly picked leaves of *Rhododendron catawbiense* 'Cunningham's White' were washed under running tap water and blotted dry on filter paper. The roots and/or soil were placed in a plastic tray and flooded with filtered pond water and the intact rhododendron leaves floated on the top of water. Enough water was added in order to make sure there was no direct contact between the leaves and the soil or roots. The trays were left at room temperature (18–25°C) on the bench. After 2–8 days, sections of 5 mm^2 were aseptically removed from the margins of developing lesions, plated onto P5ARP and incubated at 20°C in the dark.

For direct isolation from necrotic tissues, sections of root, stem and leaf samples of plants with symptoms were cut into small pieces (5 × 2 mm or 5 mm^2) with a sterilized scalpel. After being left overnight in tap water, the samples were blotted dry on filter paper and plated on P5ARP medium and incubated at 20°C in the dark.

Single hyphal tip cultures were obtained by transferring individual hyphal tips from the P5ARP plates onto carrot agar (CA; Erwin & Ribeiro, 1996; 200 g carrots macerated and mixed in a blender with approximately 500 mL cold tap water and filtered through a muslin cloth, the final volume was adjusted to 1 L and 15 g of agar added and autoclaved twice at 121°C for 30 min). For long-term storage, the isolates were subcultured on oatmeal agar slopes (Sigma-Aldrich) covered with paraffin oil. The slopes were kept in the dark at 10°C.

Phytophthora isolates

The isolates used in the morphological, temperature–growth rate and phylogenetic studies are given in Table 1.

DNA isolation, amplification and sequencing

Phytophthora cultures were transferred onto CA plates, overlaid with a washed and autoclaved cellophane disc and incubated at 20°C in the dark. After 7 days, the mycelium was scraped from the cellophane and stored at −80°C until DNA extraction.

The mycelium and samples of leaves, roots and stems from plants with symptoms were ground in liquid nitrogen using a pestle and mortar and the DNA extracted using the Plant DNeasy Mini kit (QIAGEN) according to the manufacturer's instructions.

DNA was extracted from soil by bead beating in a Mini-Beadbeater (Biospec). The method was developed by Danny Cullen at SCRI (D. E. L. Cooke, The James Hutton Institute, Dundee, UK, personal communication). Dried soil samples (10 g) were suspended in 20 mL extraction buffer (0·12 M Na_2HPO_4, 1·5 M NaCl, 2% CTAB). Samples were broken up by vortex mixing and aliquots of 1 mL of each soil suspension were disrupted with 0·1 g 1-mm sterile glass beads on a bead beater at 5000 oscillations min^{-1} for 1 min. Soil debris was pelleted by centrifugation at 3800 ***g***. The supernatant was mixed by inversion for 1 min with 750 μL chloroform and the mixture spun at 17 900 ***g*** for 5 min. The upper layer was transferred into a fresh tube containing 750 μL isopropanol and incubated for 30–60 min at room temperature. The precipitated DNA was collected by centrifugation at 17 900 ***g*** for 5 min. The resulting pellet was air dried and resuspended in 100 μL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8·0). DNA solutions of plant material and soil were further purified in Micro-Biospin columns (Bio-Rad) containing PVP (Polyclar SB100; Merck) and stored at −20°C.

The complete ribosomal RNA (rRNA) internally transcribed spacer (ITS) regions and the 5·8S gene were amplified by a semi-nested PCR reaction using PCR beads (Amersham Pharmacia Biotech) according to the manufacturer's instructions. In the first round, 3 μL DNA sample, the primers ITS4 (White *et al.*, 1990)

Table 1 Isolates and species of *Phytophthora* used in this study

Isolate no.[a]	Identification	Host	Sample	Location	Year	References	ITS	β-tubulin	*EF-1α*	*cox1*	*nadh1*
CBS133931, B057[b]	*P. acerina*[c]	*Acer pseudoplatanus*	Plant tissue	Italy, Milan	2010	Ginetti *et al.* (2013); this study	KC855340	KC855364	KC855388	KC855436	KC855412
B080[b]	*P. acerina*	*A. pseudoplatanus*	Rhizosphere	Italy, Milan	2010	Ginetti *et al.* (2013); this study	KC855341	KC855365	KC855389	KC855437	KC855413
P1823	*P. capensis*	*Olea capensis*	n.a.	South Africa	2004–5	Bezuidenhout *et al.* (2010)	GU191231	GU191327	GU191198	GU191298	GU191256
P1819	*P. capensis*[c]	*Curtisia dentata*	Roots	South Africa	2004–5	Bezuidenhout *et al.* (2010)	GU191232	GU191328	GU191199	GU191275	GU191257
P5843.2000[de]	*P. cinnamomi*	*Taxus baccata*	Roots	UK	2000	This study	KC855342	KC855366	KC855390	KC855438	KC855414
IMI021173, CBS 221.88, 33H8	*P. citricola s. str.*[c]	*Citrus sinensis*	Fruit	Taiwan	1927	Scott *et al.* (2009); Hong *et al.* (2011)	FJ237526	FJ665256	GQ247662	FJ237512	GQ247671
CBS295.29[b]	*P. citricola s. str.*[f]	*Citrus* sp.	Leaf	Japan	1929	This study	KC855336	KC855360	KC855384	KC855432	KC855408
ATCC64811, P1814	*P. citricola s.str.*	*Citrus* sp.	n.a.	South Africa	n.a.	Bezuidenhout *et al.* (2010)	GU191217	GU191321	GU191192	GU191278	GU191263
P1815	*P. citricola s. str.*	*Citrus* sp.	n.a.	South Africa	n.a.	Bezuidenhout *et al.* (2010)	GU191218	GU191322	GU191193	GU191274	GU191264
P1321	*P. citricola* E.[g]	*Rubus idaeus*	n.a.	USA, California	n.a.	Bezuidenhout *et al.* (2010)	GU191216	GU191320	GU191191	GU191296	GU191262
P6624	*P. citricola* E.[g]	*Fragaria* sp.	n.a.	Taiwan	n.a.	Bezuidenhout *et al.* (2010)	GU191230	GU191326	GU191197	GU191280	GU191255
1E1; P130; SG-R-1; MYA-3658	*P. citricola* III	n.a.	Irrigation water	USA, Oklahoma	n.a.	Hong *et al.* (2011)	FJ392326	GU071235	GU071242	GU071239	GU071246
15C9	*P. citricola* III	*Acer saccharum*	n.a.	USA, Wisconsin	1985	Hong *et al.* (2011)	FJ392327	GU071236	GU071243	GU071240	GU071247
P0513	*P. mengei*	*Persea americana*	n.a.	Mexico	n.a.	Bezuidenhout *et al.* (2010)	GU191234	GU191332	GU191203	GU191295	GU191261
P1165	*P. mengei*	*P. americana*	n.a.	Guatemala	n.a.	Bezuidenhout *et al.* (2010)	GU191235	GU191331	GU191202	GU191299	GU191260
CBS 54996	*P. multivesiculata*	*Cymbidium* sp.	Stem base	Netherlands, Mijdrecht	Deposited in 1996	Kroon *et al.* (2004); Robideau *et al.* (2011)	HQ643288	AY564080	AY564136	AY564195,	AY564022
CBS 124094, WAC 13201	*P. multivora*[c]	*Eucalyptus marginata*	Rhizosphere	Western Australia, Yalgorup	2007	Scott *et al.* (2009)	FJ237521	FJ665260	n.a.	FJ237508	KF233986
RHS226.2001[e]	*P. multivora*	*Ceanothus* sp.	Soil	UK	2001	This study	KC855319	KC855343	KC855367	KC855415	KC855391
P1817	*P. multivora*	*Medicago sativa*	n.a.	South Africa	n.a.	Bezuidenhout *et al.* (2010)	GU191221	GU191323	GU191194	GU191297	GU191265
IMI502404, RHS53593.1[bdeh]	*P. pachypleura*[c]	*Aucuba japonica*	Roots	UK, Cheshire	2008	This study	KC855330	KC855354	KC855378	KC855426	KC855402
RHS2474.2001[bh]	*P. pachypleura*	*A. japonica*	Roots	UK, Glamorgan	2001	This study	KC855321	KC855345	KC855369	KC855417	KC855393
RHS4187S.2006	*P. pachypleura*	*A. japonica*	Soil	UK, Essex	2006	This study	KC855322	KC855346	KC855370	KC855418	KC855394

(continued)

Table 1 (continued)

Isolate no.[a]	Identification	Host	Sample	Location	Year	References	ITS	β-tubulin	*EF-1α*	*cox1*	*nadh1*
RHS5955.2006	*P. pachypleura*	*A. japonica*	Soil	UK, Surrey	2006	This study	KC855323	KC855347	KC855371	KC855419	KC855395
RHS4187.2006[bh]	*P. pachypleura*	*A. japonica*	Stem base	UK, Essex	2006	This study	KC855324	KC855348	KC855372	KC855420	KC855396
RHS7540	*P. pachypleura*	*A. japonica*	Soil	UK, Dorset	2007	This study	KC855325	KC855349	KC855373	KC855421	KC855397
RHS20408[bdh]	*P. pachypleura*	*A. japonica*	Roots	UK, Hampshire	2007	This study	KC855326	KC855350	KC855374	KC855422	KC855398
RHS38027[bdh]	*P. pachypleura*	*A. japonica*	Roots	UK, Surrey	2007	This study	KC855327	KC855351	KC855375	KC855423	KC855399
RHS20408S	*P. pachypleura*	*A. japonica*	Soil	UK, Hampshire	2007	This study	KC855328	KC855352	KC855376	KC855424	KC855400
RHS38027ST[bdh]	*P. pachypleura*	*A. japonica*	Stem	UK, Surrey	2007	This study	KC855329	KC855353	KC855377	KC855425	KC855401
RHS92615.1[bdh]	*P. pachypleura*	*A. japonica*	Stem base	UK, West Yorkshire	2009	This study	KC855331	KC855355	KC855379	KC855427	KC855403
RHS105415.1	*P. pachypleura*	*A. japonica*	Roots	UK, Berkshire	2009	This study	KC855332	KC855356	KC855380	KC855428	KC855404
RHS15432.2004[bh]	*P. pachypleura*	*A. japonica*	Stem base	UK, London	2004	This study	KC855333	KC855357	KC855381	KC855429	KC855405
RHS14165	*P. pachypleura*	*A. japonica*	Roots	UK, Surrey	2007	This study	KC855334	KC855358	KC855382	KC855430	KC855406
RHS29131	*P. pachypleura*	*A. japonica* 'Variegata'	Roots	UK, Exeter	2007	This study	KC855335	KC855359	KC855383	KC855431	KC855407
CBS181.25, 045F1, P343	*P. pini*[c]	*Pinus resinosa*	Roots	USA, Minnesota	1925	Hong *et al.* (2011); C. X. Hong (unpublished data)	FJ392322	GQ247656	GQ247666	GQ247650	GQ247675
CIT-US1[b]	*P. pini*[i]	*Fagus sylvatica*	Canker	USA	2003	Jung & Burgess (2009); this study	KC855337	KC855361	KC855385	KC855433	KC855409
CIT-US9[b]	*P. pini*[i]	*F. sylvatica*	Canker	USA	2003	T. Jung & T. I. Burgess (unpublished data); this study	KC855338	KC855362	KC855386	KC855434	KC855410
CBS 124093, PLU-A5[be]	*P. plurivora*[c]	*F. sylvatica*	Roots	Germany	2004	Jung & Burgess (2009); this study	KC855339	KC855363	KC855387	KC855435	KC855411
RHS3783.2006	*P. plurivora*	*Viburnum plicatum*	Soil	UK, Swansea	2006	This study	KC855320	KC855344	KC855368	KC855416	KC855392
STE-U 6269	*P.* taxon 'emzansi'	*Agathosma betulina*	n.a.	South Africa	2004–5	Bezuidenhout *et al.* (2010)	GU191228	GU191317	GU191188	GU191270	GU191250
STE-U 6272	*P.* taxon 'emzansi'	*A. betulina*	n.a.	South Africa	2004–5	Bezuidenhout *et al.* (2010)	GU191220	GU191316	GU191187	GU191269	GU191249

[a]Abbreviations of isolates and culture collections: ATTC: American Type Culture Collection, USA; CBS: Centraalbureau voor Schimmelcultures Utrecht, The Netherlands; IMI: CABI Bioscience, UK; RHS: Royal Horticultural Society, UK; STE-U Department of Plant Pathology, University of Stellenbosch, South Africa; WAC: Department of Agriculture and Food Western Australia Plant Pathogen Collection, Perth, Australia; VHS: Vegetation Health Service of the Department of Environment and Conservation, Perth, Australia. Other isolate names and numbers are as given on GenBank or in the listed references.

[b]Isolates used in the growth–temperature studies.

[c]Ex-type isolate.

[d]Isolates used in the *A. japonica* pathogenicity trials.

[e]Isolates used in the host range pathogenicity trials.

[f]Authentic type.

[g]Designated as *P. citricola* CIT2 by Oudemans *et al.* (1994).

[h]Isolates used in the morphological studies.

[i]Designated as *P. citricola* I by Jung & Burgess (2009).

and DC6 (Bonants *et al.*, 1997) were used. One microlitre of the resulting PCR product was then diluted 10-fold in sterile PCR water and amplified with primers ITS4 and ITS6 (Cooke *et al.*, 2000) in the second round. The PCR conditions for both rounds were the same as those described by Cooke *et al.* (2000) except for the annealing temperature, which was 62°C in the first round and 63°C in the second round.

For selected isolates, four additional gene regions were amplified: (i) the mitochondrial gene *cox1* was amplified with the primers FM83 and FM84 as described by Martin & Tooley (2003); (ii) the β-tubulin nuclear region was amplified as described by Blair *et al.* (2008) using the primers Btub-F1 (Blair *et al.*, 2008) and TUBUR1 (Kroon *et al.*, 2004); (iii) the mitochondrial region *nadh1* was amplified with primers NADHF1 and NADHR1 as described by Kroon *et al.* (2004); (iv) the nuclear region *EF-1α* was amplified with the primers ELONGF1 and ELONGR1 primers as described by Kroon *et al.* (2004).

All PCR reactions were carried out in 25 μL total volume with 10 pmol of each primer using PCR beads as above. Reactions were performed in a Progene thermocycler (Techne). Products were visualized under UV light with 5% (v/v) ethidium bromide (10 mg mL^{-1}) in 1% agarose gels in TBE. PCR products were purified using the QIAquick Gel Extraction kit (QIAGEN) following the manufacturer's instructions and sequenced by a commercial sequencing service (Macrogen, Korea). Templates were sequenced in both directions with the primers used in the amplification stage, except for *cox1* which was additionally sequenced with the primers FM85 and FM50 (Martin & Tooley, 2003).

A consensus sequence was computed from the forward and reverse sequences with SEQMAN from the LASERGENE v. 8.0.2 package (DNAstar). The sequences were edited and aligned using BIOEDIT v. 7.0.5 (Hall, 1999). Additional reference sequences were obtained from GenBank (NCBI) for species comparison and identification. Adjustments were made manually when necessary.

Phylogenetic analysis

The ITS, *cox1*, β-tubulin, *nadh1* and *EF-1α* DNA regions were analysed independently. Phylogenetic analyses of species from ITS Clade 2 were performed using Bayesian inference of maximum likelihood with MRBAYES v. 3.1.2 (Ronquist & Huelsenbeck, 2003), applying a general time reversible (GTR) substitution model with gamma (G) and proportion of invariable (I) site parameters to accommodate variable rates across sites, as determined by MRMODELTEST v. 2.2 (Nylander, 2004). Two simultaneous runs of Markov chain Monte Carlo (MCMC) using four chains were run over 10 000 000 generations. Trees were sampled every 1000 generations and the first 2500 trees were discarded as burn-in for calculation of clade posterior probabilities. The 50% majority rule consensus phylogeny and posterior probability (PP) were calculated from the remaining sample, and trees were visualized using FIGTREE v. 1.4 (http://tree.bio.ed.ac.uk/software/figtree/). The alignments and trees resulting from each individual analysis were lodged in TreeBASE (S14700).

Colony morphology, growth rates and cardinal temperatures

Growth rates of eight isolates of *P. pachypleura* from *A. japonica* in the UK, i.e. RHS2474.2001, RHS15432.2004, RHS 4187.2006, RHS20408, RHS38027, RHS38027ST, RHS92615 and IMI50240, two isolates of *P. acerina* from *Acer pseudoplatanus* in Italy (ex-type CBS 133931 and B080) and *P. pini* from *Fagus sylvatica* in the USA (CIT-US1 and CIT-US9), the ex-type isolate of *P. plurivora* (CBS 124093) and the authentic type isolate of *P. citricola s. str.* (CBS295.29) (Table 1) were examined at 5, 10, 15, 20, 25, 30, 32, 35°C. Agar plugs (5 mm diameter) from 6-day-old colonies of each isolate were placed centrally on V8 juice agar plates (V8A: 2 g $CaCO_3$, 200 mL V8 juice, and 15 g agar in 800 mL distilled water) and incubated in the dark. Three replicates for each isolate and temperature were prepared and the experiment was repeated once. All plates were incubated at 20°C in the dark for 24 h before transferring to the different temperatures. Diameters of each colony were measured in two directions (at right angles) after 3 or 5 days and then the mean daily radial growth rate and the standard deviation calculated. Plates from temperatures where growth was arrested were incubated at 25°C to determine whether the isolates were still viable. The temperature–growth relations of different species were analysed by ANOVA and Tukey's HSD multiple comparison tests using GENSTAT v. 13 (2010, VSN International Ltd.).

Colony growth patterns were described from 7-day-old cultures grown at 20°C in the dark on clarified V8A, malt extract agar (MEA; Oxoid Ltd.) and half-strength potato dextrose agar [1/2 PDA: 19·5 g PDA (Biokar-Diagnostics), 7·5 g agar to 1 L distilled water].

Morphology of sporangia and gametangia

The same eight isolates of *P. pachypleura* that were included in the temperature–growth rate studies were also used for detailed morphological studies and measurements of sporangia, oogonia and antheridia, and for comparisons with known species reported in the literature (Table 2). Sporangia were produced by flooding 10 × 10 mm agar squares from growing margins of 3–5-day-old colonies with non-sterile soil extract (Jung & Burgess, 2009) in Petri dishes and incubating them at 20°C in the dark. For each isolate, dimensions and characteristic features of 50 mature sporangia chosen at random were determined at ×400 and ×1000 magnification (Nikon Eclipse Ni-U). Dimensions and characteristic features of 50 mature oogonia and oospores and at least 10 antheridia, due to their uniformity, were measured as above on 10 × 10 mm agar squares taken from the centre of 14–21-day-old clarified V8A (CV8A; 100 mL V8 filtered through cloth, 2 g $CaCO_3$, 900 mL distilled water, 15 g agar) cultures grown at 20°C in the dark. For each isolate, the oospore wall index was calculated as the ratio between the volume of the oospore wall and the volume of the entire oospore (Dick, 1990).

Pathogenicity tests

Shoots (25 cm long) of *A. japonica*, *Taxus baccata*, *Rosa* 'Ausprior', *Viburnum tinus* 'Israel', *Cornus sericea* 'Bud's Yellow', and *Rhododendron argyrophyllum* subsp. *nankingense* were collected at Wisley gardens, UK. Freshly cut shoots were inoculated with 3 mm plugs from 7-day-old *Phytophthora* cultures grown on CA. A bark incision was made using a sterile 3 mm cork borer to expose the cambium and the plug placed in the incision. The wounds were wrapped in damp sterile cotton wool, Parafilm and foil. Controls used sterile CA. The pathogenicity trial on *A. japonica* was performed three times on 8 October, 8 November and 27 November 2012 with five isolates of *P. pachypleura* (Table 1) and one isolate of the polyphagous *P. cinnamomi* (P5843/2000) as a reference. *Phytophthora cinnamomi* is also recorded through the RHS Advisory Service on *A. japonica*. The host range experiment was performed twice on 8 and 27 November and one isolate of *P. pachypleura* (IMI502404), *P. cinnam-*

Table 2 Morphological characters, dimensions (μm) and temperature–growth relations of *Phytophthora pachypleura*, *P. acerina*, *P. capensis*, *P. citricola s. str.*, *P. multivora*, *P. pini*, *P. plurivora* and *P.* taxon 'emzansi'

	P. pachypleura	*P. acerina*	*P. capensis*	*P. citricola s. str.*	*P. multivora*	*P. pini* [a]	*P. plurivora*	*P.* t. 'emzansi'
No. of isolates investigated	8	15[b]	3[c]	2[d]	6[e]	2[d]	7[d]	2[c]
Sporangia	Ovoid, limoniform, obpyriform, ellipsoid, distorted	Ovoid, limoniform, obpyriform, ellipsoid, distorted	Ovoid, limoniform	Ovoid, limoniform, obpyriform, ellipsoid, distorted	Ovoid, limoniform, obpyriform, ellipsoid, distorted	Ovoid, limoniform, ellipsoid, distorted	Ovoid, limoniform, obpyriform, ellipsoid, distorted	Highly variable
l × b mean	59·8 ± 1·6 × 33·0 ± 0·7	52 ± 13 × 32·8 ± 7·7	39·1 ± 6 × 24 ± 3·3	52 ± 7·9 × 29·9 ± 5·1	51 ± 10·4 × 30 ± 5·1	53·7 ± 6·5 × 33·8 ± 3·9	47·4 ± 7·7 × 33·5 ± 5·9	46·9 ± 8·6 × 27·4 ± 5·7
Range of isolate means	41·7–62·8 × 27·3–33·6	42·5–61·6 × 26·8–38·3		50·9–52 × 29·9	44·2–62·1 × 26·2–34·2	51·2–56·2 × 33·5–34·1	39·6–52·3 × 28·9–38·8	
Total range	30·2–83·8 × 22·0–43·6	20·3–105·7 × 11·1–51·3	27·5–50 × 17·5–32·5	36–75 × 21–40	25–97 × 13–63	39–70 × 20–42·1	27·5–80·5 × 16·7–69·6	35–67·5 × 17–47·5
l/b ratio	1·82 ± 0·05	1·6 ± 0·26	1·6–1·7	1·73 ± 0·28	1·7 ± 0·22	1·6 ± 0·16	1·43 ± 0·19	1·8–1·9
Oogonia								
Mean diameter	29·8 ± 0·12	32·0 ± 4·4	24 ± 2·5	30·0 ± 3·0	26·5 ± 1·9	31·2 ± 2·6	28·5 ± 3·3	30·7 ± 3·1
Range of isolate means	29·5–31·7	28·1–36·3		29·7–30·3	25·5–27·8	30·9–31·4	27·5–29·9	
Total range	20·9–39·4	19·2–45·5	20–27·5	16·7–35·9	19–37	21·3–36	15–37·5	25–37·5
Oospores								
Plerotic oospores (%)	89 (74–98)	30·4 (4–60)	Mainly plerotic	56 (44–68)	55 (48–64)	57 (52–62)	55·7 (38–78)	53
Mean diameter	24·7 ± 0·1	28·4 ± 3·9	22·7 ± 2	27·1 ± 2·8	23·6 ± 1·8	27·7 ± 2·3	25·9 ± 3·1	27·9 ± 2·6
Total range	17·6–30·0	15·9–39·3	20–27·5	15·3–30·9	17·3–33·1	18·4–33·2	14–35·8	22·5–32·5
Wall diameter	2·6 ± 0·02	2·0 ± 0·4	2·7	1·68 ± 0·35	2·6 ± 0·5	1·8 ± 0·36	1·45 ± 0·35	<2·5
Oospore wall index	0·71 ± 0·004	0·38 ± 0·09	0·56[g]	0·33 ± 0·05	0·52 ± 0·07	0·34 ± 0·05	0·3 ± 0·06	0·45[g]
Abortion rate (%)	6·4	38·5 (10–99)						42–46[h]
Antheridia	Paragynous	Paragynous	Paragynous	Paragynous	Paragynous	Paragynous	Paragynous	Amphigynous
Hyphal swellings in water	–	Globose to irregular, some catenulate	–	–	–	–	Few, globose, small	–
Hyphal aggregations	–	+	–	–	–	–	–	–
Maximum temperature (°C)	32	32	27·5	32	30–32·5	32	32	27·5
Optimum temperature (°C)	25	25	22·5	25	25	30	25	20
Growth rate on V8A at optimum (mm day^{-1})	6·9 ± 0·03	7·7 ± 0·19		6·9 ± 0·1	6·5 ± 0·02	9·2 ± 0·74	8·1 ± 0·18	
Growth rate on V8A at 20°C (mm day^{-1})	6·2 ± 0·03	6·52 ± 0·20	6·6[i]	6·2 ± 0·04	4·8 ± 0·6	6·3 ± 0·23	6·3 ± 0·1	5[i]

[a]Designated as *P. citricola* I in Jung & Burgess (2009).
[b]Five of the 15 isolates were included in the growth tests; data from Ginetti *et al.* (2013).
Data from: [c]Bezuidenhout *et al.* (2010); [d]Jung & Burgess (2009); [e]Scott *et al.* (2009); [f]Hong *et al.* (2011).
[g]Values calculated from data in Bezuidenhout *et al.* (2010).
[h]Aborted plus immature oospores.
[i]Growth rate on CMA at 20°C.

omi (P5843/2000), *P. plurivora* (P3783/2006) and *P. multivora* (P226/2001) were included (Table 1). For both experiments, 10 shoots per isolate were inoculated and incubated in a plastic bag at 20°C in the dark. After 15 days, lengths of phloem lesions were measured. Random reisolations from the margins of lesions of three stems per isolate and control using P5ARP were made to confirm the pathogenicity of the species tested.

Differences in lesion lengths between isolates and species were analysed using ANOVA and Tukey's HSD multiple comparison tests using GENSTAT v. 13. The lesion sizes were log transformed in ANOVA to equate variances between *Phytophthora* species.

Results

Isolates

In total, 23 *P. pachypleura* isolates were obtained from 14 different plants of *A. japonica* received by the Advisory Service at Wisley, UK. In addition, *P. pachypleura* was detected by direct ITS sequencing from 37 tissue samples with symptoms or soil samples of 27 different plant species including *Buxus sempervirens*, *Meconopsis grandis*, *Robinia pseudoacacia* 'Frisia', *Rubus idaeus*, *Salvia officinalis*, *Tagetes erecta*, *Taxus baccata* and *Vaccinium myrtillus*, and taxa that (on the basis of the data provided by the RHS Advisory Service) could only be identified to the level of the following genera: *Begonia*, *Bougainvillea*, *Ceanothus*, *Chaenomeles*, *Cornus*, *Crocus*, *Eryngium*, *Ilex*, *Lathyrus*, *Osmanthus*, *Prunus*, *Pyrus*, *Rhododendron*, *Rosa* and *Viburnum*.

Phylogenetic position of *P. pachypleura*

Fifteen out of the 23 isolates of *P. pachypleura* were used for DNA analysis (Table 1) and all of them had nearly identical ITS, β-tubulin, *EF-1α*, *cox1* and *nadh1* sequences (TreeBASE S14700). There were 18 fixed polymorphisms across the five gene regions unique to *P. pachypleura* and separating it from *P. acerina*, *P. citricola s. str.*, *P. pini*, *P. plurivora*, *P. citricola* III and *P citricola* E. In total, the numbers of polymorphisms that separated the different isolates of *P. pachypleura* from the tested isolates of *P. acerina*, *P. citricola s. str.*, *P. pini*, *P. plurivora*, *P. citricola* III and *P citricola* E were 4–5, 3–4, 5–7, 5–7, 4–5 and 5–6 in ITS (Table S1); 4, 4–5, 5–6, 5, 5 and 3 in β-tubulin (Table S1); 7–17, 4–17, 5–19, 6–14, 7–15 and 4–18 in *EF-1α* (Table S2); 13–15, 9–18, 12–19, 16–19, 12–18 and 8–11 in *cox1* (Table S3); and 14, 14, 12–13, 10, 12 and 16 in *nadh1* (Table S4), respectively. As the *cox1* sequences of the tested isolates of *P. citricola* E and *P. citricola* III were shorter than those of the other species and missed seven highly polymorphic sites at the beginning and five polymorphic sites at the end (Table S3), the numbers of polymorphisms separating *P. pachypleura* from these two species have probably been underestimated. In the separate phylogenetic analyses of the five gene regions, all *P. pachypleura* isolates grouped together in a well-defined clade with PP support higher than 0·5 (PP values: ITS 1·0; *EF-1α* 0·87; β-tubulin 0·61, *cox1* 1·0; *nadh1* 1·0). The phylogenetic trees based on the ITS and the *cox1* analyses are presented in Figures 1 and 2. Among the three nuclear gene regions, the ITS analysis produced the best-resolved tree showing a strong support (PP = 0·93) for the grouping of *P. pachypleura* as a sister group to *P. citricola s. str.* (Fig. 1). In the analyses using mitochondrial genes, the closest relative of *P. pachypleura* was *P. plurivora* (PP = 0·94) in the *nadh1*-based analysis and *P. citricola* E and *P. acerina* in the analysis based on *cox1* (PP = 0·78) (TreeBASE S14700).

Taxonomy

Phytophthora pachypleura B. Henricot, A. Pérez Sierra & T. Jung, sp. nov – Fungal name registration FN570084; Figures 3 and 4; Tables 2 and 3.

Etymology: name refers to the thick wall of the oospores ('*pachy*' = 'thick' and '*pleura*' = 'wall' in Greek).

Sporangia were produced abundantly in non-sterile soil extract. Sporangia of *P. pachypleura* were borne terminally (Fig. 3a) on mostly unbranched sporangiophores or were less frequently laterally attached (Fig. 3e,i). External proliferation close to the sporangial base (Fig. 3b,f) resulting in loose sympodia was infrequently observed in all isolates. Hyphal swellings were sometimes formed close to the sporangial base (Fig. 3i). Sporangia were non-caducous and semipapillate (Fig. 3a–i), infrequently bipapillate or bilobed (<1% over all isolates; Fig. 3h). No basal plug protruding into the empty sporangium was observed. Within all *P. pachypleura* isolates, sporangial shapes showed a wide variation, with the more common shapes being ovoid (all isolates 32·2%; Fig. 3a,d), ellipsoid (27·1%; Fig. 3b,f), obpyriform (15·1%; Fig. 3d, e), mouse-shaped (8·3%; Fig. 3c) and limoniform (1·3% Fig. 3g). A small proportion of the sporangia were also distorted (Fig. 3h,i). Sporangia with lateral attachment were rare (1·8%; Fig. 3e) while sporangia with curved apices were common (over all isolates 10%; Fig. 3c).

The mean sporangial dimensions of eight isolates of *P. pachypleura* averaged 59·8 ± 1·6 × 33·0 ± 0·7 μm (overall range 30·2–83·8 × 22·0–43·6 μm) with a range of isolate means of 41·7–62·8 × 27·3–33·6 μm (Table 2). The mean length/breadth ratio was 1·82 ± 0·05 (range of isolate means 1·53–1·91). The exit pore for the release of the zoospore was 9·2 μm wide in average. Chlamydospores were not observed.

Oogonia, oospores and antheridia (Fig. 3j–o): *P. pachypleura* is homothallic with gametangia readily produced on V8A by all isolates within 7 days. The percentage of oogonial or oospore abortion was low (6·4%). Oogonia were borne terminally, had smooth walls and were usually globose to slightly subglobose (Fig. 3j–m,o). Elongated oogonia with long tapering base occurred rarely (Fig. 3n). The oogonia had a mean diameter of 29·8 ± 0·12 μm (overall range 20·9–39·4 μm; range of isolate means 29·5–31·7 μm; Table 2). The mean proportion of plerotic oospores in *P. pachypleura* was 89% (Fig. 3j–l,o; range of isolate means 74–98%); sometimes oospores were slightly aplerotic but rarely clearly aplerotic (Fig. 3m). Oospores measured 24·7 ± 0·1 μm (overall range 17·6–30·0 μm

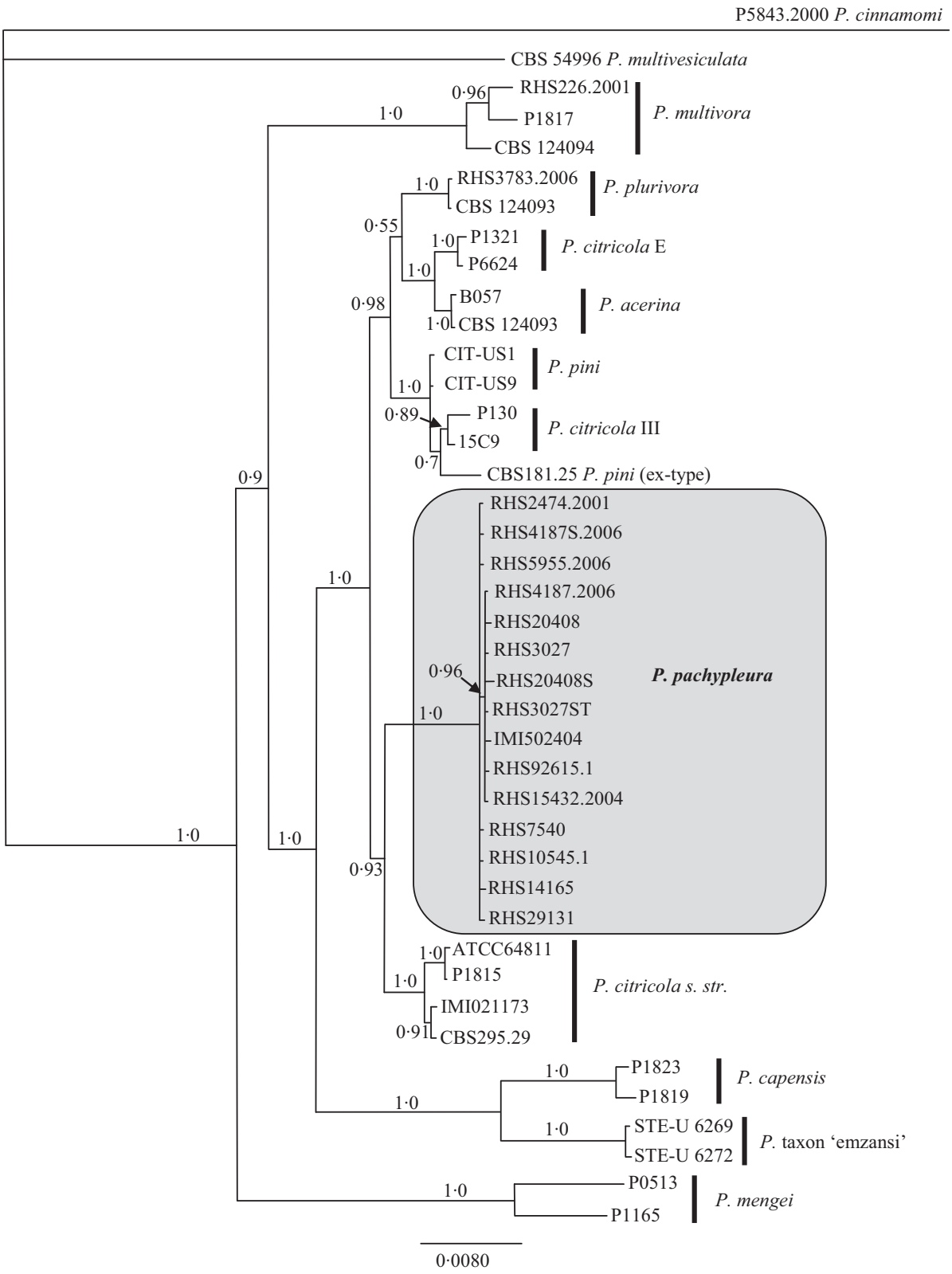

Figure 1 Bayesian inference tree based on rDNA ITS sequences, showing phylogenetic relationships between *Phytophthora pachypleura* and other species in ITS Clade 2. Numbers above the branches represent posterior probability based on Bayesian analysis. *Phytophthora cinnamomi* was used as the out-group taxon.

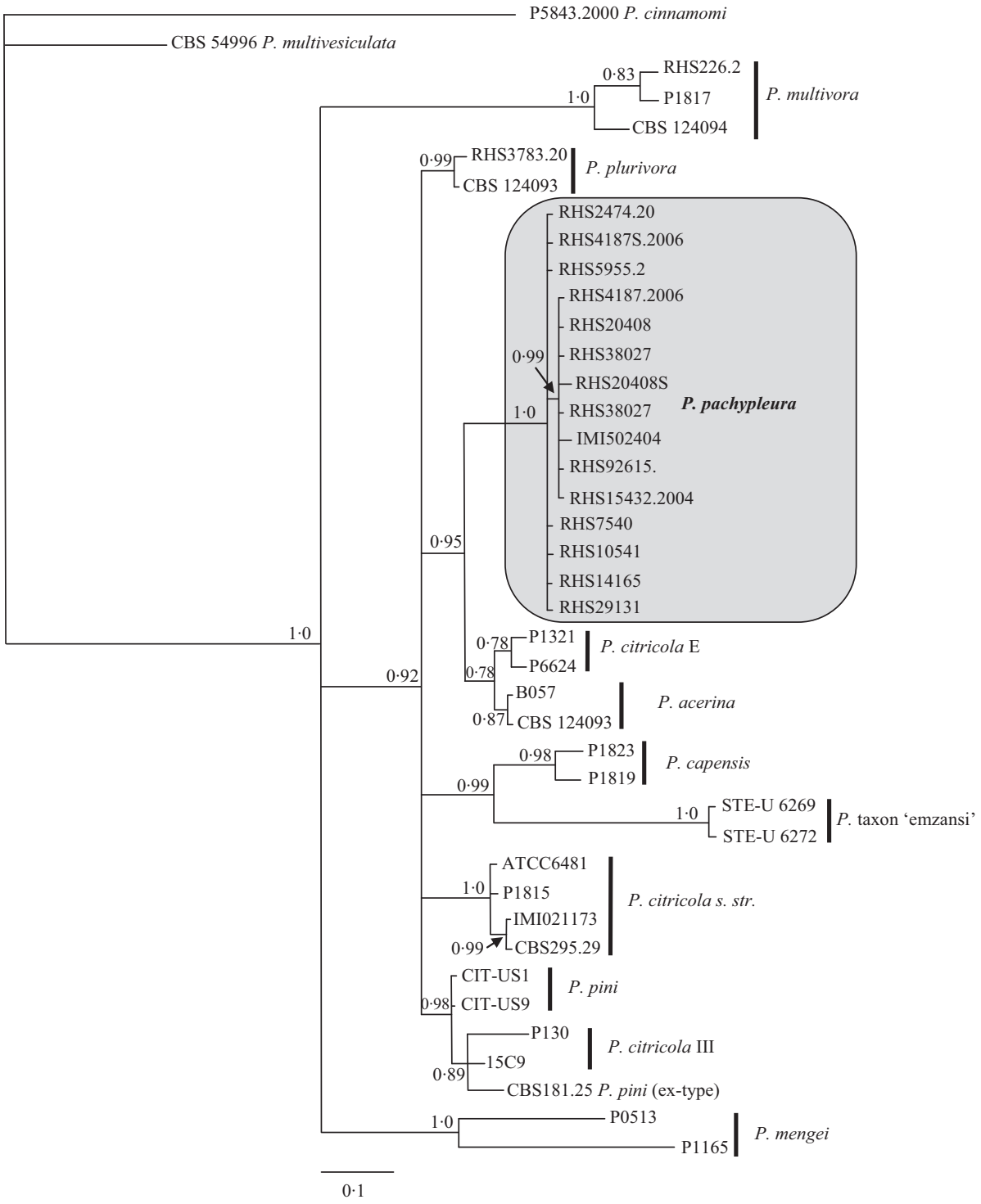

Figure 2 Bayesian inference tree based on *cox1* sequences, showing phylogenetic relationships between *Phytophthora pachypleura* and other species in ITS Clade 2. Numbers above the branches represent posterior probability based on Bayesian analysis. *Phytophthora cinnamomi* was used as the out-group taxon.

and range of isolate means 24·2–26·2 μm). The oospore walls were thick (Fig. 3j–o), averaging 2·6 ± 0·02 μm in diameter (overall range 1·4–4·2 μm) with a high oospore wall index of 0·71 ± 0·004 (Table 2).

The antherida of *P. pachypleura* were obovoid, club-shaped or irregular, almost exclusively paragynous and usually attached close to the oogonial stalk (Fig. 3j–n). They measured 12·1 ± 0·23 × 8·0 ± 0·11 μm

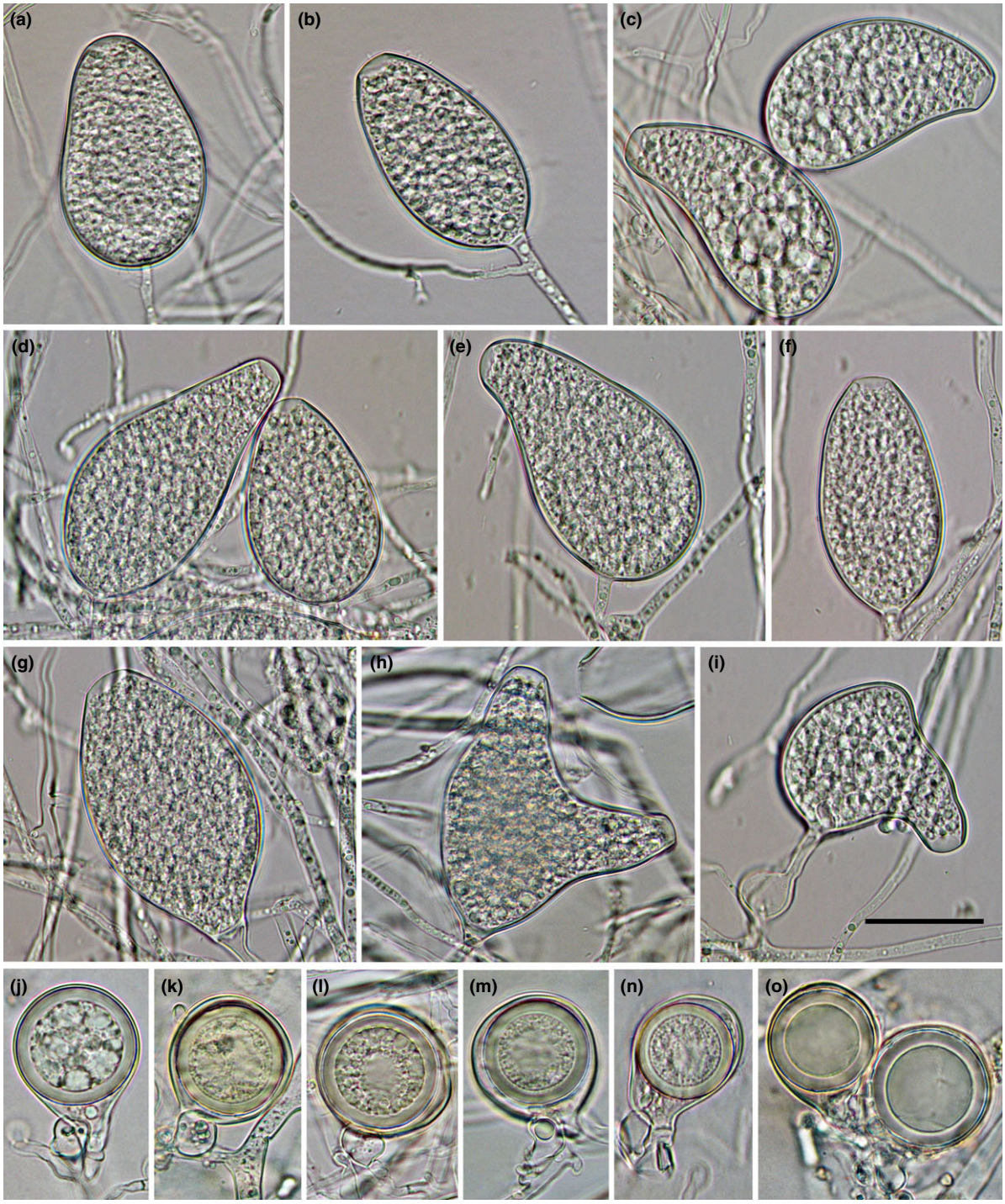

Figure 3 Morphological structures of *Phytophthora pachypleura*. (a–i) Semipapillate persistent sporangia formed in non-sterile soil extract; (a) ovoid to obpyriform; (b) ellipsoid with external proliferation; (c) mouse-shaped with markedly curved apices; (d) obpyriform (left) and ovoid (right); (e) obpyriform, laterally attached; (f) ellipsoid with external proliferation; (g) limoniform; (h) bipapillate to bilobed; (i) distorted with hyphal swelling close to sporangial base. (j–o) Oogonia; (j) juvenile oogonium containing thick-walled oospore with undifferentiated cytoplasm; (k–o) mature oogonia containing thick-walled oospores with ooplasts, paragynous antheridia; (k–l) plerotic oospores; (m) aplerotic oospore; (n) elongated oogonium with long tapering base; (o) oogonia with plerotic oospores and thick oospore walls. Bar = 25 μm.

(overall range 6·7–21·3 × 5·4–10·5 μm). In some cases more than one antheridium per oogonium were observed.

Colony morphology, growth rates and cardinal temperatures. Colony growth patterns of one isolate each of *P. pachypleura* (ex-type IMI502404), *P. citricola s. str.*

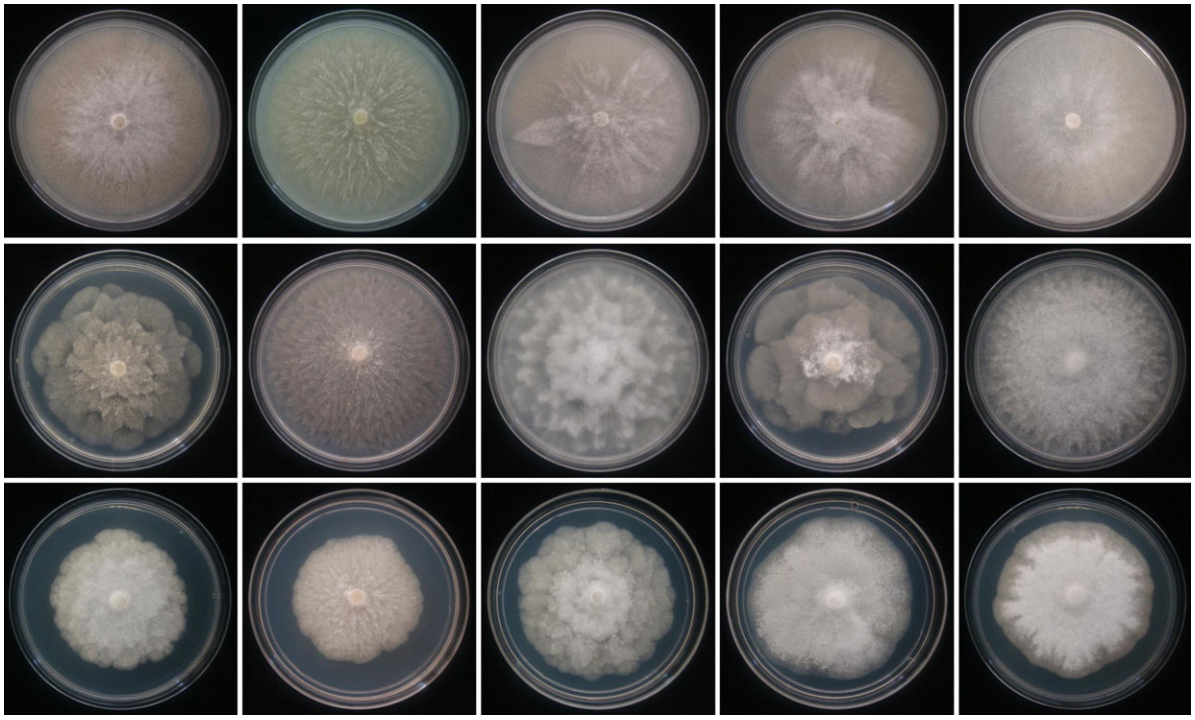

Figure 4 Colony morphology of (from left to right) *Phytophthora pachypleura* (ex-type IMI502404), *P. citricola s. str.* (authentic type CBS 295.29), *P. plurivora* (ex-type CBS 124093), *P. pini* (CIT-US1) and *P. acerina* (ex-type CBS 133931) after 7 days' growth at 20°C on (from top to bottom) V8 juice agar, malt extract agar and potato dextrose agar.

Table 3 Temperature–growth relations of eight isolates of *Phytophthora pachypleura*, one isolate each of *P. citricola s. str.* and *P. plurivora*, and two isolates each of *P. pini* and *P. acerina* on V8 juice agar (growth rate at optimum temperature shown in bold)

	Average radial growth rate (mm day^{-1}) at temperature (°C)							
	5	10	15	20	25	30	32	35
P. pachypleura	1·0	2·3	4·5	6·2	**7·0**	3·7	0·8	0·3
P. citricola s. str.	1·6	2·8	5·4	6·8	**7·1**	6·8	1·6	0·8
P. plurivora	1·7	3·4	6·0	7·1	**8·1**	7·4	3·5	1·0
P. pini	1·0	2·4	5·0	6·9	7·4	**8·0**	3·8	0·8
P. acerina	1·2	2·9	5·9	7·5	**7·9**	7·2	1·5	0·6

(authentic type CBS 295.29), *P. plurivora* (ex-type CBS 124093), *P. pini* (CIT-US1) and *P. acerina* (ex-type CBS 133931) are shown in Figure 4. All *P. pachypleura* isolates formed similar colony growth patterns with limited aerial mycelium on the three different agar media. On V8A colonies were striate while colonies on MEA were petaloid to chrysanthemum-like. On PDA felty colonies with a faint petaloid pattern were produced.

Temperature–growth relations of eight isolates of *P. pachypleura*, one isolate of *P. citricola s. str.* (authentic type CBS 295.29) and *P. plurivora* (ex-type CBS 124093), and two isolates of *P. pini* (CIT-US1 and CIT-US9) and *P. acerina* (ex-type CBS 133931 and B080) are shown in Table 3. All eight isolates of *P. pachypleura* had identical cardinal temperatures and showed growth rates that did not differ from each other at any temperature tested (Tukey's HSD test, $P < 0{\cdot}05$). The maximum growth temperature for *P. pachypleura* was above 35°C. All eight *P. pachypleura* isolates had a clear growth optimum at 25°C with a growth rate of $7{\cdot}0 \pm 0{\cdot}03$ mm day^{-1}. *Phytophthora citricola s. str.*, *P. plurivora* and *P. acerina* also had a growth optimum at 25°C with growth rates of $7{\cdot}1 \pm 0{\cdot}2$ mm day^{-1}, $8{\cdot}1 \pm 0{\cdot}5$ mm day^{-1} and $7{\cdot}9 \pm 0{\cdot}3$ mm day^{-1}, respectively. Interestingly, *P. citricola s. str.* growth rates at 20°C and 30°C differed from growth at optimum only by 0·28 and 0·32 mm day^{-1}. Only *P. pini* differed from the other isolates by having an optimum at 30°C with $8{\cdot}0 \pm 0{\cdot}3$ mm day^{-1}. Analysis at individual temperatures (5–35°C) generally showed significantly lower growth rates for *P. pachypleura* in comparison to the other *Phytophthora* species tested (Tukey's HSD test, $P < 0{\cdot}05$). This difference was most pronounced at 30°C.

Typus: United Kingdom, Cheshire, isolated from roots of *Aucuba japonica*, June 2008, B. Henricot. Holotype IMI502404 (preserved in a metabolically inactive state

by deep-freezing in CABI, Egham, Surrey, UK). Ex-type culture IMI502404.

Additional specimens examined. United Kingdom, Glamorgan, isolated from roots of *A. japonica*, 2001, RHS Advisory Service, RHS2474.2001; UK, Essex, isolated from stem base of *A. japonica*, 2006, RHS Advisory Service, RHS4187.2006; UK, Hampshire, isolated from roots of *A. japonica*, 2007, RHS Advisory Service, RHS20408 and RHS20408S; UK, Surrey, isolated respectively from roots and rhizosphere soil of *A. japonica*, 2007, RHS Advisory Service, RHS38027; UK, West Yorkshire, isolated from stem base of *A. japonica*, 2009, RHS Advisory Service; RHS92615.1; UK, London, isolated from stem base of *A. japonica*, 2004, RHS Advisory Service, RHS15432.2004.

Distribution: United Kingdom.

Notes: Main morphological characters, morphometric data, cardinal temperatures of growth and growth rates at optimum and at 20°C of *P. pachypleura* and other described taxa of the '*P. citricola* complex' are presented in Table 2. *Phytophthora pachypleura* morphologically resembles other species in the '*P. citricola* complex' including *P. citricola s. str.*, *P. plurivora*, *P. multivora*, *P. acerina* and *P. pini* but can be easily distinguished from all taxa by its high oospore wall index of 0·71 (Table 2). The species with the second highest oospore wall index is *P. capensis* (0·56; calculated from data in Bezuidenhout *et al.*, 2010) followed by *P. multivora* (0·52; Jung & Burgess, 2009).

Pathogenicity

Five isolates of *P. pachypleura* were used in the first pathogenicity test on *A. japonica* twigs in comparison with *P. cinnamomi* (RHS5843.2000). All *P. pachypleura* isolates were pathogenic to *A. japonica* causing necrotic phloem lesions with average lengths (15 days after inoculation) of 22–70 mm in October, 78–160 mm in November and 122–158 mm in December 2012. Lesions caused by *P. pachypleura* on *A. japonica* were significantly longer than those caused by *P. cinnamomi* ($P < 0{\cdot}001$) at all three inoculation dates (data not shown). In the host range trial (Fig. 5), all plant species except *C. sericea* showed susceptibility to at least two *Phytophthora* species. In addition to *A. japonica*, *P. pachypleura* was able to infect *V. tinus* and *R. argyrophyllum* causing necrotic lesions with average lengths of $22{\cdot}6 \pm 8{\cdot}4$ mm and $33{\cdot}9 \pm 6{\cdot}22$ mm, respectively, after 15 days. On *Rosa*, *T. baccata* and *C. sericea*, infections by *P. pachypleura* were limited to the tissue surrounding the inoculation points. On *A. japonica*, all the *Phytophthora* species tested caused necrotic lesions longer than the control and significantly different from each other ($P < 0{\cdot}001$), with *P. pachypleura* being the most aggressive pathogen followed by *P. multivora*, *P. plurivora* and *P. cinnamomi*. *Phytophthora pachypleura*, *P. cinnamomi*, *P. plurivora* and *P. multivora* were successfully reisolated from the margins of the lesions in all tests from each plant species tested.

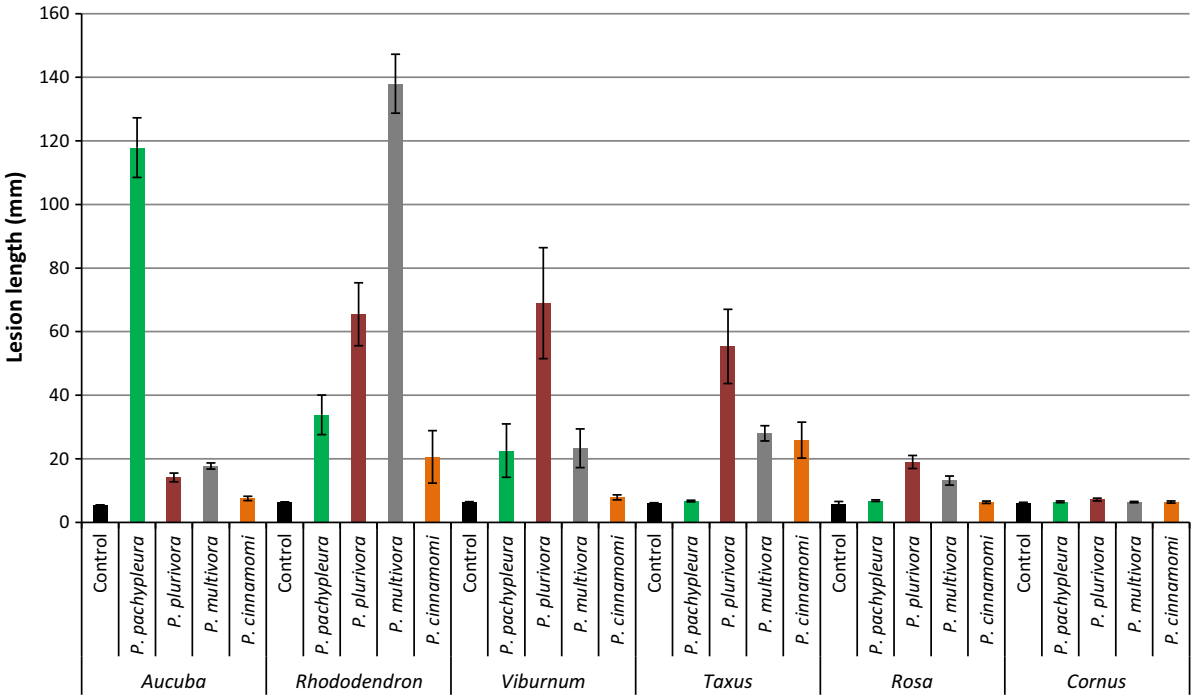

Figure 5 Mean lesion lengths on *Aucuba japonica*, *Rhododendron argyrophyllum*, *Viburnum tinus*, *Taxus baccata*, *Rosa* 'Ausprior' and *Cornus sericea* 15 days after inoculation with *Phytophthora pachypleura* (ex-type IMI502404), *P. plurivora* (RHS3783.2006), *P. multivora* (RHS226.2001), *P. cinnamomi* (RHS5843.2000) and a sterile carrot agar control. Bars represent SE.

Discussion

This paper reports a new homothallic species belonging to the '*P. citricola* complex' of *Phytophthora* ITS Clade 2, which is described here as *P. pachypleura*. The discovery of this new species came as a result of an investigation initiated in 1999 by the RHS into the threat of *Phytophthora* species to ornamentals grown in UK gardens.

The ex-type and authentic type isolates of *P. citricola* were recovered by Sawada from brown rot of *Citrus* in Taiwan in 1927 and from *Citrus* leaves in Japan in 1929 (Erwin & Ribeiro, 1996; Jung & Burgess, 2009). In the following six decades, apart from isolates from pit canker of elms in the USA that were described by Caroselli and Tucker in 1949 as *P. inflata* (Erwin & Ribeiro, 1996), all homothallic isolates with paragynous antheridia and semipapillate, variable and persistent sporangia from more than 100 host species in different continents were designated as *P. citricola*. The study of Oudemans *et al.* (1994) demonstrated for the first time that the morphospecies *P. citricola* comprised a complex of cryptic taxa. Jung & Burgess (2009) showed that isolates designated in recent years as *P. inflata* were conspecific with the newly described *P. plurivora* and that the original *P. inflata* is a lost species. Subsequently, many isolates in the *P. citricola* complex have been reassigned to new species, including *P. multivora* (Scott *et al.*, 2009), *P. mengei* (Hong *et al.*, 2009), *P. capensis* (Bezuidenhout *et al.*, 2010) and *P. pini* (Hong *et al.*, 2011). *Phytophthora mengei* and the recently described *P. elongata* from Australia belong to ITS subclade 2b and are more distantly related to the taxa of the '*P. citricola* complex', which resides in ITS subclade 2a (Hong *et al.*, 2009; Rea *et al.*, 2010). Other new taxa from the '*P. citricola* complex' that were recently described or await formal description are *P. acerina* (Ginetti *et al.*, 2013), the subgroup Cil III (*P. citricola* III of Jung & Burgess, 2009) (Hong *et al.*, 2011) and *P.* taxon 'emzansi' (Bezuidenhout *et al.*, 2010).

Phytophthora pachypleura displays a lot of sporangial shapes, a character shared by other species in the complex including *P. plurivora*, *P. multivora* and *P.* taxon 'emzansi'. The sporangia, oogonia and oospore dimensions also overlap between the species in the '*P. citricola* complex' (Table 2). However, due to its particularly high oospore wall index of 0·71, *P. pachypleura* can be easily differentiated from the other known species and designated taxa in the '*P. citricola* complex' (see Notes and Table 2). The status of *P. pachypleura* as a distinct species was clearly demonstrated by DNA analysis. The phylogenetic analyses based on three nuclear (ITS, β-tubulin, *EF-1α*) and two mitochondrial gene regions (*cox1*, *nadh1*) demonstrated that *P. pachypleura* forms a unique cluster within the '*P. citricola* complex', with *P. citricola s. str.*, *P. plurivora*, *P. citricola* E and *P. acerina* as its closest relatives. The phylogenetic analysis of the present study confirmed the phylogenies presented by Jung & Burgess (2009), Bezuidenhout *et al.* (2010) and Hong *et al.* (2011). The five tested DNA regions of the 17 studied isolates of *P. pachypleura* are nearly identical. To ascertain whether its population is clonal and therefore likely to be non-native, genetic variation over the whole genome should be assessed. However, the high oospore wall index which is characteristic of species that are adapted to dry environments indicates that *P. pachypleura* might have evolved in a dry climate rather than in the humid environment of the UK. Examples of other species with a high oospore wall index include *P. arenaria* (Clade 4), *P. multivora* and *P. elongata* (Clade 2), and *P. gibbosa* (Clade 6), all of which are associated with episodic dieback of native vegetation in the dry Mediterranean southwest of Western Australia (Scott *et al.*, 2009; Rea *et al.*, 2010, 2011; Jung *et al.*, 2011); *P. alticola* (Clade 4) causing collar and root rot of *Eucalypus* in South Africa (Maseko *et al.*, 2007); *P. capensis* (Clade 2) causing root rot of ornamentals in South Africa and *P. quercina* (Clade 4) causing fine root losses and decline in oak stands in southern Europe and on dry sites in central Europe. As these species belong to several different phylogenetic clades, this morphological adaptation must have evolved in a convergent way several times in the genus under the selective force of a dry climate or an otherwise dry environment. As almost all known *Phytophthora* species with a high oospore wall index thrive under Mediterranean climates, it is probable that the origin of *P. pachypleura* also lies in a Mediterranean region.

With an optimum temperature for growth of 25°C and reasonable growth even at 5°C, the climate in the UK with temperatures ranging from 11–23°C in summer and 3–12°C in winter (data from Met Office for the period 1980–2010; http://www.metoffice.gov.uk/) is suitable for *P. pachypleura*.

Phytophthora pachypleura, *P. plurivora* and *P. multivora* have been present in UK gardens for at least 10 years (RHS data, unpublished). With 35 recorded cases, *P. pachypleura* appears to be more limited in its impact in gardens than *P. plurivora*, which has been isolated from 249 plant or soil samples. However, *P. pachypleura* is significantly more aggressive to *A. japonica* than *P. plurivora* and *P. multivora* as demonstrated by the pathogenicity trials of this study. In addition to *A. japonica*, *P. pachypleura* has been detected by direct DNA sequencing in rhizosphere soil and tissues of 27 different plant species with symptoms which might be potential hosts. Five of these species were included in the pathogenicity trials of the present study and *P. pachypleura* was found to be pathogenic to *V. tinus* and *R. argyrophyllum* but not to *T. baccata*, *C. sericea* and *Rosa* 'Ausprior'. There are several factors that might explain the lack of pathogenicity to the latter three species. First, information about plant species was not always available from the advisory samples and the wrong species of *Cornus* and *Rosa* might have been included in the pathogenicity tests. Secondly, as indicated by the root rot symptoms of *A. japonica* in UK gardens, *P. pachypleura*, like many other *Phytophthora* species,

probably infects through the roots. Though shoot inoculation tests are widely accepted as a rapid method to test pathogenicity of a *Phytophthora* isolate to a certain plant species (Brasier & Kirk, 2001), it is known that the results do not necessarily correlate with root infections. There are also potential problems with records made from environmental samples such as the likelihood of false positives due to the presence of dead cells or cross contamination problems. Finally, it cannot be excluded that *P. pachypleura* is affected by seasonal resistance of certain host species. This phenomenon has been reported for other *Phytophthora*–host combinations including *P. alni* and *Alnus glutinosa* (Brasier & Kirk, 2001) or *P. ramorum* and *Quercus* spp. (Moralejo *et al.*, 2009). In general, plants are less susceptible to *Phytophthora* during the dormant season and the cause for this phenomenon is poorly understood. Factors that may be responsible for seasonal changes in susceptibility include growth stage, the nutritional and water status of the plant, complex interaction between pathogen and host at the gene level, and the seasonal variation of toxic compounds such as phenolics in the attacked tissues (Brasier & Kirk, 2001; Moralejo *et al.*, 2009).

In conclusion, through the survey work of the RHS Advisory Service, a new *Phytophthora* species was found and described here under the name *P. pachypleura*. This species is the main cause of mortality of *A. japonica* in UK gardens. This work has also highlighted the potential threat of this species to other ornamentals and further research is needed to assess the full extent of its host range and its susceptibility to seasonal resistance.

Acknowledgements

The authors would like to thank Ian Waghorn for his excellent technical assistance and his help with the preparation of the figures, Dr Andy Peace for his help with the statistical analyses, Beatrice Ginetti for sharing isolates of *P. acerina* and Dr Treena Burgess for sharing isolates and DNA sequences of several species within the '*P. citricola* complex'. T. J. and A. P. S. thank the COST office and the European Council for enabling stimulating discussions of the authors on the '*P. citricola* complex' during their participation in the European COST Action FP0801: Established and Emerging *Phytophthora*: Increasing Threats to Woodland and Forest Ecosystems in Europe (http://www.cost.eu/domains_actions/fps/Actions/FP0801). Cultures were held at the RHS under licence PHL 257/6581.

References

Bezuidenhout CM, Denman S, Kirk SA, Botha WJ, Mostert L, McLeod A, 2010. *Phytophthora* taxa associated with cultivated *Agathosma*, with emphasis on the *P. citricola* complex and *P. capensis* sp. nov. *Persoonia* **25**, 32–49.

Blair JE, Coffey MD, Park SY, Geiser DM, Kang S, 2008. A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genetics and Biology* **45**, 266–77.

Bonants PJM, Hagenaar-de Weerdt M, van Gent-Pelzer MPE, Lacourt I, Cooke DEL, Duncan JM, 1997. Detection and identification of *Phytophthora fragariae* Hickman by the polymerase chain reaction. *European Journal of Plant Pathology* **103**, 345–55.

Brasier CM, 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* **57**, 792–808.

Brasier CM, 2009. *Phytophthora* biodiversity: How many *Phytophthora* species are there. In: Goheen EM, Frankel SJ, eds. *Phytophthoras in Forests and Natural Ecosystems. Proceedings of the Fourth International Union of Forest Research Organisations (IUFRO) Working Party 7.02.09*. General Technical Report PSW-GTR-221. Albany, CA: USDA Forest Service, 101–15.

Brasier CM, Kirk SA, 2001. Comparative aggressiveness of standard and variant hybrid alder phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts. *Plant Pathology* **50**, 218–29.

Brasier CM, Vettraino AM, Chang TT, Vannini A, 2010. *Phytophthora lateralis* discovered in an old growth *Chamaecyparis* forest in Taiwan. *Plant Pathology* **59**, 595–603.

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30**, 17–32.

Davies ZG, Fuller RA, Loram A, Irvine KN, Sims V, Gaston KJ, 2009. A national scale inventory of resource provision for biodiversity within domestic gardens. *Biological Conservation* **142**, 761–71.

Dick MW, 1990. *Keys to* Pythium. Reading, UK: University of Reading Press.

Erwin DC, Ribeiro OK, 1996. *Phytophthora Diseases Worldwide*. St Paul, MN: APS Press.

Ginetti B, Moricca S, Squires JN, Cooke DEL, Ragazzi A, Jung T, 2013. *Phytophthora acerina* sp. nov., a new species causing bleeding cankers and dieback of *Acer pseudoplatanus* trees in planted forests in northern Italy. *Plant Pathology*. doi: 10.1111/ppa.12153.

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–8.

Hansen EM, Reeser PW, Sutton W, 2012. *Phytophthora* beyond agriculture. *Annual Review of Phytopathology* **50**, 359–78.

Hong CX, Gallegly ME, Browne GT, Bhat RG, Richardson PA, Kong P, 2009. The avocado subgroup of *Phytophthora citricola* constitutes a distinct species, *Phytophthora mengei* sp. nov. *Mycologia* **101**, 833–40.

Hong C, Gallegly ME, Richardson PA, Kong P, 2011. *Phytophthora pini* Leonian resurrected to distinct species status. *Mycologia* **103**, 351–60.

Huai WX, Tian G, Hansen EM *et al.*, 2013. Identification of *Phytophthora* species baited and isolated from forest soil and streams in northwestern Yunnan province, China. *Forest Pathology* **43**, 87–103.

Jung T, 2009. Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. *Forest Pathology* **39**, 73–94.

Jung T, Burgess TI, 2009. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* **22**, 95–110.

Jung T, Stukely MJC, Hardy GStJ *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.

Jung T, Vettraino AM, Cech TL, Vannini A, 2013. The impact of invasive *Phytophthora* species on European forests. In: Lamour K, ed. *Phytophthora: A Global Perspective*. Wallingford, UK: CABI, 146–58.

Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM, Flier WG, 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* **41**, 766–82.

Martin FN, Tooley PW, 2003. Phylogenetic relationships of *Phytophthora ramorum*, *P. nemorosa*, and *P. pseudosyringae*, three species recovered from areas in California with sudden oak death. *Mycological Research* **107**, 1379–91.

Maseko B, Burgess TI, Coutinho TA, Wingfield MJ, 2007. Two new *Phytophthora* species from South African *Eucalyptus* plantations. *Mycological Research* **111**, 1321–38.

Moralejo E, García-Muñoz JA, Descals E, 2009. Susceptibility of Iberian trees to *Phytophthora ramorum* and *P. cinnamomi*. *Plant Pathology* **58**, 271–83.

Nylander JAA, 2004. MrModeltest v. 2.2. Program distributed by the author. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University.

Oudemans P, Förster H, Coffey MD, 1994. Evidence for distinct isozyme subgroups within *Phytophthora citricola* and close relationships with *P. capsici* and *P. citrophthora*. *Mycological Research* **98**, 189–99.

Rea AJ, Jung T, Burgess TI, Stukely MJC, Hardy GEStJ, 2010. *Phytophthora elongata* sp. nov., a novel pathogen from the *Eucalyptus marginata* forest of Western Australia. *Australasian Plant Pathology* **39**, 477–91.

Rea AJ, Burgess TI, Hardy GEStJ, Stukely MJC, 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**, 1055–68.

Reader's Digest, 2001. *Field Guide to the Trees and Shrubs of Britain*. London, UK: Reader's Digest Association Limited.

Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Koike ST, 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Disease* **86**, 205–14.

Robideau GP, de Cock AWAM, Coffey MD *et al.*, 2011. DNA barcoding of oomycetes with cytochrome *c* oxidase subunit I and internal transcribed spacer. *Molecular Ecology Resources* **11**, 1002–11.

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–4.

Scott PM, Burgess TI, Barber PA *et al.*, 2009. *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia* **22**, 1–13.

Vettraino AM, Brasier CM, Brown AV, Vannini A, 2011. *Phytophthora himalsilva* sp. nov. an unusually phenotypically variable species from a remote forest in Nepal. *Fungal Biology* **115**, 275–87.

Webber JF, Mullett M, Brasier CM, 2010. Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum*. *New Disease Reports* **22**, 19.

White TJ, Bruns T, Lee SB, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR Protocols: A Guide to Methods and Applications*. San Diego, USA: Academic Press, 315–22.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Polymorphic nucleotides from aligned sequence data of ITS and β-tubulin showing the variation between isolates of *Phytophthora pachypleura*, *P. acerina*, *P. citricola s. str.*, *P. citricola* III and *P. citricola* E (*P. citricola* CIT2), *P. pini* and *P. plurivora*.

Table S2. Polymorphic nucleotides from aligned sequence data of *EF-1α*.

Table S3. Polymorphic nucleotides from aligned sequence data of *cox1*.

Table S4. Polymorphic nucleotides from aligned sequence data of *nadh1*.