Multiple new cryptic pathogenic *Phytophthora* species from *Fagaceae* forests in Austria, Italy and Portugal

Thomas Jung1,2,3, Marília Horta Jung1,2,3, Santa Olga Cacciola4, Thomas Cech5, József Bakonyi6, Diána Seress6, Saveria Mosca7, Leonardo Schena7, Salvatore Seddaiu8, Antonella Pane4, Gaetano Magnano di San Lio5, Cristiano Maia2, Alfredo Cravador2, Antonio Franceschini6, and Bruno Scanu9

1Phytophthora Research Centre, Mendel University, 613 00 Brno, Czech Republic
2Laboratory of Molecular Biotechnology and Phytopathology, Centre for Mediterranean Bioresources and Food, University of Algarve, 8005–130 Faro, Portugal
3Phytophthora Research and Consultancy, 83131 Nusdorf, Germany
4Department of Agriculture, Food and Environment, University of Catania, 95123 Catania, Italy
5Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Seckendorff-Gudent-Weg 8, A-1131 Vienna, Austria
6Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary
7Dipartimento di Agraria, University Mediterranea of Reggio Calabria, 89122 Reggio Calabria, Italy
8Dipartimento della ricerca per il sughero e la silvicoltura, Agris Sardegna, Via Limbara 9, 07029 Tempio Pausania, Italy
9Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università degli Studi di Sassari, Viale Italia 39, 07100 Sassari, Italy; corresponding author e-mail: bscanu@uniss.it

**Abstract:** During surveys of *Phytophthora* diversity in natural and semi-natural *Fagaceae* forests in Austria, Italy and Portugal, four new cryptic species were isolated from rhizosphere soil samples. Multigene phylogeny based on nuclear ITS, β-tubulin and HSP90 and mitochondrial cox1 and NADH1 gene sequences demonstrated that two species, *P. tyrrenica* and *P. vulcanica* spp. nov., belong to phylogenetic Clade 7a, while the other two species, *P. castaneorum* and *P. tubulina* spp. nov., clustered together with *P. quercina* forming a new clade, named here as Clade 12. All four new species are homothallic and have low optimum and maximum temperatures for growth and very slow growth rates at their respective optimum temperature. They differed from each other and from related species by a unique combination of morphological characters, cardinal temperatures, and growth rates. Pathogenicity of all *Phytophthora* species to the root system of their respective host species was demonstrated in soil infestation trials.

**Key words:** Clade 7, cryptic species, evolution, homothallic, phylogeny, *Phytophthora quercina* species radiation

**Article info:** Submitted: 11 May 2017; Accepted: 20 September 2017; Published: 28 September 2017.

**INTRODUCTION**

The family *Fagaceae* comprises about 1000 species belonging to eight to ten genera widely distributed across the Northern Hemisphere (Manos & Stanford 2001). In Europe, species from the genera *Castanea*, *Fagus*, and *Quercus* dominate a wide variety of habitats ranging from Mediterranean sclerophyllous evergreen communities to temperate, deciduous lowland and mountainous forests (http://www.euforgen.org). Besides species with wide geographical distributions and ecological amplitudes, such as sweet chestnut (*Castanea sativa*) and European beech (*Fagus sylvatica*), some evergreen oaks (e.g. *Quercus ilex* and *Q. suber*) are geographically restricted and adapted to particular environmental conditions such as prolonged summer drought and fire (Schirone *et al.* 2016). Although species in these genera constitute primarily forest resources important for their wide range of uses (biomass, fibre, wood products, cork and food), they are also keystone species in forest ecosystems and considered main drivers of terrestrial biodiversity (Kremer *et al.* 2012). In some countries, they are major patrimonial and cultural resources (Logan 2005).

Since the early 1990s, numerous surveys in declining and healthy European *Fagaceae* forests have revealed a wealth of resident *Phytophthora* species (Jung 2009, Perez-Sierra *et al.* 2013, Scanu *et al.* 2013). Several *Phytophthora* species, including *P. cactorum*, *P. x cambivora* (previously known as *P. cambivora*), *P. cinnamomi*, *P. plurivora*, and *P. quercina* were strongly associated with the decline and dieback of forests, while other species, such as *P. cryptogea*, *P. europaea*, *P. gallica*, *P. gonapodyides*, *P. megasperma*, *P. pseudosyringae*, *P. psychrophila*, *P. syringae*, *P. uliginosa*, *P. sp. forestsoil*, and *P. sp. riversoil*, were more cryptic, and their role in forest ecosystems is still not fully understood.

Between 2010 and 2016, surveys of *Phytophthora* diversity were independently performed in forests in Austria, Italy, and Portugal during which four new cryptic *Phytophthora* species were recovered from the rhizosphere of *Fagaceae*
species. All four species showed scattered distribution, very slow growth in culture, and were difficult to isolate using traditional soil baiting methods. Preliminary analysis of sequence data from the ITS region of the nrDNA and part of the mitochondrial cox1 gene showed that two taxa were closely related to *P. uliginosa* from subclade a of phylogenetic Clade 7 (in the following Clade 7a) whereas the other two taxa clustered with *P. quercina* and the informally designated taxon *Nothophytophthora* sp. ohiensis.

In this study, morphological and physiological characteristics are used in combination with DNA sequence data from the ITS, part of the nuclear heat shock protein 90 (HSP90) and β-tubulin (Btub), and the mitochondrial cox1 and NADH1 genes to characterize the four putative new *Phytophthora* taxa isolated from *Fagaceae* forests in Austria, Italy, and Portugal, and compare them to their closest relatives. The results of this study are presented and the new taxa described as *P. quercina* and *P. vulcanica* spp. nov. Pathogenicity of all new *Phytophthora* species against their respective hosts was also tested in soil infestation trials to confirm Koch’s postulates.

**MATERIAL AND METHODS**

**Phytophthora** isolation and culture maintenance

Isolates were obtained from rhizosphere soil of mature trees (Supplementary Table 1) using the sampling and isolation methods described by Jung et al. (1996), Jung (2009), and Scanu et al. (2015). Additional isolates used in the phylogenetic, morphological, and physiological studies and in the pathogenicity tests were sourced from the culture collections of the authors (Supplementary Table 1). In addition, isolates of *P. quercina* were obtained between 2010 and 2014 from various *Quercus* species in Portugal and Poland for comparative studies (Supplementary Table 1). For all isolates, single hyphal tip cultures were produced under the stereomicroscope from the margins of fresh cultures on V8-juice agar (V8A; 16 g agar, 3 g CaCO₃, 900 mL distilled water). Stock cultures were maintained on carrot agar (CA; 16 g agar, 3 g CaCO₃, 200 g carrot, 1000 mL distilled water; Scanu et al. 2014a) at 10 °C in the dark. All isolates of the four new species are preserved in the culture collections maintained at Mendel University (BD numbers, referring to the BioDiversA project RESIPATH), the University of Sassari (P and PH numbers, both referring to *Phytophthora*) and the University of Catania (Roman numbers). Dried culture holotypes were lodged with the CBS Herbarium, and ex-type and isotype cultures were deposited at the Westerdijk Fungal Biodiversity Institute (CBS; Utrecht, The Netherlands) (Supplementary Table 1).

**DNA isolation, amplification and sequencing**

Extraction of mycelial DNA was performed according to Jung et al. (2017b) using the DNeasy Plant Mini kit (QIAGEN, Hilden) or the E.Z.N.A.® Fungal DNA Mini Kit (OMEGA Bio-tek, Norcross, GA) following the manufacturer’s instructions and checked for quality and quantity by spectrophotometry. DNA was stored at −20 °C until required. For 29 isolates of the four new *Phytophthora* species and five isolates of *P. quercina*, three nuclear and two mitochondrial loci were amplified and sequenced (Supplementary Table 1). The ITS1-5.8S-ITS2 region of the nrDNA was amplified using the primer-pair ITS1/ITS4 (White et al. 1990) and the PCR reaction mixture and cycling conditions described by Nagy et al. (2003) with an annealing temperature of 57 °C for 30 s. Partial heat shock protein 90 (HSP90) gene was amplified with the primer-pair HSP90F1int/HSP90R1 as described previously (Blair et al. 2008). Segments of the β-tubulin (Btub) and the mitochondrial genes cytochrome c oxidase subunit 1 (cox1) and NADH dehydrogenase subunit 1 (NADH1) were amplified using primer-pairs TUBUF2/TUBUR1, COXF4N/COXR4N and NADHF1/NADHR1, respectively, and the PCR reaction mixture and cycling conditions as described by Kroon et al. (2004). Products of Thermo Fisher Scientific (Waltham, MA) and Bio-Rad C1000™ or Applied Biosystems® 2720 Thermal Cyclers were used for the PCR reactions. Amplicons were purified and sequenced in both directions using the primers of the PCR reactions by Macrogen Inc. (Amsterdam) and BMR Genomics DNA sequencing service (www.bmrgenomics.it).

Electrophoregrams were quality checked and forward and reverse reads were compiled using Pregap4 version 1.5 and Gap v.4.10 of the Staden software package (Staden et al. 2000). Heterozygous sites were labelled according to the IUPAC coding system. All sequences derived in this study were deposited in GenBank and accession numbers are given in Supplementary Table 1.

**Phylogenetic analysis**

DNA sequences generated were combined with sequences of *Phytophthora* species obtained from GenBank. Sequences of each locus were aligned using the online MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) by the E–INS–I strategy (ITS) or the G–INS–i option (all other loci). The phylogenetic analyses of *P. tyrrenica* and *P. vulcanica* from Clade 7a, and of *P. castanetorum* and *P. tubulina* from the *P. quercina* clade were performed separately.

For the phylogenetic analyses of *P. tyrrenica* and *P. vulcanica* all 16 previously known taxa from Clade 7a were included and *P. cinnamomi* (CBS 144.22) and *P. niederhauserii* (CBS 124086), both from Clade 7b, were used as outgroups (Abad et al. 2014, Jung et al. 2017b). The datasets of the five loci were first analysed separately and then combined. The 5-loci dataset comprised 4257 characters (ITS=842, Btub=911, HSP90=840, cox1=867, NADH1=797) and included 43 *Phytophthora* isolates.

To resolve the phylogenetic positions of *P. castanetorum* and *P. tubulina* within the *P. quercina* clade, and of the latter within the genus *Phytophthora*, 24 isolates from seven putative taxa of the *P. quercina* clade, including *P. castanetorum*, *P. tubulina*, *P. quercina*, *P. versiformis* (Paap et al. 2017), and the undescribed taxa *P. sp. ohiensis*, *P. sp. quercina-like* (only ITS sequence available), and *P. sp. versiformis-like*, and representative species from all phylogenetic *Phytophthora* clades were included in the analyses. The designated ex-type isolate (CBS 142348) of *Nothophytophthora amphigynosa*, the type species of a new sister genus of *Phytophthora* (Jung et al. 2017c) was
used as outgroup taxon in all analyses. The datasets of the five loci were first analysed separately and then combined. The cox1 sequences of *P. sp. ohiensis*, *P. versiformis* and *P. sp. versiformis*-like from GenBank were generated using primer pair FMT77/FMB8 (Martin & Tooley 2003) and, hence, had insufficient overlap with those of *P. castanetorum*, *P. quercina* and *P. tubulina* generated in this study with primer pair COX4F/COX4R. Therefore, cox1 was excluded from the two multigene analyses of the *P. quercina* clade. The combined four locus dataset comprised 3539 characters (ITS=992, Btub=915, HSP90=843, NADH1=789). For 11 of the 24 *Phytophthora* species from other clades no isolates with sequences for all four loci were available at GenBank or at Phytophthora database, hence sequences from two different isolates per species were combined (Supplementary Table 1). Since for *P. illii* no NADH1 sequence was available a separate analysis of a combined nuclear ITS-Btub-HSP90 dataset was performed in order to clarify whether *P. illii* constitutes a distinct phylogenetic clade as suggested by Rahman et al. (2015) and whether the inclusion of *P. illii* has any effect on the grouping of the *P. quercina* clade. This three locus dataset comprised 2768 characters (ITS=1010, Btub=915, HSP90=843) and included 49 *Phytophthora* taxa.

For all individual gene and all multigene alignments, both Maximum Likelihood (ML) phylogenetic and Bayesian Inference (BI) analyses were performed. ML analyses of the datasets were carried out with RAxML (Stamatakis 2014) in the raxmlGUI version 1.5b1 (Silvestro & Michalak 2012) implemented with the GTR+G model. There were 10 runs of the ML and bootstrap (“thorough bootstrapping”) analyses with 1000 replicates used to test the support of the branches. Phylogenetic BI analyses were performed with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2014) using the GTR+G model. For multigene BI analyses the individual gene alignments were divided into five (Clade 7a) and four or three (*P. quercina* clade) partitions, respectively. Four Markov chains were run for 10 million generations with three heated chains (temperature = 0.2) and one cold chain. Trees were sampled every 1000 steps after removing the first 6000 generations as burn in. For the multigene BI analysis of Clade 7a 20 million, generations with a burn in of the first 6000 generations were used. Phylogenetic trees were visualized in MEGA6 (Tamura et al. 2013) and edited in figure editor programs. All alignments and trees deriving from BI and ML analyses were deposited in TreeBASE (20982).

**Morphology of asexual and sexual structures**

Morphological features and morphometric data of sporangia, oogonia, oospores, antheridia, chlamydospores, hyphal swellings, and aggregations of the four new species were compared with each other and with those of related species from published studies.

Formation of sporangia was induced by submerging two 12–15 mm square discs cut from the growing edge of a 3-7-d-old V8A colony in a 90 mm diam Petri dish in non-sterile soil extract (50 g of filtered oak forest soil in 1000 mL of distilled water, filtered after 24 h; Jung et al. 1996). The Petri dishes were incubated at 20 °C in natural daylight and the soil extract changed after ca 6 h (Jung et al. 2017b). Shape, type of apex, caducity and special features of sporangia and the formation of hyphal swellings and aggregations were recorded after 24–48 h. For each isolate 50 sporangia were measured at x400 using a compound microscope (Zeiss Imager.Z2), a digital camera (Zeiss AxioCam ICc3) and a biometric software (Zeiss AxiosVision).

The formation of gametangia (oogonia and antheridia) and their characteristic features were examined on V8A after 21-30 d growth at 20 °C in the dark. For each isolate each 50 oogonia, oospores and antheridia chosen at random were measured under a compound microscope at x400 as described before. The oospore wall index was calculated according to Dick (1990).

For comparisons, morphometric, morphological and physiological data of *P. quercina* and *Phytophthora* species from Clade 7a, respectively, were taken from published descriptions (Jung et al. 1999, 2002, 2017b).

**Colonies morphology, growth rates and cardinal temperatures**

Colonies of *Phytophthora* species were described from 15-d-old (Clade 7a species) and 10-d-old (*P. castanetorum*, *P. quercina*, and *P. tubulina*) cultures grown at 20 °C in the dark on CA, V8A, malt-extract agar (MEA; Oxoid, Basingstoke, UK) and potato-dextrose agar (PDA; Oxoid) according to Scanu et al. (2014a), Jung et al. (1999, 2017b), and Erwin & Ribeiro (1996).

For temperature-growth relationships, representative isolates of the four new *Phytophthora* species and *P. quercina* (Supplementary Table 1) were subcultured onto 90 mm V8A plates and incubated for 24 h at 20 °C to stimulate onset of growth (Jung et al. 1999). Then, three replicate plates per isolate were transferred to 5, 10, 15, 20, 25, 30, and 35 °C. Radial growth was recorded after 6 d, along two lines intersecting the centre of the inoculum at right angles and the mean growth rates (mm/d) were calculated. Plates showing no growth at 30 or 35 °C were returned to 20 °C to determine isolate viability.

**Soil infestation trials**

In order to fulfil Koch’s postulates, pathogenicity of all four new *Phytophthora* species was tested using the soil infestation method described by Jung et al. (1996). *Phytophthora tubulina* and *P. vulgarica* were tested in trial 1 with 3-year-old saplings of *Fagus sylvatica*, while *P. castanetorum* and *P. tyrhenica* were tested in trial 2 with 1-year-old seedlings of *Castanea sativa*, and *Quercus ilex* and *Q. suber*, respectively. Saplings and seedlings were sourced from local nurseries and confirmed to be non-infested by *Phytophthora* using the soil baiting method described before. Two isolates for each new *Phytophthora* species were used, and isolates of *P. xambivora* and *P. cinnamomii* (both primary pathogens of *Fagaceae*) were included as positive controls (Supplementary Table 1). Inocula consisted of 4-wk-old cultures of the respective *Phytophthora* isolate grown at 20 °C in 500 mL Erlenmeyer flasks on an autoclaved mixture of 250 cm3 of vermiculite and 20 cm3 of millet seeds thoroughly moistened with 175 mL of V8-juice broth (200 mL/L juice, 800 mL/L distilled water amended with 3 g/L CaCO3) (Jung et al. 1996). Before use, the colonized medium was rinsed with...
Fig. 1. Fifty percent majority rule consensus phylogram derived from Bayesian inference analysis of four-locus (ITS, Btub, HSP90, NADH1) dataset of the Phytophthora Clade 12 and representative species from phylogenetic Clades 1–10. Bayesian posterior probabilities and Maximum Likelihood bootstrap values (in %) are indicated, but not shown below 0.90 and 70 %, respectively. Nothophytophthora amphigynosa was used as outgroup taxon (not shown). Bar = 0.05 expected changes per site per branch.

distilled water to remove excess nutrients. In trial 1, 12 plants per Phytophthora isolate were planted as pseudo-replicates in 50 x 35 x 27 cm boxes in an autoclaved mixture of peat, vermiculite, and sand (1:1:1 v:v:v). Tubes initially inserted as placeholders in the soil between the individual plants were removed and the holes were filled with the inoculum (ca 40 cm3 of inoculum per plant) (Jung et al. 2017b). Controls received only rinsed non-infested vermiculite/millet seed/V8-juice mixture at the same rate. In trial 2, ten plants of each Phytophthora isolate/host plant combination were infested in individual pots with the same rate of inoculum as described before. In both trials, plants were incubated for five months in a walk-in growth chamber at 18–20 °C, 65 % relative humidity, a natural daylight regime, and flooded every 3 wk for 72 h. At the end of the trial, roots were thoroughly washed free from soil. Then, specific symptoms such as root and collar rot lesions and chlorosis and wilting of foliage were recorded.

Two different root damage assessments were used. In trial 1 with Fagus sylvatica saplings, the proportion of root damage was assessed visually after spreading the roots uniformly on trays etched with 2 x 2 cm squares according to a scale of five root damage classes: 4 = healthy root system with dense fine root system and well developed tap roots; 3 = < 25 % fine root losses, beginning taproot dieback and small necrotic lesions on woody roots and/or the collar; 2 = 26–50 % fine root losses, beginning taproot dieback and large necrotic lesions on tap roots and/or the collar; 1 = 51–75 % fine root losses, extensive taproot dieback and girdling necrotic lesions on tap roots and/or the collar. Then roots were dried for 72h at 65 °C and the dry weights of small woody roots (diam 2–10 mm) and fine roots (diam <2 mm) were recorded for each plant. Data were analysed using one-way ANOVA followed by Dunnett’s multiple comparisons test using the programme package Prism 6 (Graphpad, San Diego, CA). In trial 2, Castanea sativa, Quercus ilex, and Q. suber seedlings were removed from the pots and the root system gently washed under tap water (Scanu et al. I M A F U N G U S
New pathogenic *Phytophthora* species from Europe

**RESULTS**

**Phylogenetic analysis**

Sequence datasets of Clade 7a species and species related to *Phytophthora quercina* were analysed separately. All phylogenetic analyses of individual nuclear (ITS, Btub, and HSP90) and mitochondrial (cox1 and NADH1) DNA sequences resulted in similar overall tree topologies (data not shown), thus indicating that the individual loci could be combined.

To resolve the phylogenetic positions of *P. castanetorum* and *P. tubulina* within the *P. quercina* clade, and of the latter within the genus *Phytophthora*, a 4-loci dataset (ITS–Btub–HSP90–NADH1) and a nuclear 3-loci dataset (ITS–Btub–HSP90) were analysed separately. Compared to the ML analyses, the BI analyses provided higher support for both terminal clades and the deeper branches. Since the topologies of the BI and ML trees were congruent, the Bayesian terminal clades and the deeper branches. Since the topologies of the BI and ML trees were congruent, the Bayesian posterior probabilities and Maximum Likelihood bootstrap values (in %) are indicated, but not shown below 0.90 and 70 %, respectively. *Nothophytophthora amphigynosa* was used as outgroup taxon (not shown). Bar = 0.05 expected changes per site per branch.

Fig. 2. Fifty percent majority rule consensus phylogram derived from Bayesian inference analysis of nuclear three–locus (ITS, Btub, HSP90) dataset of *Phytophthora* Clade 12 and representative species from phylogenetic Clades 1–11. Bayesian posterior probabilities and Maximum Likelihood bootstrap values (in %) are indicated, but not shown below 0.90 and 70 %, respectively. *Nothophytophthora amphigynosa* was used as outgroup taxon (not shown). Bar = 0.05 expected changes per site per branch.

2014a). Single roots were then cut off at the collar, scanned and total root length for each plant was calculated using the APS Assess 2.0 software (The American Phytopathological Society, St Paul, MN).

In all cases, re-isolations of *Phytophthora* from necrotic tissues were made using selective PARPNH agar (Jung 2009). During each flooding cycle, soils were baited using young leaves of *Ceratonia siliqua*, *F. sylvatica* and *Q. suber* as baits in order to test whether the respective *Phytophthora* species was still active. After each flooding cycle, the water was collected and autoclaved. At the end of the trial boxes were sterilised with bleach and infested substrates and the plants were autoclaved.

2014a). Single roots were then cut off at the collar, scanned and total root length for each plant was calculated using the APS Assess 2.0 software (The American Phytopathological Society, St Paul, MN).

In all cases, re-isolations of *Phytophthora* from necrotic tissues were made using selective PARPNH agar (Jung 2009). During each flooding cycle, soils were baited using young leaves of *Ceratonia siliqua*, *F. sylvatica* and *Q. suber* as baits in order to test whether the respective *Phytophthora* species was still active. After each flooding cycle, the water was collected and autoclaved. At the end of the trial boxes were sterilised with bleach and infested substrates and the plants were autoclaved.
Table 1. Pairwise numbers of different positions along alignments of ITS (842 bp), Btub (911 bp), HSP90 (840 bp), cox1 (867 bp) and NADH1 (797 bp) among the taxa from Phytophthora Clade 12.

| Species                  | P. castanetorum | P. quercina | P. sp. ohioensis | P. sp. versiformis-like | P. tubulina |
|--------------------------|-----------------|-------------|------------------|-------------------------|-------------|
| Nucleotides missing from the terminal part(s) of partial sequences and undetermined bases (N) were not considered as polymorphisms. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Btub–HSP90               | 1               | 0           | 2                | 0                       | 0           |
| cox1                     | 1               | 0           | 2                | 0                       | 0           |
| ITS                      | 1               | 0           | 2                | 0                       | 0           |
| NADH1                    | 1               | 0           | 2                | 0                       | 0           |

Nucleotides missing from the terminal part(s) of partial sequences and undetermined bases (N) were not considered as polymorphisms. and P. tubulina and the three taxa P. sp. ohioensis, P. versiformis, and P. sp. versiformis-like, allowed to resolve the phylogenetic position of the P. quercina clade within Phytophthora. Together with P. quercina, P. sp. ohioensis, P. versiformis, and P. sp. versiformis-like, P. castanetorum and P. tubulina formed a fully supported monophyletic group (BI posterior probability = 1.00, ML bootstrap = 100 %; Figs 1–2).

Since in the analysis of the nuclear 3-loci dataset P. lili formed a distinct clade (Fig. 2), confirming Rahman et al. (2015), the names Clade 11 and Clade 12 are proposed here for P. lili and the new P. quercina clade, respectively. In the analyses of both datasets, Clade 12 formed a fully supported group together with Clades 1, 2 and 4, while Clade 3 was basal to this group (Figs 1–2). Within Clade 12, isolates of P. castanetorum grouped in a well-supported cluster together with P. quercina (posterior probability = 1.00, ML bootstrap = 100 %). Phytophthora sp. ohioensis from North America appeared in a basal position of this cluster, while P. versiformis and P. sp. versiformis-like formed an Australian cluster which grouped in a sister position to the P. quercina–P. castanetorum–P. sp. ohioensis clade (Figs 1–2). Phytophthora tubulina appeared in a basal position of Clade 12 (Figs 1–2). In the separate BI and ML analyses of an ITS sequence alignment, P. sp. quercina-like, for which only an ITS sequence was available, grouped within Clade 12 (data not shown, but available from TreeBASE: S20982). Phytophthora castanetorum differed from the closest taxon P. quercina and from P. sp. ohioensis in three nuclear (ITS–Btub–HSP90) and the mitochondrial NADH1 gene regions by 9–20 and 23–28 characters, respectively, and from the more distantly related P. versiformis, P. sp. versiformis-like, and P. tubulina by 45–52, 45–51 and 97–102 characters, respectively (Table 1). In addition, in cox1 P. castanetorum showed differences to P. quercina at 5–14 positions (Table 1). Isolates of P. castanetorum showed intraspecific variability, with the Sardinian isolates forming a distinct cluster (Figs 1–2). DNA sequences of Sardinian isolates differed from those of P. castanetorum from Portugal in Btub and NADH1 by 3 bp and 2 bp, respectively (Table 1). In contrast, P. tubulina isolates were identical across all five loci examined. Phytophthora versiformis and P. sp. versiformis-like showed differences to each other at 6–9 positions, and to P. castanetorum and P. quercina at 45–52 and 46–53 positions, respectively (Table 1). The basal species P. tubulina differed from P. castanetorum, P. quercina, P. sp. ohioensis, P. versiformis and P. sp. versiformis-like by 97–102, 92–106, 57, 51–53 and 52–53 characters, respectively (Table 1). In addition, P. tubulina was separated from P. castanetorum and P. quercina in cox1 by 37 and 33–42 characters, respectively (Table 1).

Bayesian and ML analyses of the combined five locus dataset for Clade 7a generated trees with essentially the same topology. The Bayesian analysis provided more support for deeper branches, while support for terminal clades and their clustering was equivalent in both analyses. The Bayesian tree is shown in Fig. 3 with both BI posterior probability and ML bootstrap values provided at the nodes (Fig. 3). The resolution was good at terminal clades and allowed for the differentiation of 17 distinct lineages within Clade 7a corresponding to 14 described species, P. attenuata, P. europaea, P. flexuosa, P. formosa, P. intricata, P. fragariae, P. rubi, P. uliginosa, P.
uniformis, P. xambivora, P. xheterohybrida, P. xincrassata, P. xalni i P. xmultiformis, the two informally designated taxa P. sp. xambivora-like and P. sp. xmultiformis-like, and the two new species P. tyrrhenica and P. vulcanica described in this study (Fig. 3). Isolates of P. tyrrhenica grouped together with P. uliginosa in a well-supported clade (posterior probability = 1.00, ML bootstrap = 100 %), both species being separated by 2, 4 and 1–2 characters in ITS, Btub and HSP90, respectively, and by 22–24 and 8–10 characters in cox1 and NADH1, respectively. The relative phylogenetic position

P. vulcanica

P. uliginosa

P. tyrrhenica

P. attenuata

P. fragariae

P. rubi

P. uniformis

P. xambivora

P. sp. xambivora-like

P. xmultiformis-like

P. xalni + P. xmultiformis

P. xheterohybrida

P. xincrassata

P. formosa

P. intricata

P. europaea

P. flexuosa

Fig. 3. Fifty percent majority rule consensus phylogram derived from Bayesian phylogenetic analysis of five-locus (ITS, Btub, HSP90, cox1, NADH1) dataset of Clade7a. Bayesian posterior probabilities and Maximum Likelihood bootstrap values (in %) are indicated, but not shown below 0.90 and 70 %, respectively. Phytophthora cinnamomi and P. niederhauserii from Clade 7b were used as outgroup taxa (not shown). Bar = 0.05 expected changes per site per branch.
of *P. tyrrenica* and *P. uliginosa* was also well-supported (Fig. 3). The *P. tyrrenica*–*P. uliginosa* clade grouped in a sister position to the remaining in-group taxa representing 15 taxa from Clade 7a (Fig. 3). Small intraspecific variation of up to seven characters across the five gene regions was found amongst *P. tyrrenica* isolates, which was related to different oak species and geographic origin. Isolate PH103 (from *Quercus suber* in Sardinia) represented a distinct basal lineage (Fig. 3). In contrast, *P. vulcanica* was monomorphic across all five loci examined, suggesting that the tested isolates could belong to the same clone of the species. Across the five DNA regions, *P. vulcanica* showed differences to its closest relatives *P. uliginosa* and *P. tyrrenica* at 109 and 109–114 positions, respectively, and resided in a well-supported basal position of the whole Clade 7a (Fig. 3).

**Hosts and geographic distribution**

*Phytophthora castaneatorum* was exclusively isolated from *Castanea sativa* forests in Portugal, at Monchique (N 37° 18.85¹, W 8° 32.455¹; 496 m above sea level, asl) and in the Parque Natural Serra da Estrela (N 40° 25.489, W 7° 22.523¹; 901 m asl), and in Sardinia at Gennargentu montain (N 39° 56.946¹, E 9° 11.387¹; 858 m asl). It usually co-occurred with *P. xambivora* and *P. cinnamomi*, associated with severe ink disease symptoms. *Phytophthora xambivora* was isolated alongside *P. xambivora* and *P. plurivora* from mildly declining *Fagus sylvatica* trees in the Dunkelsteiner Forest in Austria (N 48° 14.56¹, E 15° 28.276°; 497 m asl), while *P. vulcanica* was recovered from relatively healthy *F. sylvatica* trees at Timpa Rossa on Mount Etna in Sicily (N 37° 48.849¹, E 15° 1.407¹; 1862 m asl). *Phytophthora tyrrenica* was associated with declining *Quercus ilex* and *Q. suber* trees in different forest stands on the Italian islands Sardinia (N 37° 52.492¹, E 9° 2.720¹; 183 m asl) and Sicily (N 37° 54.341¹, E 14° 4.490°; 1110 m asl).

**Soil infestation trials**

*Pathogenicity on Fagus sylvatica seedlings (trial 1)*

At the end of the trial shoots and root systems of control plants of *F. sylvatica* were generally healthy and well developed with a fine root/main root weight (frw/mrw) ratio of 2.2 and a root damage class of 3.6 ± 0.67 (Fig. 4). *Phytophthora xambivora* was the most aggressive species causing after 5 months 50 % mortality, a frw/mrw ratio of 0.78 ± 0.36 (64.5 % reduction compared to the control), 54.4 % reduction of fine.
root weight compared to control plants and a root damage class of 1.3 ± 0.14 (i.e. 67.5 % fine root losses and advanced dieback and necrotic lesions of tap roots and root collars) (Fig. 4). Phytophthora tubulina isolates (TUB1= CBS 141212 and TUB2= CBS 141213) caused 8.3% and 16.6% mortality, a frw/mrw ratio of 1.22 ± 0.49 and 1.39 ± 0.1 (44.5 and 42.3% reduction compared to the control), and a root damage class of 2.5 ± 1.0 and 2.7 ± 1.0, respectively (Fig. 4). Beech plants in soil infested by isolates X3d and X3e of P. vulcanica had a frw/mrw ratio of 1.55 ± 0.52 and 1.27 ± 0.41 (29.8 and 42.3% reduction compared to the control), and a root damage class of 2.0 ± 0.95 and 2.4 ± 0.9, respectively (Fig. 4). Both isolates caused 8.3% mortality and, chlorosis and wilting in 58.3% (X3d) and 75% (X3e) of the plants. Differences of root damage class and frw/mrw ratio to the control were statistically significant for P. x cambivora, both isolates of P. vulcanica and isolate TUB1 of P. tubulina (Fig. 4). For P. tubulina isolate TUB 2, the difference to the control was only significant for the frw/mrw ratio but not for the root damage class (Fig. 4).

Pathogenicity on Castanea sativa seedlings (trial 2)

At the end of the experiment seedlings inoculated with P. x cambivora were mostly dead (mortality rate above 80%) or showed severe wilting associated with collar and root necrosis. In contrast, all seedlings inoculated with P. castanetorum were alive and showed only weak symptoms of wilting and chlorosis of leaves. Control plants did not show any aboveground symptoms and exhibited overall faster growth. Phytophthora x cambivora was extremely aggressive to the root systems causing more than 60% reduction of total root length (P < 0.0001). In contrast, seedlings inoculated with P. castanetorum showed only mild fine root infections but no symptoms of woody root and collar infections, and total root length did not differ statistically from the control plants (Fig. 5).

Pathogenicity on Quercus ilex and Quercus suber seedlings (trial 2)

Oak seedlings inoculated with P. tyrrenhica started to show symptoms of leaf chlorosis and shoot dieback 45 d post-inoculation. Mortality of seedlings occurred between the third and fourth month after inoculation. Based on mortality rates after five months, P. cinnamomi was more aggressive than P. tyrrenhica on both Q. ilex and Q. suber, killing 80% and 63.5% of seedlings, respectively, whereas P. tyrrenhica caused an average mortality on Q. ilex and Q. suber of 48.5% and 25%, respectively. On Q. ilex, both Phytophthora species caused fine root losses and necrosis of mother roots, and a significant reduction in root length compared to control seedlings (P < 0.0001) (Fig. 5). Phytophthora cinnamomi caused significantly higher root reduction than P. tyrrenhica for which Dunnett’s test revealed no significant differences in total root length of inoculated seedlings. Also on Q. suber, both P. cinnamomi and P. tyrrenhica caused significant total root length reduction, but the reduction by P. cinnamomi was significantly higher from that caused by both isolates of P. tyrrenhica (P < 0.0001) (Fig. 5).

At the end of all pathogenicity trials, each Phytophthora species could be re-isolated from both infested soils using young leaves of C. siligua, F. sylvatica and Q. suber as baits and necrotic fine roots or necrotic root lesions by direct plating onto PARPNH agar. No Phytophthora species could be isolated from soil and roots of control plants.

TAXONOMY

Morphological and physiological characters and measurements of the four new Phytophthora taxa and related species are given in the comprehensive Table 2.
Table 2. Morphological characters and dimensions (µm), cardinal temperatures (°C) and temperature-growth relations (mm/d) on V8A of *Phytophthora castanetorum*, *P. tyrrenica*, *P. tubulina*, *P. vulcanica* and their closest relatives in phylogenetic Clades 7a and 12, respectively. Most discriminating characters are highlighted in bold.

| Character                  | *P. castanetorum* | *P. tubulina* | *P. quercina* | *P. tyrrenica* | *P. vulcanica* | *P. uliginosa* | *P. europaea* | *P. flexuosa* |
|----------------------------|-------------------|---------------|--------------|---------------|---------------|---------------|---------------|--------------|
| **Sporangia**              |                   |               |              |               |               |               |               |              |
| Ovoid, subglobose, obpyriform, globose, (ellipsoid, distorted) | elongated ellipsoid, elongated ovoid, elongated limoniform | ovoid, ellipsoid, pear-shaped, (obpyriform, limoniform) | ovoid, ellipsoid, pear-shaped, (obpyriform, limoniform) | ovoid, ellipsoid, pear-shaped, (obpyriform, limoniform) | ovoid, ellipsoid, pear-shaped, (obpyriform, limoniform) | ovoid, ellipsoid, pear-shaped, (obpyriform, limoniform) | ovoid, ellipsoid, pear-shaped, (obpyriform, limoniform) | ovoid, ellipsoid, pear-shaped, (obpyriform, limoniform) |
| **apex**                   |                   |               |              |               |               |               |               |              |
| 77.4% papillate, 14.9% semi-papillate; 7.7% non-papillate, 4.9% curved | all papillate, some bi- and tripapillate; often curved | non-papillate | non-papillate | non-papillate | non-papillate | non-papillate | non-papillate | non-papillate |
| **lxb mean**               | 47.0±5.7 x 36.6±3.7 | 51.6±9.8 x 37.3±7.0 | 42.4±11.5 x 29.3±13.8 | 68.4±17.8 x 28.9±1.9 | 57.3±6.8 x 34.5±4.2 | 67.0±6.5 x 42.4±6.4 | 63.7±16.9 x 44.6±8.3 | 56.1±7.4 x 36.7±5.2 |
| **Range of isolates means**| 44.2–48.1 x 35.4–43.8 | 43.3–57.8 x 31.2–45.0 | 36.0–49.2 x 23.3–33.7 | 66.1–70.0 x 27.5–31.9 | 53.6–61.0 x 31.8–36.4 | 65.7–70.3 x 30.9–44.4 | 50.0–78.9 x 36.7–51.2 | 53.3–58.6 x 33.9–39.4 |
| **Total range**            | 27.1–74.5 x 18.1–47.9 | 29.5–98.5 x 19.2–59.2 | 18.8–112.5 x 13.8–47.5 | 45.3–110.2 x 16.2–39.4 | 35.5–80.7 x 22.5–42.9 | 41.1–85.0 x 24.8–56.9 | 23.6–124.3 x 21.9–67.3 | 34.9–74.8 x 22.8–49.7 |
| **l/b ratio**              | 1.29±0.15         | 1.40±0.18     | 1.45±0.37     | 2.40±0.18     | 1.67±0.22     | 1.60±0.19     | 1.42±0.19     | 1.54±0.18     |
| **Caducity**               | –                 | –             | –             | –             | –             | –             | –             | –             |
| **Lateral insertion**      | 37.7%             | 22.4%         | frequent      | frequent      | frequent      | frequent      | frequent      | frequent      |
| **Exit pores**             | 7.0±1.1           | 6.4±1.2       | 6.5±1.2       | 12.1±2.4      | 17.0±3.0      | 15.4±3.4      | 19.3±4.7      | 19.7±3.4      |
| **Zoospore cysts**         | 9.2±1.0           | 11.7±1.8      | 9.5 (7.1–12.9)| 14.6±2.3      | 10.0±1.2      | 11.9±1.8      | 15.3±2.0      | 13.3±1.3      |
| **Breeding system**        | Homothallic       | Homothallic   | Homothallic   | Homothallic   | Homothallic   | Homothallic   | Homothallic   | Homothallic   |
| **Oogonia**                |                   |               |              |               |               |               |               |              |
| Mean diam                  | 32.9±3.0          | 29.2±4.4      | 29.4±4.1      | 37.2±3.3      | 35.8±3.6      | 46.2±7.5      | 37.3±5.4      | 36.8±3.3      |
| Range of isolate means     | 30.5–34.4         | 25.4–30.1     | 25.8–32.0     | 36.8–40.9     | 33.6–36.5     | 41.6–53.3     | 34.4–39.9     | 36.0–36.3     |
| Total range                | 24.3–41.7         | 16.5–42.5     | 19.0–45.0     | 25.0–39.3     | 23.0–46.0     | 17.9–65.5     | 19.2–48.4     | 25.7–42.8     |
| Tapering base              | 61.3% (40–86%)    | 48.7% (30–78%)| n.a.          | –             | 37.3% (8–60%) | –             | 60.0% (52–81%)| 47.9% (32–58%)|
| Elongated                  | 65.4% (50–82%)    | 59.5% (36–86%)| 45% (10–86%)  | –             | 44.8% (8–80%) | –             | 21.3% (12–34%)| 19.7% (4–36%) |
| Tubular                    | –                 | 24.5% (21–28%)| –             | –             | –             | –             | –             | –             |
| Excentric                  | 6.7% (2–20%)      | 5.0% (2–8%)   | n.a.          | –             | 20.9% (9–46%) | –             | 4.0% (0–8%)   | 5.3% (2–10%)  |
| Smooth-walled              | 100%              | 100%          | 100%          | 100%          | 100%          | 100%          | 100%          | 100%          |
| **Oospores**               |                   |               |              |               |               |               |               |              |
| Plerotic                   | 64.3% (50–83%)    | 44% (24–76%)  | mostly aplerotic | >99% (99–100%)| 27.3% (10–45%)| 30.0% (12–46%)| 46.3% (38–53%)| >99% (99–100%)|
| Aplerotic                  | 35.7% (57–80%)    | 56% (24–76%)  | mostly aplerotic | >99% (99–100%)| 72.7% (10–45%)| 70.0% (12–46%)| 53.7% (38–53%)| >99% (99–100%)|
| Phytophthora species | mean diam (microns) | wall diam (microns) | oospore wall index | Abortion rate | Antheridia | Chlamydospores | Hyphae | Hyphal aggregations | Maximum hyphal swellings | Optimum temperature | Growth rate at optimum | Growth rate at 20°C |
|---------------------|---------------------|--------------------|-------------------|--------------|------------|---------------|----------|-------------------|-------------------------|----------------------|----------------------|-------------------|
| Phanerochaete tans | 28.7 ± 3.7 | 23.7 ± 1.3 | 3.1 ± 0.3 | 21.5 % (18–28 %) | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Phyllachora castaneum | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Phyllachora vulcanica | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces castaneus | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces elegans | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces trichophila | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces uliginosus | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces tans | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces vulcanica | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces tans | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces vulcanica | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces tans | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces vulcanica | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces tans | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces vulcanica | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
| Pithomyces tans | 24.7 ± 2.6 | 18.0 ± 0.6 | 2.0 ± 0.05 | 99 % paragynous | 99 % paragynous | Globose – rare, in some isolates | Sympodial branching with protruding tip of mother hypha | – | 17–35 |
Phytophthora castanetorum T. Jung, M. Horta Jung, Bakonyi & Scanu, sp. nov. (Fig. 6)

Etymology: Referring to the association of this species with forests of Castanea sativa (Castanetum is the phytosociological term for a chestnut forest).

Diagnosis: Phytophthora castanetorum differs from all other Phytophthora species from Clade 12 by regularly producing chlamydospores, and from P. quercina also by producing a low proportion of semipapillate and nonpapillate sporangia.

Type: Portugal: Algarve: Monchique, isolated from rhizosphere soil of a mature Castanea sativa tree, March 2015, T. Jung (CBS H-22983—holotype, dried culture on V8A; CBS 142299 = BD 292—ex-type culture). ITS and cox1 sequences GenBank MF036182 and MF036266, respectively.

Description: Sporangia, hyphal swellings and chlamydospores (Fig. 6A–G): Sporangia commonly observed in solid agar of older cultures (Fig. 6A) and produced abundantly in nonsterile soil extract; typically borne terminally on unbranched sporangiphores or in irregular lax or regular dense sympodia, and some formed on short lateral hyphae (Fig. 6H) or intercalary (Fig. 6I); non-caducous, monopapillate (77.4 %; Fig. 6A–G), very rarely bipapillate (over all isolates <1 %), semipapillate (14.9 %; Fig. 6H) or non papillate (7.7 %; Fig. 6I), and often forming a conspicuous basal plug that protrudes into the empty sporangium (Fig. 6J); in solid agar with conspicuously protruding papillae (Fig. 6A); sporangial shapes very variable, ovoid (over all isolates 77.4 %; Fig. 6B–D, I–J) or subglobe to globose (11.7 %; Fig. 6G) to obpyriform (4.6 %; Fig. 6E), ellipsoid (2.0 %), limoniform (1.0 %; Fig. 6A, F), or distorted (3.3 %); unusual features such as lateral attachment of the sporangiophere (over all isolates 37.7 %; Fig. 6D, G), hyphal extensions (9.1 %; Fig. 6B, D), markedly curved apices (4.9 %) or the presence of a vacuole (8.0 %; Fig. 6B–G) common in all isolates. Small subglobe to limoniform hyphal swellings sometimes formed close to the sporangial base (Fig. 6C). Zoospores discharged through an exit pore 4.0–9.5 µm wide (av. 7.0 ± 1.1 µm) (Fig. 6J), limoniform to reniform whilst motile, becoming spherical (av. diam. = 9.2 ± 1.0 µm) on encystment, direct germination common in older water cultures (Fig. 6L). Sporangia of seven isolates averaged 47.0 ± 5.7 × 36.6 ± 3.7 µm (overall range 27.0–74.5 × 18.0–48.0 µm) with a range of isolate means of 44.2–48.1 × 35.4–38.3 µm and a length/breadth ratio of 1.29 ± 0.15 (range of isolate means 1.25–1.36). Swellings commonly observed in liquid culture; 27.1 ± 9.9 µm diameter (total range 10.0–47.0 µm), subglobe to globose, limoniform, pyriform or distorted; sometimes catenulate and often with individual or radiating hyphal extensions, (Fig. 6P–Q). Chlamydospores globose, produced on both CA and V8A, 29.5 ± 4.9 µm diam (total range 18.0–47.0 µm) (Fig. 6R).

Oogonia, oosporas, antheridia and hyphae (Fig. 6H–O): Gametangia readily produced in single culture by all isolates on V8A within 7–10 d. Oogonia terminal, smooth-walled, and elongated pyriform to ellipsoid (on av. 65.4 %; Fig. 6N–O) or globose to slightly subglobe (34.6 %; Fig. 6K–M); bases often tapering (61.3 %; Fig. 6K, N–O) and sometimes slightly curved (4.9 %; Fig. 6O); mean 32.9 ± 3.0 µm diam (overall range 24.5–41.5 µm and range of isolate means 30.5–34.5 µm) and an average length of 40.5 ± 4.6 µm; almost plerotic (53.1 %) or aplerotic (46.9 %). Oospores usually globose (98.3 %; Fig. 6K–N) but could be slightly elongated in elongated oogonia (1.7 %; Fig. 6O); thick-walled, wall thickness 3.1 ± 0.5 µm (range 1.8–4.5 µm), and oospore wall index 0.51 ± 0.05%; abortion 19–24 % after 4 wk increasing to 58–91 % after 12 months. Antheridia almost exclusively paragynous and club-shaped to subglobe (Fig. 6K–L, N), sometimes with one or more finger-like projections (4.3 %), but a few amphigynous antheridia observed in all isolates (Fig. 6M). Primary hyphae often branched in a mono- or dichasium with the mother hypha ending in a short protruding tip (Fig. 6S–T).

Figures (Figs 11, 13): Colonies on CA stellate with limited aerial mycelium and on V8A uniform and woolly; on PDA and MEA isolates formed uniform colonies, dense feltly on PDA and mostly submerged on MEA. All isolates with very slow growth on PDA and MEA (Fig. 11). Temperature-growth relations are shown in Fig. 13. All six isolates included in the growth test had similar growth rates. The maximum growth temperature was 25–30 °C, with no growth when plates incubated for 5 d at 30 °C were transferred to 20 °C. Average radial growth rate at the optimum temperature of 25 °C 3.7 mm/d.

Additional material examined: Portugal: Beira Alta: Parque Natural da Serra da Estrela, isolated from rhizosphere soil of a mature Castanea sativa tree.
New pathogenic *Phytophthora* species from Europe
**Phytophthora tubulina** T. Jung, T. Cech, Scanu, Horta Jung & Bakonyi, *sp. nov.*

*MycoBank MB 819701* (Figs 7–8)

**Etymology**: Name refers to the tubular shape of many oogonia (tubulina Lat., tubular).

**Diagnosis**: *Phytophthora tubulina* differs from all other known *Phytophthora* species by having partially extremely elongated, often tubular oogonia with high oospore abortion rates, and from *P. quercina* by producing a low proportion of semipapillate and nonpapillate sporangia.

**Type**: Austria: Lower Austria; Dunkelsteiner Forst, isolated from rhizosphere soil of a mature *Fagus sylvatica* tree, Sept. 2010, T. Jung (CBS H-22557—holotype, dried culture on V8A; CBS 141212 = TUB1—ex-type culture). ITS and *cox1* sequences GenBank MF036196 and MF036277, respectively.

**Description**: Sporangia, hyphal swellings and chlamydospores (Fig. 7A–U): Sporangia infrequently observed in solid agar, abundantly produced in non-sterile soil extract; borne terminally on unbranched sporangiophores, in lax sympodia, on short lateral sporangiophores (Fig. 7B, O) or intercalary (3.4 %; Fig. 7L); sometimes formed on short hyphal appendices growing from mature sporangia (Fig. 7N); usually non-caducous (Fig. 7A–L, N–S) but a low proportion (<1 %) of caducous sporangia without preformed pedicels (Fig. 7O–Q) present in most isolates; apices highly variable ranging from monopapillate (56.6 %; Fig. 7A–G, N, P), bi- or tripapillate (21.4 %; Fig. 7H–I, Q), semipapillate (13.3 %; Fig. 7J) to nonpapillate (8.9 %; Fig. 7K–L, O); sometimes curved (4.9 %; Fig. 7C, G); sporangial shapes ranging from ovoid or elongated ovoid (69.4 %; Fig. 7A–B, E), obpyriform (15.3 %; Fig. 7C), limoniform (3.0 %; Fig. 7D), subglobose (3.7 %) or less frequently ellipsoid and globose (2.3 %) to distorted shapes (6.3 %; Fig. 7H–I, Q); hyphal appendices (6.7 %; Fig. 7K, O) and laterally attached sporangiophores (22.4 %; Fig. 7B, E, N–O) commonly observed; dimensions of six isolates of *P. tubulina* averaging 51.6 ± 9.8 x 37.3 ± 7.0 µm (overall range 29.5–98.5 x 19.0–59.0 µm) with a range of isolate means of 43.5–58.0 x 31.0–45.0 µm; length/breadth ratio 1.4 ± 0.18 with a range of isolate means of 1.27–1.49; sporangial germination directly (Fig. 7R) or more often indirectly with zoospores discharged through an exit pore 4.0–9.0 µm wide (av. 6.4 ± 1.2 µm) (Fig. 7S). Zoospores limoniform to reniform whilst motile, becoming spherical (av. diam = 11.7 ± 1.8 µm) on encystment. **Swellings** subglobose to limoniform, often catenate and with radiating hyphae, infrequently produced on sporangiophores (Fig. 7M, T–U). Chlamydospores not observed.

**Oogonia, oospores, antheridia and hyphae** (Fig. 8A–S): Gametangia readily produced in single culture by all isolates of *P. tubulina* on V8A within 10 d. Oogonia borne terminally or laterally, with smooth walls and tapering, often long bases (on av. 53.2 %; Fig. 8A–E, G, K); globose to subglobose (35.8 %; Fig. 8A), elongated to tubular (59.2 %; Fig. 8B–K) or excentric (5 %; Fig. 8L); diameters averaging 29.2 ± 4.4 µm (overall range 16.5–42.5 µm and range of isolate means 25.4–30.1 µm). Oogonial length 44.8 ± 11.6 µm; tubular viable oogonia (Fig. 8A–J) up to 109.0 µm long, aborted tubular oogonia (Fig. 8M–Q) reaching lengths of 300.0 µm. Oospores with a mean diameter of 26.1 ± 3.7 µm (total range 16.0–36.5 µm), perithecoid or apiculiform, usually globose (89 %) or less frequently elongated (11 %) assuming the shape of the elongated or excentric oogonium (Fig. 8E–H). Some oogonia with two oospores of which one was usually aborted (Fig. 8J). Oospores with medium-thick walls (2.0 ± 0.6 µm) and a mean oospore wall index of 0.38 ± 0.08; containing either one large ooplast (Fig. 8A–D) or several smaller lipid vacuoles (Fig. 8E–H); abortion high (on. av. 72.3 %; 40–91 %) occurring either before (Fig. 8M–Q) or after the formation of the oospore (Fig. 8F, J–L). Antheridia exclusively paragynous (Fig. 8A–Q) averaging 14.9 ± 3.1 x 10.9 ± 1.7 µm, with shapes ranging from subglobose to club-shaped. Primary hyphae often branching in a mono- or dichasium with the mother hyphae ending in a short protruding tip (Fig. 8R–S).

**Cultures** (Figs 11, 13): Colonies of all five *P. tubulina* isolates similar on the four different types of media (Fig. 11); largely submerged with limited felty aerial mycelium around the inoculum plug on all four agar media, uniform on CA and V8A, and irregular to dendroid on PDA; almost no growth on MEA. Temperature–growth relations on V8A are shown in Fig. 13. All isolates with identical cardinal temperatures and similar growth rates at all temperatures. The maximum growth temperature was between 25 and 30 °C. All isolates with slow growth, unable to grow at 30 °C and not resuming growth when plates incubated for 5 d at 30 °C were transferred to...
Fig. 8. Phytophthora tubulina (CBS 141212 — ex-type), structures formed in solid V8 agar. A–H. Mature oogonia with paragynous antheridia, formed in single culture. A. Subglobose with tapering base and aplerotic, globose thick-walled oospore. B–C. Elongated ellipsoid with tapering bases and aplerotic, globose thick-walled oospores. D. Elongated pyriform with tapering base and aplerotic, globose thick-walled oospore. E. Elongated tubular with tapering base and plerotic, elongated tubular, thick-walled oospore. F–G. Elongated excentric with almost plerotic elongated oospores containing two ooplasts. H. Elongated tubular containing a globose, viable thin-walled oospore. I. Elongated tubular containing a subglobose, viable thin-walled oospore and a second, ellipsoid aborted oospore (arrow). J–L. Oogonia aborted after oospore formation. M. Elongated tubular. N. Elongated pyriform. O. Excentric. P–Q. Extremely elongated, tubular oogonia, aborted before oospore formation, with paragynous antheridia (arrows). R–S. Primary hyphae branching in a dichasium (R) or monochasium (S) with the mother hypha ending in a short protruding tip. Bar A–S = 25 µm.
New pathogenic Phytophthora species from Europe

20 °C. Phytophthora tubulina had a broad growth optimum between 15 and 25 °C with an average radial growth rate of 2.8 ± 1.1 mm/d at 20 °C.

Additional material examined: Austria: Lower Austria: Dunkelsteiner Forest, isolated from rhizosphere soil of a mature Fagus sylvatica tree, Sept. 2010, T. Jung (CBS 141213 = TUB2, TUB3, TUB4, TUB5).

Phytophthora tyrrhenica B. Scanu, S.O. Cacciola, Seddaiu, Bakonyi & T. Jung, sp. nov.
MycoBank MB819700 (Fig. 9)

Etymology: Name refers to the origin of all known isolates in the Tyrrhenian islands Sardinia and Sicily (tyrrhenica Lat., Tyrrhenian).

Diagnosis: Phytophthora tyrrhenica is distinguished from its closest relatives P. uliginosa and P. vulcanica by the exclusive production of elongated and mostly ellipsoid sporangia, and from P. vulcanica also by the absence of amphiigenous antheridia.

Type: Italy: Sardinia; Tergu, isolated from rhizosphere soil of a mature Quercus ilex tree, May 2012, B. Scanu (CBS H-22984–holotype, dried culture on V8A; CBS 142301 = PH154–ex-type culture). ITS and cox1 sequences GenBank KU899188 and KU899343, respectively.

Description: Sporangia, hyphal swellings and chlamydospores (Fig. 9A–I): Sporangia not observed on solid agar but produced abundantly after 24 h in non-sterile soil extract; borne terminally on unbranched sporangiophores, non-caducous, and non-papillate (Fig. 9A–I); ellipsoid to elongated ellipsoid (55.6 %; Fig. 9A, C, I), limoniform to elongated limoniform (18 %; Fig. 9B), ovoid to elongated ovoid (18.8 %; Fig. 9D) and elongated obpyriform (7.6 %); sporangial proliferation usually internal, mainly in a nested (Fig. 9D, F–J) but also extended way (Fig. 9E); dimensions of five isolates 68.4 ± 1.7 x 28.9 ± 1.9 µm (overall range 45.5–110.0 x 16.0–39.5 µm) and range of isolate means 33.7 ± 1.3 µm (total range 24.5–40.0 µm), thick walls (av. 2.9 ± 1.5 µm, total range 1.5–4.5 µm), with a mean oospore wall index of 0.41 ± 0.03. Mean oogonial abortion rate low (5 %). Antheridia formed terminally or laterally (Fig. 9M), exclusively paragynous, averaging 16.9 ± 4.6 x 12.8 ± 0.13 µm, with shapes ranging from clavate, globose to subglobose.

Cultures (Figs 12–13): Colonies uniform with dense-felt to woolly aerial mycelium and regular margin on CA and V8A, and uniform appressed on PDA. All isolates with very limited and irregular growth on MEA (Fig. 12). Temperature-growth relations are shown in Fig. 13. All five isolates included in the growth test with similar growth rates and cardinal temperatures. Maximum growth temperature was 25–30 °C; all isolates resuming growth when plates incubated for 5 d at 30 °C were transferred to 20 °C. Average radial growth rate at the optimum temperature of 20 °C 1.5 ± 0.3 mm/d.

Additional material examined: Italy: Sicily: Madonie mountains, isolated from rhizosphere soil of mature Quercus ilex trees, May 2015, T. Jung (TYR1, TYR2, TYR3, TYR4, TYR5, TYR6); Sardinia; Tergu, isolated from rhizosphere soil of a mature Q. ilex tree, May 2012, B. Scanu (PH155); Sardinia; Alà dei Sardi, isolated from rhizosphere soil of a mature Quercus suber tree, June 2012, B. Scanu and S. Seddaiu (CBS 142303 = PH103).

Phytophthora vulcanica T. Jung, M. Horta Jung, Scanu, Bakonyi & Cacciola, sp. nov.
MycoBank MB819702 (Fig. 10)

Etymology: Name refers to the origin of all known isolates from volcanic soil (vulcanica Lat., volcanic).

Diagnosis: Phytophthora vulcanica differs from its closest relatives P. uliginosa and P. tyrrhenica by the production of both paragynous and amphiigenous antheridia, by its low optimum temperature of 15 °C and by slower growth rates at 15 and 20 °C.

Type: Italy: Sicily; Mount Etna, isolated from rhizosphere soil of a mature Fagus sylvatica tree, May 2013, T. Jung (CBS H-22566–holotype, dried culture on V8A; CBS 141216 = X3a–ex-type culture). ITS and cox1 sequences GenBank MF036209 and MF036287, respectively.

Description: Sporangia, hyphal swellings and chlamydospores (Fig. 10A–I): Sporangia not formed on solid agar but produced in non-sterile soil extract; non-caducous and non-papillate with a flat apex (Fig. 10A–E, G); borne terminally, on unbranched sporangiophores or in lax sympodia, or less frequently laterally (Fig. 10D), proliferating internally in both a nested (Fig. 10G, H) and extended way (Fig. 10I). Sporangioles often branching close to the sporangial base forming lax sympodia (Fig. 10F–G). Sporangial shapes ovoid (52.5 %; Fig. 10A–B, F–G, I), elongated ovoid (14.0 %; Fig. 10C), ellipsoid to elongated ellipsoid (32.5 %; Fig. 10E–F, H) or less frequently limoniform (Fig. 10D), pyriform and obpyriform (1.0 %); dimensions of six isolates 57.3 ± 8.7 x 34.5 ± 4.2 µm (overall range 35.5–81.0 x 22.5–43.0 µm)
Fig. 9. *Phytophthora tyrrenica* (CBS 142301 — ex-type). A–I. Sporangia formed on V8 agar (V8A) flooded with soil extract. A–D. Nonpapillate with flat apex. A. Ellipsoid. B. Limoniform with tapering base. C. Elongated ellipsoid. D. Mature ovoid (right) and empty with wide exit pore and internal nested proliferation (left). E–I. Empty with wide exit pores and internal extended (E) or internal nested proliferation (F–I). J–N. Mature, golden-brown, smooth-walled, globose oogonia formed in single culture in V8A, containing thick-walled plerotic oospores with big ooplasts, with paragynous antheridia. K–L. Antheridia with finger-like hyphal extensions. O–P. Globose, limoniform and angular catenulate hyphal swellings formed on V8A flooded with soil extract. Bar A–O = 25 µm, P = 50 µm.
New pathogenic *Phytophthora* species from Europe

**Fig. 10.** *Phytophthora vulcanica* (CBS 141216 — ex-type). A–I. Sporangia formed on V8 agar (V8A) flooded with soil extract. A–E. Nonpapillate with flat apex. A–B. Ovoid. C. Elongated ovoid. D. Limoniform, on short lateral hypha. E. Elongated ellipsoid. F. Empty sporangia after release of zoospores through wide exitpores, showing external proliferation. G. Empty sporangium with both internal nested proliferation (arrow) and external proliferation forming a mature ovoid sporangium. H. Empty, elongated ellipsoid sporangium with internal nested proliferation. I. Empty ovoid sporangium with internal extended proliferation. J–N. Mature, golden-brown oogonia formed in single culture in V8A, with medium thick-walled oospores. J. Subglobose with aplerotic globose oospore and amphigynous antheridium. K. Elongated, slightly excentric with aplerotic subglobose oospore and paragynous antheridium. L. Obovoid with aplerotic globose oospore and paragynous antheridium. M. Elongated ellipsoid with aplerotic ellipsoid oospore and paragynous antheridium. N. Elongated pyriform with plerotic ellipsoid oospore. O. Globose and triangular, catenulate hyphal swellings. P. Dense hyphal aggregation. Q. Tubular to elongated club-shaped hyphae. Bar A–O = 25 µm, P–Q = 40 µm.
with a range of isolate means of 53.6–61.0 x 31.8–36.4 µm, and a length/breadth ratio of 1.67 ± 0.22 (range of isolate means 1.58–1.72). Zoospores of *P. vulcanica* discharged through wide exit pores averaging 17.0 ± 3.0 µm (12.0–25.0 µm), limoniform to reniform whilst motile, becoming spherical (av. diam = 10.0 ± 1.2 µm) on encystment; cysts usually germinating directly but diplanetism also observed in all isolates. Swellings formed on sporangiophores by all isolates, subglobose, angular or irregular, often catenulate (Fig. 10O), with an average diameter of 21.1 ± 5.5 µm. Chlamydospores not observed.

Oogonia, oospores, antheridia and hyphae (Fig. 10J–Q): Oogonia produced by all five isolates on V8A in single culture; borne terminally or laterally, with smooth walls and often tapering (37.3 %; Fig. 10L–N) or curved bases (7.2 %; Fig. 10M); elongated, ellipsoid to pyriform or excentric (on av. 65.7 %; Fig. 10K–N) or less frequently globose to subglobose (34.3 %; Fig. 10J), turning golden-brown during ageing (Fig. 10J–N); mean 35.8 ± 3.6 µm diam. with an overall range of 23.0–46.0 µm and isolate means ranging from 33.6–36.5 µm. Oospores with a mean diameter of 30.5 ± 3.2 µm (total range 20.0–38.0 µm), aplerotic (72.7 %) or less frequently plerotic (27.3 %), containing a large ooplast (Fig. 10J–N); medium thick-walled (2.4 ± 0.4 µm), with a mean oospore wall index of 0.40 ± 0.06. High oogonial abortion rate in most isolates (av. 52.8 %; 0–80 %). Antheridia 16.3 ± 3.1 x 12.6 ± 2.3 µm, predominantly paragynous (95.3 %; Fig. 10K–N) but few amphigynous antheridia observed in all isolates (4.7 %; Fig. 10J). Hyphal aggregations regularly formed by all isolates (Fig. 10P). Hyphae often inflated-tubular to club-shaped (Fig. 10Q).

*Cultures* (Figs 12–13): Colonies of all *P. vulcanica* isolates uniform felty on CA and V8A, with irregular margins on CA and dome-shaped with regular margins on V8A; very limited and irregular growth on PDA and MEA (Fig. 12). Temperature–growth relations on V8A are shown in Fig. 13. All isolates with very slow growth and similar growth rates at all temperatures; maximum growth temperature between 25 and 30 °C. Isolates with no growth when plates incubated for 5 d at 30 °C were transferred to 20 °C. Average radial growth rate at the optimum temperature of 15 °C 0.7 ± 0.1 mm/d.

*Additional material examined: Italy:* Sicily; Mount Etna, isolated from rhizosphere soil of mature *F. sylvatica* trees, May 2013, T. Jung (CBS 141217 = X3b, X3c, X3d, X3e).
DISCUSSION

Four new cryptic Phytophthora species recently isolated from Fagaceae trees in forest stands in Austria, Italy, and Portugal were characterized. Phylogenetic analyses of the DNA sequence data for three nuclear (ITS, Btub and HSP90) and two mitochondrial (cox1 and NADH1) gene regions together with detailed morphological and physiological studies allowed the description of these four taxa as *P. castanetorum*, *P. tubulina*, *P. tyrrhenica*, and *P. vulcanica*.

Multigene phylogenetic analyses demonstrated that *P. castanetorum* and *P. tubulina* are distinct species closely related to *P. quercina*. In previous studies, the phylogenetic position of *P. quercina* was ambiguous and, depending on the gene regions analysed, this species loosely clustered with Clade 3 (ITS; Cooke et al. 2000), Clade 4 (seven nuclear loci; Blair et al. 2008), Clade 5 (four mitochondrial loci; Martin et al. 2014), and Clade 1 (seven nuclear loci; Martin et al. 2014). Due to the inclusion of *P. castanetorum*, *P. tubulina* and the three taxa *P*. sp. ohiensis, *P*. versiformis and *P*. sp. versiformis-like in the phylogenetic analyses of both a four-locus (ITS, Btub, HSP90, NADH1) and a nuclear three-locus (ITS, Btub, HSP90) dataset in this work the phylogenetic position of the *P. quercina* clade within *Phytophthora* could be resolved. *Phytophthora quercina*, *P. castanetorum*, *P. tubulina*, *P*. sp. ohiensis, *P*. versiformis and *P*. sp. versiformis-like, formed a fully supported monophyletic group. Due to the unique phylogenetic position of *P. lilii*, which was confirmed in both the phylogenetic analysis of the three-locus dataset here, and a new expanded phylogeny of *Phytophthora* with more than 150 taxa included (Yang et al. 2017), Rahman et al. (2015) proposed that *P. lilii* constituted an eleventh *Phytophthora* clade. Consequently, the *P. lilii* clade and the *P. quercina* clade are named here as Clades 11 and 12, respectively.

All Clade 12 species in Europe and North America are associated with Fagaceae. *Phytophthora quercina* is involved in decline syndromes of natural and planted Quercus stands (*Q. cerris*, *Q. coccinea*, *Q. faginea*, *Q. ilex*, *Q. palustris*, *Q. petraea*, *Q. pubescens*, *Q. pyrenaica*, *Q. robur*, *Q. rubra*, *Q. suber*, and *Q. vulcanica*) across Europe causing a progressive destruction of fine root systems, which predisposes affected oaks to climatic extremes and secondary pathogens and pests (Jung et al. 1999, 2000, 2013, 2016, Balci & Halmschlager 2003a, b, Perez-Sierra et al. 2013). Interestingly, *P*. sp. ohiensis and *P*. sp.
Quercina-like were also found associated with oak forests in the USA, the latter taxon with *Q. ellipsoides* and *Q. rubra* in Minnesota and Wisconsin, respectively (Balci et al. 2007), and *P. sp. ohioensis* with declining *Q. alba* in southern Ohio (Balci et al. 2010). *Phytophthora* sp. quercina-like was also recovered from oak seedlings in woody ornamental nurseries in Minnesota (Schwingle et al. 2007). *Phytophthora castanetorum* and *P. tubulina* were isolated from the other two genera of *Fagaceae* in Europe, *Castanea* and *Fagus*, respectively. Interestingly, in Australia, which lacks any native *Fagaceae*, *P. versiformis* and *P. sp. versiformis-like* were both frequently isolated from the rhizosphere of a *Myrtaceae* tree (*Corymbia calophylla*) in remnant bushland, parks and gardens in the southwest of Western Australia (Barber et al. 2013, Paap et al. 2017). In a metagenomic *Phytophthora* survey of rhizosphere soils, *P. versiformis* was detected in more than 10 % of the 640 sites sampled across Australia, and due to its consistent association with native vegetation it was considered as a native species (Burgess et al. 2017).

Both *P. castanetorum* and *P. tubulina* share basic phenotypic features with their closest relative *P. quercina*, such as the homothallic breeding system, production of elongated oogonia and persistent papillate sporangia, sympodial hyphal branching with the mother hypha ending with a protruding tip, slow growth in culture and uniform woolly colony growth patterns, but differed in a number of characters (Table 2). *Phytophthora castanetorum* and *P. tubulina* produce a low proportion of semipapillate and nonpapillate sporangia, whereas sporangia of *P. quercina* are exclusively papillate. *Phytophthora tubulina* also forms markedly longer sporangia than the other two species and partially extremely elongated, often tubular oogonia with high oospore abortion rates, suggesting genetic instability. *Phytophthora castanetorum* differed from the other two species in the regular production of chlamydospores, and by having on average higher oogonial diameters and a higher percentage of elongated oogonia. *Phytophthora quercina* grew faster at all temperatures tested and had a higher optimum temperature for growth than *P. castanetorum* and *P. tubulina* (25 vs 20 °C).

Concerning *P. tyrrenica* and *P. vulcanica*, multigene phylogenetic analyses placed both species in Clade 7a (Cooke et al. 2000, Blair et al. 2008), with *P. tyrrenica* forming a sister clade to *P. uliginosa* and *P. vulcanica* residing in a basal position of the subclade. Recently, six new *Phytophthora* species from Clade 7a occurring in natural ecosystems in Taiwan were described and two other new Clade 7a taxa were informally designated (Jung et al. 2017a, b) expanding the number of extant taxa in

![Fig. 13. Mean radial growth rates of *Phytophthora castanetorum* (6 isolates), *P. tubulina* (5 isolates), *P. quercina* (5 isolates), *P. tyrrenica* (5 isolates), *P. vulcanica* (5 isolates), *P. uliginosa* (2 isolates), *P. europaea* (5 isolates) and *P. flexuosa* (3 isolates) on V8 agar at different temperatures.](image-url)
Clade 7a to 16. Besides the new Taiwanese species, Clade 7a contains several important plant pathogens like the multivorous *P. x cambivora* and the host-specific *P. fragariae*, *P. rubi*, *P. uniformis*, *P. xalni* and *P. xmultiformis*, but also cryptic species such as *P. europaea* and *P. uliginosa* (Jung et al. 2002, 2017b). Their scattered distribution and putative host specialization also suggests a cryptic nature for both *P. tyrrhenica* and *P. vulcanica*. Morphologically these two species are similar to their closest relative *P. uliginosa* (Table 2). The main distinguishing characters are the exclusive production of elongated and mostly ellipsoid sporangia in *P. tyrrhenica*, the occurrence of both amphigynous and paragynous antheridia in *P. vulcanica* and the average largest oogonial and oospore diameters in *P. uliginosa*. All three species show similar slow growth rates, but differ in their optimum temperatures for growth and their growth rates at 25 °C, with *P. vulcanica* being the most psychrophilic and *P. tyrrhenica* the most thermophilic species.

The lack of previous records of all four new species from other continents, their apparent absence from European nurseries (Jung et al. 2016) and their exclusive occurrence in natural and semi-natural *Fagaceae* forest ecosystems in Europe indicate that *P. castanetorum*, *P. tubulina*, *P. tyrrhenica*, and *P. vulcanica* may be endemic to Europe or were introduced in historic times and have evolved in situ. These hypotheses are also supported by the intraspecific variability in both mitochondrial and nuclear DNA, observed particularly in *P. castanetorum* and *P. tyrrhenica*. This is in contrast to genetically uniform populations of recently introduced *Phytophthora* species which originated from relatively small and genetically impoverished founder populations (Goodwin et al. 1992). One well-known example is *P. cinnamomi*, whose global invasion was mainly achieved by the clonal spread of two genotypes from the A2 mating type (Oudemans & Coffey 1991, Dobrowski et al. 2003). The other two new species, *P. tubulina* and *P. vulcanica*, were monomorphic across all five loci examined resulting in two clonal population structures. However, this is most likely due to their scattered distribution and the origin of all isolates per species from one site. More isolates from different geographic locations are needed to confirm the endemic origin also for these two species. That the four new species and their closest European relatives *P. quercina*, *P. europaea* and *P. uliginosa* are all exclusively associated with *Fagaceae* suggests sympatric species radiation driven by the adaptation to different genera of *Fagaceae* in Europe.

All four new species from *Fagaceae* forests showed a low maximum temperature for growth and an optimum temperature between 15 °C and 25 °C. This is similar to that of other possibly endemic low-temperature *Phytophthora* species such as *P. iillicis*, *P. pseudosyringae*, *P. psychrophila*, and *P. quercina* (Jung et al. 1999, 2002, Pérez-Sierra et al. 2013, Scanu et al. 2014b, Scanu & Webber 2016). All four new species are homothallic, with a high oospore wall index (range 0.4–0.5) enabling them to survive both the long hot and dry summers typical of Mediterranean regions (*P. castanetorum*, *P. tyrrhenica*, and *P. vulcanica*) and cold winters in Austria (*P. tubulina*), on Mount Etna (*P. vulcanica*), and in the Gennargentu Mountain and Serra da Estrela (*P. castanetorum*).

While *P. vulcanica* was isolated alone and from relatively healthy beech trees, *P. castanetorum*, *P. tyrrhenica*, and *P. tubulina* co-occurred with other *Phytophthora* species in the rhizosphere of declining trees. Infections by multiple *Phytophthora* species have been previously reported in *Fagaceae* (Jung 2009, Jung et al. 2013, Pérez-Sierra et al. 2013, Scanu et al. 2015). Because of their co-occurrence with aggressive pathogens of chestnut, beech, and oaks, such as *P. x cambivora*, *P. cinnamomi*, and *P. plurivora*, it is not possible to establish whether the new species were directly involved in the decline of *Fagaceae* in Europe. Pathogenicity tests, however, showed that all four new species are able to damage the roots of young seedlings of their respective hosts: beech, chestnut, and oak. In comparison to the aggressiveness of invasive *Phytophthora* species co-occurring with them, they must nevertheless be considered as relatively weak pathogens. For example, in a soil infestation trial during this study, *P. x cambivora* caused much higher mortality of chestnut seedlings than *P. castanetorum* (90 % vs <20 %). In addition, *P. tubulina* and *P. vulcanica* killed only a low proportion of beech seedlings in artificially infested soil whereas the mortality rate caused by *P. x cambivora* exceeded 50 %. The only exception was *P. tyrrhenica*, which showed considerable aggressiveness to *Quercus ilex* seedlings, although less virulent than *P. cinnamomi*. Their relatively low aggressiveness to their native *Fagaceae* hosts supports the endemism hypothesis.

The continuously increasing diversity of both invasive and potentially endemic *Phytophthora* species in Mediterranean *Fagaceae* forests (Scanu et al. 2010, Jung et al. 2013, Pérez-Sierra et al. 2013; this study) necessitates future studies to address the ecology of these cryptic species in *Fagaceae* ecosystems. Most plausibly, they live as fine root “nibblers” as demonstrated for *P. quercina* (Jung et al. 1999, 2000, Pérez-Sierra et al. 2013) causing a slow chronic decline which in interaction with climatic extremes and secondary pathogens and pests might cause episodic dieback and mortality (Jung et al. 2000, Jung 2009, Pérez-Sierra et al. 2013). A similar life-style was also suggested for the potentially native *P. arenaria* and *P. constricta* in Western Australia (Rea et al. 2011). In addition, the four new species may act as damping-off pathogens affecting seedling recruitment, as reported previously for other *Phytophthora* species (Cohen & Coffey 1986, Simamora et al. 2015). This is particularly plausible for *P. tyrrhenica* considering the high susceptibility of germinating *Q. ilex* acorns to *Phytophthora*, due to the poor suberisation and lignification of tissues and restricted allocation of defence compounds (Herms & Mattson, 1992, Martín-Garcia et al. 2015). B. Scanu & G. Hardy, unpubl.). However, these hypotheses need to be further investigated, for both the new species described here and other *Phytophthora* species occurring in *Fagaceae* forest ecosystems. It would also be important to test whether co-infections of trees by endemic and invasive *Phytophthora* species are causing synergistic or antagonistic interactions. In a recent study conducted by Corcobado et al. (2017), no synergistic interaction was found when *Q. ilex* seedlings were grown in soil artificially infested with two different *Phytophthora* species. On the contrary, mortality of *Q. ilex* seedlings was delayed if the less virulent and potentially endemic European pathogens *P.
gonapodyides and P. quercina were inoculated prior to the aggressive invasive P. cinnamomii (Corcobado et al. 2017), most likely due to the priming of induced resistance. The putatively endemic, new species may also have a beneficial effect in maintaining plant diversity in these ecosystems. In the kwongan shrublands in Western Australia, the putatively endemic P. arenaria and P. consticta (Rea et al. 2011) can affect the growth of non-mycorrhizal plants while they do not affect co-occurring ectomycorrhizal (ECM) ones, thus conferring an advantage to ECM species in terms of accessing scarce phosphorus resources (Albornoz et al. 2016). Consequently, the occurrence of P. castanetorum and P. tyrhenica in old impoverished soils in Portugal and Sardinia that are particularly low in phosphorus might suggest a similar ecological contribution to the maintenance of highly diverse ecosystems by reducing differences in competitiveness between plant species of contrasting nutrient-acquisition strategies.

With the advent of molecular-based techniques, the systematics and evolutionary understanding of the genus Phytophthora has advanced and the detection and description of new Phytophthora species have become a priority. However, more studies on pathogen-host-environment interactions are urgently required to increase our understanding of the ecology of cryptic endemic Phytophthora species in natural forest ecosystems.

ACKNOWLEDGEMENTS

We are grateful to the Portuguese Science and Technology Foundation (FCT) for co-financing with Portuguese national funds the European BiodivERsA project RESIPATH: Responses of European Forests and Society to Invasive Pathogens (BIODIVERSA/0002/2012), and to the Czech Ministry for Education, Youth and Sports and the European Regional Development Fund for financing the Project Phytophthora Research Centre Reg. No. CZ.02.1.01/0.0/0.0/15_003/000045. Fieldwork in Portugal had logistic support from the Institute for the Conservation of Nature and Forestry (ICNF). DNA sequencing was partly supported by the Hungarian Scientific Research Fund (OTKA) grant K101914. This work has also received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 635646, POnTE (Pest Organisms Threatening Europe).

We thank Treena Burgess (Murdoch University, Perth) for assistance with Figs 7 and 8.

REFERENCES

Abad GZ, Ivors KL, Gallup CA, Abad JA, Shew HD (2011) Morphological and molecular characterization of Phytophthora glovera sp. nov. from tobacco in Brazil. Mycologia 103: 341–350.

Abad GZ, Abad JA, Cacciola SO, Pane A, Faedda R, et al. (2014) Phytophthora niederhauserii sp. nov., a polyphagous species associated with ornamentals, fruit trees and native plants in 13 countries. Mycologia 106: 431–447.

Albornoz FE, Burgess TI, Lambers H, Etchells H, Laliberté E (2016) Native soil-borne pathogens equalise differences in competitive ability between plants of contrasting nutrient-acquisition strategies. Journal of Ecology 105: 549–557.

Balci Y, Balci S, Eggers J, MacDonald WL, Juzwik J, et al. (2007) Phytophthora spp. associated with forest soils in Eastern and North-Central U.S. oak ecosystems. Plant Disease 91: 705–710.

Balci Y, Halsmischlager E (2003a) Incidence of Phytophthora species in oak forests in Austria and their possible involvement in oak decline. Forest Pathology 33: 157–174.

Balci Y, Halsmischlager E (2003b) Phytophthora species in oak ecosystems in Turkey and their association with declining oak trees. Plant Pathology 52: 694–702.

Balci Y, Long RP, Mansfield M, Balser D, MacDonald WL (2010) Involvement of Phytophthora species in white oak (Quercus alba) decline in southern Ohio. Forest Pathology 40: 430–442.

Barber P, Paap T, Burgess T, Dunstan W, Hardy GSJ (2013) A diverse range of Phytophthora species are associated with dying urban trees. Urban Forestry and Urban Greening 12: 569–575.

Bezuïndhout CM, Denman S, Kirk SA, Botha WJ, Mostert L, McLeod A (2010) Phytophthora taxa associated with cultivated Agathosma, with emphasis on the P. citicola complex and P. capsenis sp. nov. Persoonia 25: 32–49.

Blair JE, Coffey MD, Park S, Geiser DM, Kang S (2008) A multilocus phylogeny for Phytophthora utilizing markers derived from complete genome sequences. Fungal Genetics and Biology 45: 266–277.

Brazee NJ, Wick RL, Hulvey JP (2016) Phytophthora species recovered from the Connecticut River Valley in Massachusetts, USA. Mycologia 108: 6–19.

Burgess TI, White D, McDougall KM, Garnas J, Dunstan WA, et al. (2017) Distribution and diversity of Phytophthora across Australia. Pacific Conservation Biology 23: 1–13.

Cohen Y, Coffey MD (1986) Systemic fungicides and the control of Oomycetes. Annual Review of Phytopathology 24: 311–338.

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

Corcobado T, Miranda-Torres JJ, Martin-Garcìa J, Jung T, Solla A (2017) Early survival of Quercus ilex subspecies from different populations after infections and co-infections by multiple Phytophthora species. Plant Pathology 76: 792–804.

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

Dobrowolski MP, Tommerup IC, Blakeman HD (2003) Non-mendelian inheritance revealed in a genetic analysis of sexual progeny of Phytophthora cinnamomii with microsatellite markers. Fungal Genetics and Biology 35: 197–212.

Erwin DC, Ribeiro OK (1996) Phytophthora Diseases Worldwide. St Paul, MN: American Phytopathological Society Press.

Gallely ME, Hong C (2008) Phytophthora: identifying species by morphology and DNA fingerprints. St Paul, MN: American Phytopathological Society Press.

Goodwin SB, Spielman LJ, Matuszak JM, Bergeron SN, Fry WE (1992) Clonal diversity and genetic differentiation of Phytophthora infestans populations in northern and central Mexico. Phytopathology 82: 955–61.

Hansen EM, Reeser PW, Davidson JM, Garbelotto M, Ivors K,
New pathogenic *Phytophthora* species from Europe

Douhan L, Rizzo DM (2003) *Phytophthora nemorosa*, a new species causing cankers and leaf blight of forest trees in California and Oregon, U.S.A. *Mycotaxon* 88: 129–138.

Hansen EM, Wilcox WF, Reeser PW, Sutton W (2009) *Phytophthora rosacearum* and *P. sansomeana*, new species segregated from the *Phytophthora megasperma* “complex”. *Mycologia* 101: 129–135.

Henricot B, Pérez-Sierra A, Jung T (2014) *Phytophthora pachypleura* sp. nov., a new species causing root rot of *Aucuba japonica* and other ornamentals in the United Kingdom. *Plant Pathology* 63: 1095–1109.

Hermes DA, Mattson WJ (1992) The dilemma of plants to grow or defend. *Quarterly Review of Biology* 67: 283–335.

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

Husson C, Aguayo J, Revellin C, Frey P, Iosu R, et al. (2015) Evidence for homoploid speciation in *Phytophthora alni* supports taxonomic reclassification in this species complex. *Fungal Genetics and Biology* 77: 12–21.

Iliev E, Ilieva E, Man In’t Veld WA, Veenbaas-Rijks W, Pieters R (1998) *Phytophthora multivesiculata*, a new species causing rot in *Cymbidium*. *European Journal of Plant Pathology* 104: 677–684.

Jee H-J, Cho W-D, Kim W-G (1997) *Phytophthora* cactorum sp. nov., a new species segregating from the genus. *Mycological Research* 101: 19–32.

Jung T, Blaschke H, Neumann P (1996) Isolation, identification and taxonomic reclassification in this species complex. *Mycological Research* 100: 129–135.

Jung T, Chang TT, Bakonyi J, Seress D, Pérez-Sierra A, et al. (2017a) Diversity of *Phytophthora* species in natural ecosystems of Taiwan and association with disease symptoms. *Plant Pathology* 66: 194–211.

Jung T, Cooke DEL, Blaschke H, Duncan JM, Ólafsson W (2000) Involvement of soilborne *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. *Plant Pathology* 49: 706–718.

Jung T, Cooke DEL, Blaschke H, Duncan JM, Ólafsson W, Delatour C (2002) Diversity of *Phytophthora* species in natural ecosystems of Taiwan and association with disease symptoms. *Plant Pathology* 66: 194–211.

Jung T, Cooke DEL, Blaschke H, Duncan JM, Ólafsson W (1999) *Phytophthora quercina* sp. nov., causing root rot of European oaks. *Mycological Research* 103: 785–798.

Jung T, Hansen EM, Winton L, Ólafsson W, Delatour C (2002) Three new species of *Phytophthora* from European oak forests. *Mycological Research* 106: 397–411.

Jung T, Horta Jung M, Scarnu B, Seress D, Kovács GM et al. (2017b) Six new *Phytophthora* species from ITS Clade 7a including two sexually functional heterothallic hybrid species detected in natural ecosystems in Taiwan. *Persoonia* 38: 100–135.

Jung T, Netchwatal J, Cooke DEL, Hartmann G, Blaschke M, et al. (2003) *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. *Mycological Research* 107: 772–789.

Jung T, Orlikowska L, Henricot B, Campos P, Aday AG, et al. (2016) Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases. *Forest Pathology* 46: 134–163.

Jung T, Scarnu B, Bakonyi J, Seress D, Kovács GM, et al. (2017c) *Nothophytophthora* gen. nov., a new sister genus of *Phytophthora* from natural and semi-natural ecosystems. *Persoonia* 39: 143–174.

Jung T, Vettraino AM, Cech TL, Vannini A (2013) The impact of invasive *Phytophthora* species on European forests. In: *Phytophthora: a global perspective* (Lamour K, ed.): 146–158. Wallingford: CAB International.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Kremer A, Abbott AG, Carlson JE, Manos PS, Plomion C, et al. (2012) Genomics of *Fagaceae*. *Tree Genetics and Genomes* 8: 583–610.

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: 766–782.

Logan WB (2005) Oak: the frame of civilization. New York: W W Norton.

Man in’t Veld WA (2007) Gene flow analysis demonstrates that *Phytophthora fragariae* var. *rubri* constitutes a distinct species, *Phytophthora rubi* comb. nov. *Mycologia* 99: 222–226.

Manos PS, Stanford AM (2001) The biogeography of *Fagaceae*: tracking the Tertiary history of temperate and subtropical forests of the Northern Hemisphere. *International Journal of Plant Science* 162: S77–S93.

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

Martin FN, Blair JE, Coffey MD (2014) A combined mitochondrial and nuclear multilocus phylogeny of the genus *Phytophthora*. *Fungal Genetics and Biology* 66: 19–32.

Martín-García J, Solla A, Corcobado T, Siasou E, Woodward S, (2015) Influence of temperature on germination of *Phytophthora cactorum* and *P. gonapodyides*, *P. quercina* and *P. psychrophila* in infected soils. *Forest Pathology* 45: 215–223.

Nagy ZÁ, Bakonyi J, Érsek T (2003) Standard and Swedish variant types of the hybrid alder *Phytophthora* attacking alder in Hungary. *Pest Management Science* 59: 484–492.

Oudemans P, Coffey MD (1991) Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. *Mycolological Research* 95: 19–30.

Paap T, Croeser L, White D, Aghighi S, Barber P, St. J. Hardy GE, Burgess TI (2017) *Phytophthora versiformis* sp. nov., a new species from Australia related to *P. quercina*. *Australasian Plant Pathology: DOI 10.1007/s13313-017-0499-7.*

Pánek M, Féret M, Mráček J, Tomšovský M (2016) Evolutionary relationships within the *Phytophthora cactorum* species complex in Europe. *Fungal Biology* 120: 836–851.

Pérez-Sierra A, López-Garcia C, León M, García-Jiménez J, Abad-Campos P, et al. (2013) Previously unrecorded low temperature *Phytophthora* species associated with Quercus decline in a Mediterranean forest in Eastern Spain. *Forest Pathology* 43: 331–339.

Rahman MZ, Uematsu S, Kimishima E, Kanto T, Kusunoki M, et al. (2015) Two plant pathogenic species of *Phytophthora* associated with...
with stem blight of Easter lily and crown rot of lettuce in Japan. *Mycoscience* **56**: 419–433.

Rea AJ, Burgess TI, Hardy GESJ, Stukely MJ C, 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.

Robideau GP, de Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, et al. (2011) DNA barcoding of oomycetes with cytchrome c oxidase subunit I (COI) and internal transcribed spacer (ITS). *Molecular Ecology Resources* **11**: 1002–1011.

Ronquist F, Heuelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Saavedra A, Hansen EM, Goheen DJ (2007) First report of *Phytophthora cambivora* in Oregon and its pathogenicity to *Chrysolepis chrysophylla*. *Forest Pathology* **37**: 409–419.

Scanu B, Linaldeddu BT, Franceschini A (2010) First report of *Phytophthora pseudosyringae* associated with ink disease of *Castanea sativa* in Italy. *Plant Disease* **94**: 1068.

Scanu B, Hunter GC, Linaldeddu BT, Franceschini A, Maddau L, et al. (2014a) A taxonomic re-evaluation reveals that *Phytophthora cinnamomi* and *P. cinnamomi* var. *parvispora* are separate species. *Forest Pathology* **44**: 1–20.

Scanu B, Linaldeddu BT, Deidda A, Jung T (2015) Diversity of *Phytophthora* species from declining Mediterranean maquis vegetation, including two new species, *Phytophthora crassamura* and *P. ornamentata* sp. nov. *PLoS ONE* **10** (12): e0143234.

Scanu B, Linaldeddu BT, Franceschini A, Anselmi N, Vannini A, et al. (2013) Occurrence of *Phytophthora cinnamomi* in cork oak forests in Italy. *Forest Pathology* **43**: 340–343.

Scanu B, Linaldeddu BT, Peréz-Sierra A, Deidda A, Franceschini A (2014b) *Phytophthora ilicis* as a leaf and stem pathogen of *Ilex aquifolium* in Mediterranean islands. *Phytopathologia Mediterranea* **53**: 480–490.

Scanu B, Webber JF (2016) Dieback and mortality of *Nothofagus* in Britain: ecology, pathogenicity and sporulation potential of the causal agent *Phytophthora pseudosyringae*. *Plant Pathology* **65**: 26–36.

Schirone B, Radoglou K, Vessella F (2016) Conservation and restoration strategies to preserve the variability of cork oak *Quercus suber*–a Mediterranean forest species-under global warming. *Climate Research* **71** (2): 171–185.

Schwingle BW, Smith JA, Blanchette RA (2007) *Phytophthora* species associated with diseased woody ornamentals in Minnesota nurseries. *Plant Disease* **91**: 97–102.

Scott PM, Burgess TI, Barber PA, Shearer BL, Stukely MJC, Hardy GESJ, Jung T (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.

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms, Diversity and Evolution* **12**: 335–337.

Simamora AV, Stukely MJC, Hardy GESJ, Burgess TI (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.

Staden R, Beal KF, Bonfield JK (2000) The Staden package 1998. *Methods in Molecular Biology* **132**: 115–130.

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

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* **30**: 2725–2729.

Tang QH, Gao F, Li GY, Wang H, Zheng XB, Wang YC (2010) First report of root rot caused by *Phytophthora sansomeana* on soybean in China. *Plant Disease* **94**: 378.

Weir BS, Padersen EP, Anand N, Uchida JY, Pennycook SR, Bellgard SE, Beever RE (2015) A taxonomic revision of *Phytophthora* Clade 5 including two new species, *Phytophthora agathidicida* and *P. cocos*. *Phytotaxa* **205**: 21–38.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds): 315–322. San Diego: Academic Press.

Yang X, Copes WE, Hong C (2014) Two novel species representing a new clade and cluster of *Phytophthora*. *Fungal Biology* **118**: 72–82.
## Supplementary Table 1
Details of isolates from *Phytophthora* and *Nothophytophthora* considered in the phylogenetic, morphological, growth-temperature and pathogenicity studies. GenBank numbers for sequences obtained in the present study are printed in italics. GenBank numbers for sequences used in the multigene phylogenetic analyses are printed in bold type.

| Species                        | Isolate numbers | Origin | GenBank accession numbers |
|-------------------------------|-----------------|--------|--------------------------|
|                               | International collections | Local collections | Host | Location; year | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| *Nothophytophthora amphigynosa* b | CBS 142348 | BD268 Stream baiting; atlantic forest | Portugal; 2015 | T. Jung; Jung et al. 2017c | KY788322 KY788515 KY788555 KY788473 KY788596 |
| *Phytophthora arenaria* c | IMI 389663 | VHS 10154 Banksia littoralis | Australia (WA); 2002 | VHS; Rea et al. (2011) | EU301114 KJ372298 KJ396725 KJ396697 n.a. |
| *P. arenaria* c | VHS 19931 | Banksia attenuata | Australia (WA); 2008 | VHS; Rea et al. (2011) | HQ013217 KJ372293 KJ396720 KJ396694 n.a. |
| *P. asparagi* b | VHS 17644 PD_02752 | Lomandra sonderi | Australia (WA); 2007 | VHS; Jung et al. (2011) | EU301168 JN547592 HQ012891 HQ012845 JN547680 |
| *P. attenuata* b | CBS 141199 | TW129 Castanopsis carlesii | Taiwan; 2013 | T. Jung; Jung et al. (2017b) | KU517154 KU899277 KU899434 KU517148 KU899519 |
| *P. attenuata* b | CBS 141200 | TW118 Chamaecyparis formosensis | Taiwan; 2013 | T. Jung; Jung et al. (2017b) | KU899196 KU899274 KU899431 KU899351 KU899516 |
| *P. boodjera* c | CBS 138637 | VHS 26806 Soil dump | Australia (WA); 2012 | n.a.; Simamora et al. (2015) | KJ372244 KJ372283 KJ396710 KJ396688 n.a. |
| *P. boehmeriae* b | WPC P6950 PD_00181 | Boehmeria nivea | Taiwan; 1929 | Sawada; Blair et al. (2008) | HQ643149 EU080162 EU080165 KT183047 DQ361200 |
| *P. cactorum* b | WPC P11184 PD_00944 | n.a. | Poland; 2003 | L. Orlikowski & G. Szuka; n.a. | FJ801579 EU080292 EU080295 n.a. n.a. |
| *P. cactorum* c | WPC P0715 PD_00930 IMI 21168 | Gallegly N93 | United Kingdom; 1921 | E. Blackwell; n.a. | FJ801258 EU080285 EU080288 n.a. n.a. |
| *P. cactorum* b | | | | | |
| *P. cactorum* b | P0184 | n.a. | Poland; 2003 | L. Orlikowski & G. Szuka; n.a. | FJ801579 EU080292 EU080295 n.a. n.a. |
| *P. cactorum* c | | | | | |
| *P. cactorum* c | | | | | |
| *P. cactorum* c | | | | | |
| *P. cactorum* c | | | | | |
| *P. capsida* b | RLW2002-MS8 | Cucurbita sp. | USA (Massachusetts); n.a. | n.a.; Brazee et al. (2016) | KJ631548 KJ631575 n.a. KJ631596 KU695492 |
| Species          | Isolate numbers | Origin               | GenBank accession numbers |
|------------------|-----------------|----------------------|---------------------------|
| *P. capsici*     | WPC P10386      | Cucumis sativus      | HQ261520 EU079544 EU079547|
|                  | CBS 121666      | USA (Michigan); 1997 | n.a. n.a.                 |
| *P. capsici*     | WPC P1319       | Capsicum annuum      | HQ261519 EU079737 EU079740|
|                  | PD _00118       | USA (California); 1983| n.a. n.a.                 |
| *P. capsici*     | IMI 352321      | Piper nigrum         | HQ261519 EU079737 EU079740|
| *P. castaneae*   | WPC P10187      | Castanea crenata     | HQ261601 EU080803 EU080806|
|                  | PD _00091       | Japan; 1970          | n.a. n.a.                 |
| *P. castaneae*   | WPC P3389       | n.a.                 | HQ261601 EU080803 EU080806|
| *P. castaneae*   | IMI 98426       | n.a.                 | n.a. n.a.                 |
| *P. castanetorum*| WPC 15598       | Soil                 | HQ643255 AY564075 AY564190|
|                  | IMI 325914      | Taiwan; 1979         | n.a. AY564190 KP295350    |
| *P. castanetorum*| CBS 142299      | Castanea crenata     | MF036182 MF036214 MF036240|
|                  | BD _14299       | Portugal; 15          | MF036240 MF036266 MF036292|
| *P. castanetorum*| BD _14299       | C. sativa            | MF036183 MF036215 MF036241|
| *P. castanetorum*| BD 292          | Portugal; 15          | MF036240 MF036266 MF036292|
| *P. castanetorum*| BD 293          | C. sativa            | MF036183 MF036215 MF036241|
| *P. castanetorum*| BD 336          | C. sativa            | MF036184 n.a. n.a. n.a.   |
| *P. castanetorum*| BD 476          | C. sativa            | MF036185 MF036216 MF036242|
| *P. castanetorum*| BD 484          | C. sativa            | MF036186 MF036217 MF036243|
| *P. castanetorum*| BD 485          | C. sativa            | MF036187 n.a. n.a. n.a.   |
| *P. castanetorum*| BD 486          | C. sativa            | MF036188 n.a. n.a. n.a.   |
| *P. castanetorum*| CBS 142300      | C. sativa            | MF036189 MF036218 MF036244|
|                  | P14             | Italy (Sardinia); 2014| MF036270 MF036296         |
| Species          | Isolate numbers | Origin | GenBank accession numbers |
|------------------|-----------------|--------|--------------------------|
| *P. castanetorum* |                 |        |                          |
| a                | b               |        |                          |
| c                | d               |        |                          |
| e                | f               |        |                          |
| P. castanetorum<sup>b</sup> | P17 | C. sativa | Italy (Sardinia); 2014 | B. Scanu & S. Seddaiu; this study | MF036190 MF036219 MF036245 MF036271 MF036297 |
| P. castanetorum<sup>c</sup> | P18 | C. sativa | Italy (Sardinia); 2014 | B. Scanu & S. Seddaiu; this study | MF036191 MF036220 MF036246 MF036272 MF036298 |
| P. cinnamomi<sup>b</sup> | CBS 144.22 | P61/04 | Cinnamomum bumanni | Indonesia (Sumatra); 1922 | Rands; Scanu et al. (2014a); Jung et al. (2017b) | KU899160 KU899233 KU899390 KU899315 KU899475 |
| P. cinnamomi<sup>f</sup> | PH169 | Quercus suber | Italy (Sardinia); 2010 | B. Scanu; Scanu et al. (2013) | KC161250 n.a. n.a. n.a. n.a. |
| P. europaea<sup>b</sup> | CBS 109049 | EUR 2 | Quercus robur | France; 1998 | T. Jung; Jung et al. (2002) | HQ261556 EU079482 EU079485 KU681022 KU899469 |
| P. europaea<sup>b</sup> | CBS 109051 | EUR 3 | Quercus sp. | France; 1998 | E.M. Hansen; Jung et al. (2002) | KU899157 KU899229 KU899384 KU899312 KU899470 |
| P. flexuosa<sup>b</sup> | CBS 141201 | TW78 | Fagus hayatae | Taiwan; 2013 | T. Jung; Jung et al. (2017b) | KU517152 KU899302 KU899459 KU517146 KU899544 |
| P. flexuosa<sup>b</sup> | CBS 141202 | TW108 | F. hayatae | Taiwan; 2013 | T. Jung; Jung et al. (2017b) | KU899193 KU899271 KU899428 KU899348 KU899513 |
| P. formosa<sup>b</sup> | CBS 141203 | TW107 | Araucaria cunninghamii | Taiwan; 2013 | T. Jung; Jung et al. (2017b) | KU899201 KU899270 KU899427 KU517147 KU899512 |
| P. formosa<sup>b</sup> | CBS 141204 | TW14 | Quercus glandulifera | Taiwan; 2013 | T. Jung; Jung et al. (2017b) | KU899201 KU899280 KU899437 KU899356 KU899522 |
| P. fragariae<sup>b</sup> | - | BBA 11/94; K018 | Fragaria x ananassa | Germany; 1994 | JKI; Jung et al. (2017b) | KU899153 KU899225 KU899380 KU899308 KU899465 |
| P. fragariae<sup>b</sup> | - | BBA L1 | F. x ananassa | Germany; 1983 | JKI; Jung et al. (2017b) | KU899156 KU899228 KU899383 KU899311 KU899468 |
| P. heveae<sup>b</sup> | WPC P3428 | ICMP 19451 | Hevea brasiliensis | Malaysia; 1929 | A.W. Thomson; Weir et al. (2015) | EU045741 AY564067 KP295293 AY564182 AY564009 |
| Species       | Isolate numbers | Origin         | GenBank accession numbers |
|--------------|-----------------|----------------|---------------------------|
|              | International   | Local          | Location; year            | Collector; reference | ITS           | Btub          | HSP90         | Cox1         | NADH1        |
|              | collections a   | collections b  | Host; year                | Collector; reference |              |              |              |              |              |
| *P. heveae*  | WPC P10167      | p17            | Forest soil; USA          | W.A. Campbell; n.a. | GU259516     | EU080796     | EU080799     | n.a.         | n.a.         |
|              | PD_00073        | N331           | (Tennessee); 1964         |                           |               |              |              |              |              |
|              | IMI 131373      |                |                           |                           |               |              |              |              |              |
|              | ATCC 16701      |                |                           |                           |               |              |              |              |              |
| *P. humicola*| WPC P3826       |                | Citrus soil; Taiwan; 1977 | PJ Ann & WH Ko; Blair et al. 2008; Jung et al. (2011) | HQ643243     | EU080169     | EU080172     | KF112862     | AY564011     |
|              | PD_00018        |                |                           |                           |               |              |              |              |              |
|              | CBS 200.81      |                |                           |                           |               |              |              |              |              |
|              | IMI 302303      |                |                           |                           |               |              |              |              |              |
|              | ATCC 52179      |                |                           |                           |               |              |              |              |              |
| *P. ilicis*  | WPC P3939       |                | *Ilex* sp.; Canada; 1988 | n.a.; Blair et al. 2008 | HQ261583     | EU079860     | EU079863     | AY129172     | n.a.         |
|              | PD_00133        |                |                           |                           |               |              |              |              |              |
|              | CBS 555.88      |                |                           |                           |               |              |              |              |              |
|              | CBS 255.93      |                |                           |                           |               |              |              |              |              |
|              | ATCC 56615      |                |                           |                           |               |              |              |              |              |
| *P. ilicis*  | CBS 114348      |                | *Ilex aquifolium*; The Netherlands; | n.a. | JX524158     | n.a.         | n.a.         | AY564186     | AY564013     |
|              | PD91/595        |                | n.a.                      |                           |               |              |              |              |              |
| *P. ilicis*  | WPC P6860       |                | *I. aquifolium*; UK; before 1990 | n.a. | HQ261580     | EU080137     | EU080139     | n.a.         | n.a.         |
|              | PD_00178        |                |                           |                           |               |              |              |              |              |
| *P. infestans*| WPC P10651     |                | *Solanum tuberosum*; Uganda; 1998 | n.a. | FJ801471     | EU079633     | EU079636     | n.a.         | n.a.         |
|              | PD_00103        |                |                           |                           |               |              |              |              |              |
| *P. infestans*| WPC P10650     |                | *S. tuberosum*; Mexico; 1998 | n.a.; Blair et al. 2008 | HQ261589     | EU079626     | EU079629     | n.a.         | n.a.         |
|              | PD_00102        |                |                           |                           |               |              |              |              |              |
| *P. infestans*| -              |                | *Solanum stoloniferum*; Mexico; 1999 | n.a. | AY770731     | AY564035     | n.a.         | AY564150     | AY563977     |
|              | Pic99186        |                |                           |                           |               |              |              |              |              |
| *P. infestans*| -              |                | *Solanum tuberosum*; The Netherlands; 1998 | n.a. | AY564036     | n.a.         | AY564151     | AY563978     |              |
|              | Dr98004         |                |                           |                           |               |              |              |              |              |
| *P. insolita*| WPC P6195       |                | Soil in Citrus orchard; Taiwan; 1979 | P.J. Ann & K.W. Ko; Gallegly & Hong 2008 | GU259059     | EU080176     | EU080179     | AY564188     | DQ361206     |
|              | PD_00175        |                |                           |                           |               |              |              |              |              |
|              | CBS 691.79      |                |                           |                           |               |              |              |              |              |
|              | IMI 288805      |                |                           |                           |               |              |              |              |              |
|              | ATCC 38789      |                |                           |                           |               |              |              |              |              |
| *P. intricata*| CBS 141211     |                | *Quercus tarokoensis*; Taiwan; 2013 | T. Jung; Jung et al. (2017b) | KU517155     | KU899284     | KU899441     | KU517149     | KU899526     |
### Supplementary Table 1. (Continued)

| Species | Isolate numbers | Origin | GenBank accession numbers |
|---------|-----------------|--------|---------------------------|
| **P. intricata**<sup>b</sup> | CBS 141210 | TW7 Q. tarokoensis | Taiwan; 2013 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | | | | | KU899219 | KU899301 | KU899458 | KU899374 | KU899543 |
| **P. lateralis**<sup>b</sup> | WPC P3361 | MG 33-7 Chamaecyparis lawsoniana | USA (Oregon); 1942 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | CBS 168.42 | | | | AF266804 | AY564076 | n.a. | AY564191 | AY564018 |
| | IMI 40503 | | | | | | | | |
| | ATCC 11261 | | | | | | | | |
| **P. lateralis**<sup>b</sup> | WPC P1728 | 186-2 | C. lawsoniana | USA; before 1974 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | PD_00169 | | | | FJ801791 | EU080090 | EU080092 | n.a. | n.a. |
| | ATCC 28512 | | | | | | | | |
| **P. lilii**<sup>c</sup> | CBS 135746 | NBRC 32174 Lilium sp. | Japan; 1987 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | | MAFF 237500 | | | AB688354 | AB856782 | AB856794 | AB856785 | n.a. |
| | | EL 8701 | | | | | | | |
| **P. meadii**<sup>b</sup> | WPC P3950 | Ho H24.1 | Hevea brasiliensis | India; 1968 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | CBS 219.88 | | | | HQ643268 | AY564077 | n.a. | AY564192 | AY564019 |
| | IMI 129185 | | | | | | | | |
| | ATCC 58103 | | | | | | | | |
| **P. meadii**<sup>b</sup> | WPC P6262 | Rajalakshmy 46 | H. brasiliensis | India; before 1989 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | PD_00137 | | | | JN618785 | EU079888 | EU079891 | n.a. | n.a. |
| | | | | | | | | | |
| **P. meadii**<sup>c</sup> | WPC P6128 | ICRI-240 | Elettaria cardamomum | India; before 1989 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | PD_00135 | | | | HQ261607 | EU079874 | EU079877 | n.a. | n.a. |
| | | | | | | | | | |
| **P. megakarya**<sup>b</sup> | WPC P8518 | TG.10 | Theobroma cacao | Togo; before 1994 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | PD_00149 | | | | FJ802012 | EU079983 | EU079986 | n.a. | n.a. |
| | | | | | | | | | |
| **P. megakarya**<sup>c</sup> | ATCC MYA-4040 | 22H7 p42 | T. cacao | Africa; n.a. | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | | 203532 | | | KF317085 | n.a. | n.a. | KF317107 | n.a. |
| | | | | | | | | | |
| **P. megakarya**<sup>c</sup> | WPC P8516 | | | | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | PD_00147 | | | | | | | | | |
| | | | | | | | | | |
| **P. megakarya**<sup>b</sup> | IMI 337098 | | | | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | | | | | | | | | | |
| **P. multivesiculata**<sup>b</sup> | WPC P10410 | PD 95/8679 Cymbidium sp. | The Netherlands; 1995 | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| | PD_00001 | | | | FJ801426 | EU080066 | EU080069 | AY564195 | AY564022 |
| | CBS 545.96 | | | | | | | | |
| Species | Isolate numbers | Origin | GenBank accession numbers | Host | Location; year | Collector; reference | ITS | Btub | HSP90 |
|---------|-----------------|--------|--------------------------|------|----------------|----------------------|-----|------|-------|
| P. multivesiculata | WPC-P10525 | International collections | | | | | | | |
| | CBS 101594 | The Netherlands | | | | | | | |
| | WPC-19694 | Australia (WA) | | | | | | | |
| | P0_00097 | | | | | | | | |
| | WPC-124084 | | | | | | | | |
| | CBS 124084 | | | | | | | | |
| | WPC-P02629 | | | | | | | | |
| | RHS226.2001 | | | | | | | | |
| | ICM-P19454 | | | | | | | | |
| | Theobroma cacao | Costa Rica; before 1930 | | | | | | | |
| | CBS-P1240511 | | | | | | | | |
| | Theobroma cacao | | | | | | | | |
| | ATCC MYA-4037 | | | | | | | | |
| | ATCC 26200 | | | | | | | | |
| | P0025 | | | | | | | | |
| | P0020 | | | | | | | | |
| | P0022 | | | | | | | | |
| | P0023 | | | | | | | | |
| | P0024 | | | | | | | | |
| | P0026 | | | | | | | | |
| | P0027 | | | | | | | | |
| | P0028 | | | | | | | | |
| | P0029 | | | | | | | | |
| | P0031 | | | | | | | | |
| | P0032 | | | | | | | | |
| | P0033 | | | | | | | | |
| | P0034 | | | | | | | | |
| | P0035 | | | | | | | | |
| | P0036 | | | | | | | | |
| | P0037 | | | | | | | | |
| | P0038 | | | | | | | | |
| | P0039 | | | | | | | | |
| | P0040 | | | | | | | | |
| | P0041 | | | | | | | | |
| | P0042 | | | | | | | | |
| | P0043 | | | | | | | | |
| | P0044 | | | | | | | | |
| | P0045 | | | | | | | | |
| | P0046 | | | | | | | | |
| | P0047 | | | | | | | | |
| | P0048 | | | | | | | | |
| | P0049 | | | | | | | | |
| | P0050 | | | | | | | | |
| | P0051 | | | | | | | | |
| | P0052 | | | | | | | | |
| | P0053 | | | | | | | | |
| | P0054 | | | | | | | | |
| | P0055 | | | | | | | | |
| | P0056 | | | | | | | | |
| | P0057 | | | | | | | | |
| | P0058 | | | | | | | | |
| | P0059 | | | | | | | | |
| | P0060 | | | | | | | | |
| Species            | Isolate numbers | Origin          | GenBank accession numbers |
|--------------------|-----------------|-----------------|--------------------------|
| **P. palmivora**    |                 |                 |                          |
| c                  | WPC P0113       | Carica papaya   | GU259121 EU080465 EU080468 n.a. n.a. |
|                   | PD_00032        | USA (Hawaii);   |                          |
|                   | ATCC 26286      | before 1973     |                          |
|                    |                 |                 |                          |
| P. palmivora       |                 |                 |                          |
| c                  | WPC P10212      | T. cacao        | KF317086 n.a. n.a. KF317108 n.a. |
|                   | ATCC MYA-4038   | Costa Rica      |                          |
|                    |                 |                 |                          |
| **P. pseudosyringae** |               |                 |                          |
| c                  | WPC P01437      | Q. robur        | EU079563 EU079566 KF317105  | |
|                   | PD_00093        | Germany; 1997   |                          |
|                   | CBS 111772      |                 |                          |
|                    |                 |                 |                          |
| P. pseudosyringae |                 |                 |                          |
| c                  | WPC P10443      | Q. robur        | n.a. n.a. EU08025 n.a. n.a. |
|                   | PD00159         | Germany; 1997   |                          |
|                    |                 |                 |                          |
| P. pseudosyringae |                 |                 |                          |
| c                  | PH048           | Ailnus glutinosa | KJ458957 KJ458966 n.a. KJ458943 n.a. |
|                    |                 | Italy; 2010     |                          |
|                    |                 |                 |                          |
| **P. psychrophila** |               |                 |                          |
| c                  | WPC P10433      | Q. robur        | KF358227 EU080517 EU080520 KF358239 n.a. |
|                   | PD_00039        | Germany;        |                          |
|                   | CBS 80395       |                 |                          |
|                    |                 |                 |                          |
| P. psychrophila    |                 |                 |                          |
| c                  | WPC P10434      | PSY 2           | EU079553 n.a. n.a.       |
|                   | PD_00092        | Quercus ilex    |                          |
|                    |                 | France; 1996    |                          |
|                    |                 |                 |                          |
| P. psychrophila    |                 |                 |                          |
| c                  | PH047           | Q. ilex         | KJ458947 n.a. n.a.       |
|                   |                 | Italy; 2011     |                          |
|                    |                 |                 |                          |
| **P. quercina**    |                 |                 |                          |
| c                  | CBS 784.95      | Q. robur        | HQ261659 EU080490 EU080493 n.a. n.a. |
|                   | WPC P10439      | Germany; 1995   |                          |
|                   | PD PD_01270     |                 |                          |
|                    | QUE 3           |                 |                          |
|                    | 30A5            |                 |                          |
|                    | p290            |                 |                          |
|                    |                 |                 |                          |
| P. quercina        |                 |                 |                          |
| c                  | CBS 782.95      | Q. robur        | HQ261659 EU080592 EU080595 n.a. n.a. |
|                   | WPC P10334      | Germany; 1995   |                          |
|                   | PD PD_00035     |                 |                          |
|                    | QUE 4           |                 |                          |
|                    | L50/2           |                 |                          |
|                    |                 |                 |                          |
| P. quercina        |                 |                 |                          |
| c                  | WPC P10441      | Quercus sp.     | HQ261658 EU080592 EU080595 n.a. n.a. |
|                   | PD00049         | Serbia; 2003    |                          |
|                    |                 |                 |                          |
| P. quercina        |                 |                 |                          |
| c                  | PL 7            | Q. robur        | MF036194 MF036223 MF036249 MF036275 MF036301 |
|                   |                 | Poland; 2011    |                          |
|                    |                 |                 |                          |
| P. quercina        |                 |                 |                          |
| c                  | PL 9            | Q. robur        | MF036195 MF036224 MF036250 MF036276 MF036302 |
|                   |                 | Poland; 2011    |                          |
|                    |                 |                 |                          |
| P. quercina        |                 |                 |                          |
| c                  | PT 4-1          | Q. pyrenaica    | MF036192 MF036221 MF036247 MF036273 MF036299 |
|                   | BD10            | Portugal; 2014  |                          |
|                    | P735            |                 |                          |
|                    |                 |                 |                          |
| Species          | Isolate numbers | Origin       | GenBank accession numbers |
|------------------|-----------------|--------------|--------------------------|
|                  | International  | Local        | Host                     | Location; year | Collector; reference | ITS   | Btub | HSP90 | Cox1 | NADH1 |
|                  | collections a  | collections  |                          |               |                      |       |      |       |      |       |
| P. quercina \(a\) | Beja 5          | Q. ilex      | Portugal; 2010           | T. Jung; this study | MF036193 MF036222 MF036248 MF036274 MF036300 |
| P. quercina \(b\) | WPC P1089       | Cinchona     | Peru; before 1947        | B.S. Crandall; Blair et al. 2008 | HQ643338 AY564085 n.a. AY564200 AY564027 |
| P. quercina \(b\) | WPC P3247       | C. officinalis | Peru; before 1947      | B.S. Crandall; n.a. | HQ261661 EU079803 EU079805 n.a. n.a. |
| P. rubi \(b\)    | CBS 967.95      | R. idaeus     | UK; 1985                 | D. Kennedy; Man In’t Veld (2007) | AF139370 KU899234 KU899391 DQ674736 KU899476 |
| P. rubi \(b\)    | CBS P16899      | R. idaeus     | Sweden; n.a.             | C. Olsson; Jung et al. (2017b) | KU899180 KU899256 KU899413 KU899335 KU899498 |
| P. sansomeana \(b\) | n.a.            | Glycine max  | China; n.a.              | Tang et al. 2010 | FJ966890 FJ966879 n.a. FJ966881 FJ966877 |
| P. sansomeana \(b\) | CBS 117692      | Silene latifolia subsp. alba | USA (New York); n.a. | E.M. Hansen; Hansen et al. 2009 | HQ261669 EU080271 EU080274 n.a. n.a. |
| P. stricta \(c\) | ATCC MYA-4944   | Nursery irrigation reservoir | USA (Virginia); n.a. | Yang et al. (2014) | KF192694 n.a. n.a. n.a. n.a. n.a. |
| P. tropicalis \(b\) | WPC P10329      | Macadamia integriofila | USA (Hawaii); 1975 | M. Aragaki; Blair et al. 2008 | HQ643369 EU080306 EU080309 n.a. n.a. |
| P. tropicalis \(b\) | CBS 434.91      | Rosa spp.     | The Netherlands; 1997 | n.a. | n.a. AY564046 n.a. AY564161 AY563988 |
| P. tropicalis \(c\) | NRCPh-179       | Citrus sp.    | India; n.a.              | A.K. Das; n.a. | KP698409 n.a. n.a. KP698412 |
| P. tropicalis \(c\) | WPC P10452      | Nursery recycling water | USA (California); 2002 | D. Ferrin; n.a. | EU080620 EU080623 n.a. n.a. n.a. |
| P. tubulina \(b, e\) | CBS 141212      | Fagus sylvatica | Austria; 2010 | T. Jung; this study | MF036196 MF036225 MF036251 MF036277 MF036303 |
| Species          | Isolate numbers | Origin       | GenBank accession numbers |
|------------------|-----------------|--------------|---------------------------|
|                  | International   | Local        |                          |
|                  | collections a  | collections |                          |
| **P. tubulina**  | CBS 141213      | TUB 2        |  |
| **bdef**         | TUB 3           | F. sylvatica |  |
| **CBS 142303**   | TUB 4           | F. sylvatica |  |
| **CBS 142301**   | TUB 5           | F. sylvatica |  |
| **P. tyrrhenica**| CBS 142301      | PH154        |  |
| **bdef**         | PH155           | Q. ilex      |  |
| **CBS 142303**   | PH103           | Quercus suber |  |
| **P. tyrrhenica**| CBS 142302      | TYR 1        |  |
| **bd**           | TYR 2           | Q. ilex      |  |
| **P. tyrrhenica**| CBS 142302      | TYR 3        |  |
| **bd**           | TYR 4           | Q. ilex      |  |
| **P. tyrrhenica**| CBS 142302      | TYR 5        |  |
| **bd**           | TYR 6           | Q. ilex      |  |
| **P. tyrrhenica**| CBS 142302      | TYR 7        |  |
| **bd**           | ULI 1           | Q. robur     |  |
| **P. uliginosa** | CBS 109054      | ULI 2        |  |
| **b**            | ULI 3           | Quercus petraea |  |
| **P. uniformis** | ALN 58          | A. glutinosa |  |

| Species          | Isolate numbers | Origin       | GenBank accession numbers |
|------------------|-----------------|--------------|---------------------------|
|                  | Host            | Location; year | Collector; reference     |
| **P. tubulina**  | F. sylvatica    | Austria; 2010  | T. Jung; this study      |
| **P. tyrrhenica**| Q. ilex         | Italy (Sardinia); 2012 | B. Scanu; this study   |
| **P. uliginosa** | Q. robur        | Poland; 1998  | T. Jung; Jung et al. (2002) |
| **P. uniformis** | A. glutinosa    | Germany; 1998 | T. Jung; Jung et al. (2017b) |
### Supplementary Table 1.

| Species | Isolate numbers | Origin | GenBank accession numbers |
|---------|-----------------|--------|--------------------------|
| **P. uniformis**<sup>b</sup> | - | P876 | A. glutinosa; Sweden; 1997 | C. Olsson; Brasier et al. (2004); Jung et al. (2017b) |
| | CBS 142005 | TP13.46 | C. calophylla; Australia (WA); 2013 | T. Paap; Paap et al. (2017) |
| | - | PAB11.79 | Corymbia calophylla; Australia (WA); 2011 | P. Barber; Barber et al. (2013) |
| | - | TP13.10 | C. calophylla; Australia (WA); n.a. | T. Paap; Paap et al. (2017) |
| | - | TP13.29 | C. calophylla; Australia (WA); 2013 | T. Paap; Paap et al. (2017) |
| **P. versiformis**<sup>b</sup> | - | TP13.34 | C. calophylla; Australia (WA); n.a. | T. Paap; Paap et al. (2017) |
| **P. vulgarica**<sup>bde</sup> | CBS 141216 | X3a | F. sylvatica; Italy (Sicily); 2013 | T. Jung; this study |
| **P. vulgarica**<sup>bde</sup> | CBS 141217 | X3b | F. sylvatica; Italy (Sicily); 2013 | T. Jung; this study |
| **P. vulgarica**<sup>bde</sup> | X3c | F. sylvatica; Italy (Sicily); 2013 | T. Jung; this study |
| **P. vulgarica**<sup>bde</sup> | X3d | F. sylvatica; Italy (Sicily); 2013 | T. Jung; this study |
| **P. vulgarica**<sup>bde</sup> | X3e | F. sylvatica; Italy (Sicily); 2013 | T. Jung; this study |
| **P. xalni**<sup>b</sup> | IMI 392314 | P772 | A. glutinosa; UK; 1994 | G. Mac Askill; Brasier et al. (2004); Jung et al. (2017b) |
| **P. xalni**<sup>b</sup> | - | Reis 2 | A. glutinosa; Germany; 2014 | T. Jung; Jung et al. (2017b) |
| **P. xambivora**<sup>st</sup> | CBS 141218 | IT 5-3 | Quercus pubescens; Italy (Sicily); 2013 | T. Jung; Jung et al. (2017b) |
| **P. xambivora**<sup>b</sup> | - | 4044.1 | Chrysolepis chrysophylla; USA (Oregon); 2001 | A. Saavedra; Saavedra et al. (2007); Jung et al. (2017b) |
| **P. xheterohybrida**<sup>b</sup> | CBS 141207 | TW30 | Baiting; tributary of Ha-pen River; Taiwan; 2013 | T. Jung; Jung et al. (2017b) |
| **P. xheterohybrida**<sup>b</sup> | TW32 | Baiting; tributary of Ha-pen River; Taiwan; 2013 | T. Jung; Jung et al. (2017b) |
| **P. xinncassata**<sup>b</sup> | CBS 141209 | TW269 | Baiting; tributary of Ha-pen River; Taiwan; 2013 | T. Jung; Jung et al. (2017b) |
## Supplementary Table 1. (Continued).

| Species | Isolate numbers | Origin | GenBank accession numbers |
|---------|-----------------|--------|--------------------------|
|         | International collections | Local collections  | Host | Location; year | Collector; reference | ITS | Btub | HSP90 | Cox1 | NADH1 |
| **P. xincrassata**<sup>a</sup> | CBS 141208 | TW283 | Baiting; tributary of Ha-pen River | Taiwan; 2013 | T. Jung; Jung et al. (2017b) | KU899206 | KU899287 | KU899444 | KU899361 | KU899529 |
| **P. xmultiformis**<sup>b</sup> | IMI 392316 | P770 | A. glutinosa | Netherlands; 1964 | H. van Kesteren, Brasier et al. (2004); Jung et al. (2017b) | AF139368 | KU899239 | KU899396 | KU681018 | KU899481 |
|         | WPC P16202 | PD PD_01913 | - | - | - | - | - | - | - | - |
| **P. xmultiformis**<sup>b</sup> | - | PAM 396 | A. glutinosa | France; 2007 | T. Scordia; Husson et al. (2015); Jung et al. (2017b) | KU899184 | KU899261 | KU899418 | KU899339 | KU899503 |
| **P. sp. ohioensis**<sup>b</sup> | WPC P16050 | ST 18-37 | Quercus alba | USA (Ohio); 2006 | Y. Bald; Martin et al. (2014) | HQ261710 | KX011324 | KX011259 | GU594815 | KX011307 |
|         | PD PD_01627 | PD PD_02093 | ATCC MYA-4452 | - | - | - | - | - | - | - |
| **P. sp. quercina-like**<sup>c</sup> | ATCC MYA-4090 | MN 023 | Quercus rubra | USA; 2004 | Y. Bald; Balci et al. (2007) | DQ313224 | n.a. | n.a. | n.a. | n.a. |
| **P. sp. versiformis-like**<sup>b</sup> | - | MJ5 | C. calophylla | Australia (WA); 2013 | L. Croser; Paap et al. 2017 | KX011271 | KX011325 | KX011248 | KX011216 | KX011296 |
| **P. sp. versiformis-like**<sup>b</sup> | - | TP13.14 | C. calophylla | Australia (WA); 2013 | T. Paap; Paap et al. 2017 | KX011276 | KX011316 | KX011253 | KX011217 | KX011297 |
| **P. sp. xambivora-like**<sup>b</sup> | CBS 111329 | Malus pumila var. dulcissima | South Korea; 1996 | H-J. Jee; Jee et al. 1997; Jung et al. (2017b) | KU899158 | KU899230 | KU899386 | KU899313 | KU899472 |
| **P. sp. xmultiformis-like**<sup>b</sup> | - | 4971496 | A. glutinosa | Netherlands; 2011 | K. Rosendahl & W. Man In’t Veld; Jung et al. (2017b) | KU899170 | KU899246 | KU899403 | KU899325 | KU899488 |
| **P. sp. xmultiformis-like**<sup>b</sup> | - | P693 | A. glutinosa | - | - | - | - | - | - | - |

n.a. = not available; authentic strains, ex-types, isotypes, neotypes and paratypes are printed in bold-type.

<sup>a</sup> Abbreviations of isolates and culture collections: ATCC = American Type Culture Collection, Manassas, USA; CBS = CBS collection at the Westerdijk Fungal Biodiversity Institute (previously Centraalbureau voor Schimmelcultures), Utrecht, Netherlands; IMI = CABI Bioscience, UK; PD = Phytophthora Database (http://www.phytophthoradb.org); VHS = Vegetation Health Service Collection, Department of Environment and Conservation, Perth, Australia; WPC = World Phytophthora Collection, University of California Riverside, USA; other isolate names and numbers are as given by the collectors and on GenBank, respectively.

<sup>b</sup> Isolates used in phylogenetic studies of both multigene sequence alignments and individual genes.

<sup>c</sup> Isolates used only in phylogenetic studies of individual genes.

<sup>d</sup> Isolates used in the morphological studies.

<sup>e</sup> Isolates used in the temperature-growth studies.

<sup>f</sup> Isolates used in the soil infestation trials.