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Phytophthora quercetorum sp. nov., a novel species isolated from eastern and north-central USA oak forest soils

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ABSTRACT

Isolates belonging to an undescribed *Phytophthora* species were frequently recovered during an oak forest soil survey of *Phytophthora* species in eastern and north-central USA in 2004. The species was isolated using an oak leaf baiting method from rhizosphere soil samples collected from *Quercus rubra*, *Q. macrocarpa*, and *Q. phellos*. This species is formally described as *P. quercetorum*. It is homothallic and has aplerotic oogonia and paragynous antheridia. It produces papillate sporangia (occasionally bipapillate) of ovoid-elongated shapes. Its temperature optimum for growth is *ca* 22.5 °C with the upper limit of *ca* 32.5 °C. *P. quercetorum* differs from the morphologically related *P. quercina* in producing distinct submerged colony-patterns, different growth-temperature requirements, and oogonial shapes and sizes. Phylogenetic analyses using seven nuclear loci supported *P. quercetorum* as a novel species within clade 4, closely related to *P. arecae*, *P. palmivora*, *P. megakarya*, and *P. quercina*.

Introduction

Phytophthora species have been implicated as a factor causing oak decline, and studies have been conducted to examine their impact (Brasier *et al.* 1993; Jung *et al.* 1996, 2000; Robin *et al.* 1998; Vettraino *et al.* 2002; Balci & Halmschlager 2003a,b; Delatour 2003; Jönsson *et al.* 2005). During the course of these studies, five previously unknown taxa have been described in association with soils in European oak forests (Jung *et al.* 1999, 2002, 2003). The threat posed to forest and urban plants by the sudden oak death pathogen, *P. ramorum*, led to increased surveys in the USA and Europe, and two new taxa were discovered: *P. nemorosa* (Hansen *et al.* 2003) and *P. kernoviae* (Brasier *et al.* 2005). In the eastern and north-central USA, an oak soil survey was conducted in 2004 and 2005, which yielded seven species of *Phytophthora* (Balci *et al.* 2007). Three of these species appeared to be undescribed taxa. This paper describes the second most common species isolated during the survey. In a previous report, *P. quercetorum* was referred to as *Phytophthora* sp2 (Balci *et al.* 2007).

Materials and methods

Isolation method

Phytophthora quercetorum was isolated from rhizosphere soils collected around the base of mature oak trees at forest sites in six states in the USA. Origins and ATTC culture collection

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numbers of the principal isolates studied are listed in Table 1. At each site, four soil sub-samples were taken 1–1.5 m apart from the base of a tree in the four cardinal directions and to a soil depth of *ca* 30 cm after removing the organic layer. Soils were processed in the laboratory as described in Jung *et al.* (1996) by applying an oak leaf baiting method (*Quercus robur* leaflets) to isolate *Phytophthora* spp. from soil samples. Baitings were plated onto PARPNH selective medium [V8 juice agar (V8A) amended with 10 μg l^{-1} pimaricin, 200 μg l^{-1} ampicillin, 10 μg ml^{-1} rifampicin, 25 μg l^{-1} pentachloronitrobenzene (PCNB), 50 μg l nystatin^{-1}, and 50 μg l^{-1} hymexazol] to establish cultures (Erwin & Ribeiro 1996). Cultures were stored on V8A and Lima bean agar at 20 °C and sub-cultured every six months.

Morphology and physiology

Colony morphology was recorded on V8A (16 g agar, 3 g $CaCO_3$, 100 ml Campbell's V8 juice and 900 ml distilled water), Difco malt extract agar (MEA), Difco corn meal agar (CMA) and Difco potato-dextrose agar (PDA; Difco) after 7 d in darkness at a temperature of 20 °C. Morphological characteristics of V8A grown cultures were observed under the light microscope. A 1 cm diam plug from a 14-d-old culture was placed on a glass slide, stained with a drop of lactic acid cotton blue or distilled water and examined for hyphal and gametangial features. Because isolates produced oospores independently, crossings were not necessary. Sporangia were induced by flooding a 1 cm diam plug from the edge of an actively growing colony on V8A in a sterile soil extract. Sporangia were measured without adding lactic acid cotton blue to avoid shrinkage. The incubation period was 1 d on a laboratory bench with exposure to diffuse light for *ca* 12 h and a temperature of 20 ± 3 °C. The soil extract was prepared from 250 g forest soil dissolved in 1 l distilled water, which was then filtered through cheesecloth to remove soil particles. Then, the filtrate was autoclaved. Fifty gametangia and sporangia were measured for each isolate.

DNA extraction and phylogenetic analysis of the sequence data

Genomic DNA was extracted from 10–20 mg lyophilized mycelium. Each sample was ground using a sterile pestle in 500 μl extraction buffer [100 mM Tris–HCl, 10 mM EDTA, 1 M KCl, (pH 8)], and incubated at 75 °C for 15 min. Some samples additionally were extracted with 300 μl phenol:chloroform (1:1). The aqueous phase was collected after centrifugation for five min (16 000 ***g***), and DNA was precipitated using 300 μl isopropanol. Samples were centrifuged for 10 min (16 000 ***g***) to pellet the DNA, which was then washed with 70 % ethanol, dried, and re-suspended in 100 μl TE buffer [1×; 10 mM Tris–HCl, 1 mM EDTA (pH 8]. All DNA samples were stored at −20 °C prior to PCR amplification.

Isolates were initially screened by generating sequences from the ITS region of the ribosomal DNA. Primers ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) were used to amplify the ITS region including the 5.8S rDNA (White *et al.* 1990). PCR reaction mixtures (20 μl) contained *ca* 10 ng template DNA, 1 μM each primer, 10 μl of 2 × PCR FailSafe buffer F (Epicentre, Madison, WI), and 2 units Ampli*Taq* polymerase (PerkinElmer, Waltham, MA). Amplifications were performed with an initial denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 1 min, 48 °C for 1 min, 72 °C for 1 min; and a final extension for 5 min at 72 °C. PCR products were purified using QIAGEN spin columns (QIAGEN, Valencia, CA) and directly sequenced on both strands with the same primers used in the PCR amplification. Cycle sequencing reactions were performed at The Pennsylvania State University's Nucleic Acid Facility using the BigDye system (version 3.1 dye terminators; Applied Biosystems, Foster City, CA) and run on an ABI 3730XL DNA Analyser, using the ABI Data Collection Program (version 2.0) and ABI Sequencing Analysis software (version 5.1.1). ABI trace files were analysed using Sequencher version 4.6 (GeneCodes, Ann Arbor, MI) and consensus sequences were aligned with additional ITS accessions obtained from GenBank using ClustalX under default settings (Thompson *et al.* 1997). The ITS alignment was analysed under MP in PAUP version 4b10 (Swofford 1998) using a heuristic search with random sequence addition (nreps = 10), tree bisection–reconnection (TBR) branch swapping, and allowing multiple trees (multrees = yes); 1 K BS replicates using the same parameters were also performed to asses the level of support for each node. GenBank accession numbers for the ITS sequences generated here and the source and accession numbers for ITS sequences obtained from GenBank are listed in Supplementary Material Table S1.

In addition, from the holotype isolate seven nuclear loci originally selected to explore phylogenetic relationships within the genus (Blair *et al.* 2008) were sequenced. These

Table 1 – Origin of *Phytophthora quercetorum* isolates used for morphological and molecular characterization

Isolate no[a]	Host/substrate	Geographic location	ATCC accession no.
WV 5/6	*Quercus macrocarpa*, forest soil	N 78° 19′ 51.9″; E 39° 29′ 2.7″	MYA-4185
WV 8/3	*Q. rubra*, forest soil	N 81° 8′ 46.5″; E 37° 32′ 49.7″	MYA-4086
PA 7/3	*Q. rubra*, forest soil	N 78° 27′ 16.2″; E 39° 46′ 6.2″	MYA-4183
OH 14/1	*Q. rubra*, forest soil	N 82° 19′ 5.2″; E 39° 19′ 48.9″	MYA-4182
OH 10/4	*Q. phellos*, forest soil	N 82° 18′ 21.6″; E 40° 34′ 30.5″	MYA-4181
WI 111	*Q. rubra*, forest soil	N 89° 36′ 47.4″; E 43° 8′ 18.9″	MYA-4184
MN D4	*Q. rubra*, forest soil	N 93° 26′ 24.7″; E 46° 9′ 34.5″	MYA-4180
MD 9/2 (ex-holotype)	*Q. rubra*, forest soil	N 78° 24′ 26.7″; E 39° 42′ 0″	MYA-4186

a Isolate codes refers to the states where the soils were collected: MD, Maryland; MN, Minnesota; OH, Ohio; PA, Pennsylvania; WI, Wisconsin; WV, West Virginia.

loci include portions of six protein-coding genes (60S ribosomal protein L10, β-tubulin, elongation factor 1 alpha, enolase, heat shock protein 90 and *tigA*) and approximately 1200 bp of the 5′ end of the 28S rDNA. Primer sequences for amplification and sequencing can be found in Supplementary Material Table S2. PCR reaction mixtures (20 μl) contained *ca* 5 ng template DNA, 0.2 μM of each primer, 200 μM dNTPs, 2 μl 10 × PCR Buffer (USB, Cleveland, OH), 2.5 mM $MgCl_2$, and 1 unit Taq DNA Polymerase (USB, Cleveland, OH). Amplifications were performed with an initial denaturation at 94 °C for 2 min; 35 cycles of 94 °C for 30 s, the locus-specific annealing temperature for 30 s, 72 °C extension for 60 s (2 min for amplicons over 1 kb); and a final extension of 5 min at 72 °C. A touchdown PCR program was used when the standard amplification protocol was unsuccessful: ten cycles of 94 °C for 30 s, 65 °C for 30 s (reduced by 1 °C degree each cycle down to 56 °C), 72 °C extension for 2 min; 30 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C extension for 2 min; final extension of 5 min at 72 °C. PCR products were purified using an enzymatic system (ExoSAP-IT; USB, Cleveland, OH) following the manufacturer's instructions and sequenced as described above. GenBank accession numbers for sequences used in multi-locus analyses can be found in Table 2.

Sequences from each locus were aligned using ClustalX under default settings (Thompson *et al.* 1997). Alignments were manually adjusted for small (single bp or codon) indels, and concatenated into one matrix for phylogenetic analysis of altogether 8606 bp. A sequence for the enolase locus of *Pythium vexans* could not be obtained and was coded as missing data. Using Modeltest version 3.7 (Posada & Crandall 1998), the general time reversible nucleotide substitution model with gamma-distributed rate variation and a proportion of invariable sites was chosen based on the hierarchical likelihood ratio test and the Akaike Information Criterion. A NJ phylogeny was reconstructed with PAUP version 4b10 (Swofford 1998) using ML distances calculated with the model parameters estimated from Modeltest and 1 K BS replicates. A MP analysis was also performed using a heuristic search with random sequence addition (nreps = 10), TBR branch swapping, and allowing multiple trees (multrees-yes); 1 K BS replicates using the same parameters were performed to assess the level of support for each node. A Bayesian analysis was performed using MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003); four analyses were run simultaneously for 1 M generations with three heated chains (temperature = 0.2) and one cold chain. Flat Dirichlet priors were used for parameters of the general time reversible model; uniform priors between 0 and 1 were used for the gamma shape parameter and the proportion of invariable sites. Trees were sampled every 1 K generations, and the majority-rule consensus was calculated after removing the first 100 K generations as burn-in. The data matrix used for analyses has been deposited in TreeBASE under accession number S1908.

Table 2 – GenBank accession numbers for sequences used in multi-locus analyses

Phytophthora spp.	Isolate ID[a]	60S Ribosomal protein L10	β-tubulin	Elongation factor 1 alpha	Enolase	Heat shock protein 90	28S Ribosomal DNA	*TigA* gene fusion
P. arecae	P10213	EU080809	EU080810	EU080811	EU080812	EU080813	EU080814	EU080815
P. cactorum	P0714	EU080277	EU080278	EU080279	EU080280	EU080281	EU080282	EU080283
P. cambivora	P0592	EU080550	EU080551	EU080552	EU080553	EU080554	EU080555	EU080556
P. cinnamomi	P2159	EU079756	EU079757	EU079758	EU079759	EU079760	EU079761	EU079762
P. citricola	P7902	EU080235	EU080236	EU080237	EU080238	EU080239	EU080240	EU080241
P. clandestina	P3942	EU079866	EU079867	EU079868	EU079869	EU079870	EU079871	EU079872
P. drechsleri	P10331	EU079506	EU079507	EU079508	EU079509	EU079510	EU079511	EU079512
P. europaea	P10324	EU079481	EU079482	EU079483	EU079484	EU079485	EU079486	EU079487
P. gonapodyides	P10337	EU080530	EU080531	EU080532	EU080533	EU080534	EU080535	EU080536
P. heveae	P10167	EU080795	EU080796	EU080797	EU080798	EU080799	EU080800	EU080801
P. hibernalis	P3822	EU079513	EU079514	EU079515	EU079516	EU079517	EU079518	EU079519
P. ilicis	P3939	EU079859	EU079860	EU079861	EU079862	EU079863	EU079864	EU079865
P. katsurae	P10187	EU080802	EU080803	EU080804	EU080805	EU080806	EU080807	EU080808
P. megakarya	P8516	EU079969	EU079970	EU079971	EU079972	EU079973	EU079974	EU079975
P. megasperma	P3136	EU080058	EU080059	EU080060	EU080061	EU080062	EU080063	EU080064
P. multivesiculata	P10410	EU080065	EU080066	EU080067	EU080068	EU080069	EU080070	EU080071
P. nemorosa	P10288	EU079474	EU079475	EU079476	EU079477	EU079478	EU079479	EU079480
P. palmivora	P0255	EU080338	EU080339	EU080340	EU080341	EU080342	EU080343	EU080344
P. pseudosyringae	P10437	EU079562	EU079563	EU079564	EU079565	EU079566	EU079567	EU079568
P. psychrophila	P10433	EU080516	EU080517	EU080518	EU080519	EU080520	EU080521	EU080522
P. quercetorum	P15555	EU080900	EU080901	EU080902	EU080903	EU080904	EU080905	EU080906
P. quercina	P10334	EU080489	EU080490	EU080491	EU080492	EU080493	EU080494	EU080495
P. ramorum	P10301	EU080683	EU080684	EU080685	EU080686	EU080687	EU080688	EU080689
P. syringae	P10330	EU080557	EU080558	EU080559	EU080560	EU080561	EU080562	EU080563
P. uliginosa	P10328	EU079692	EU079693	EU079694	EU079695	EU079696	EU079697	EU079698
Pythium vexans	P3980	EU080483	EU080484	EU080485	N/A	EU080486	EU080487	EU080488

N/A, not available.
a Local identification numbers from the World *Phytophthora* Genetic Resource Collection.

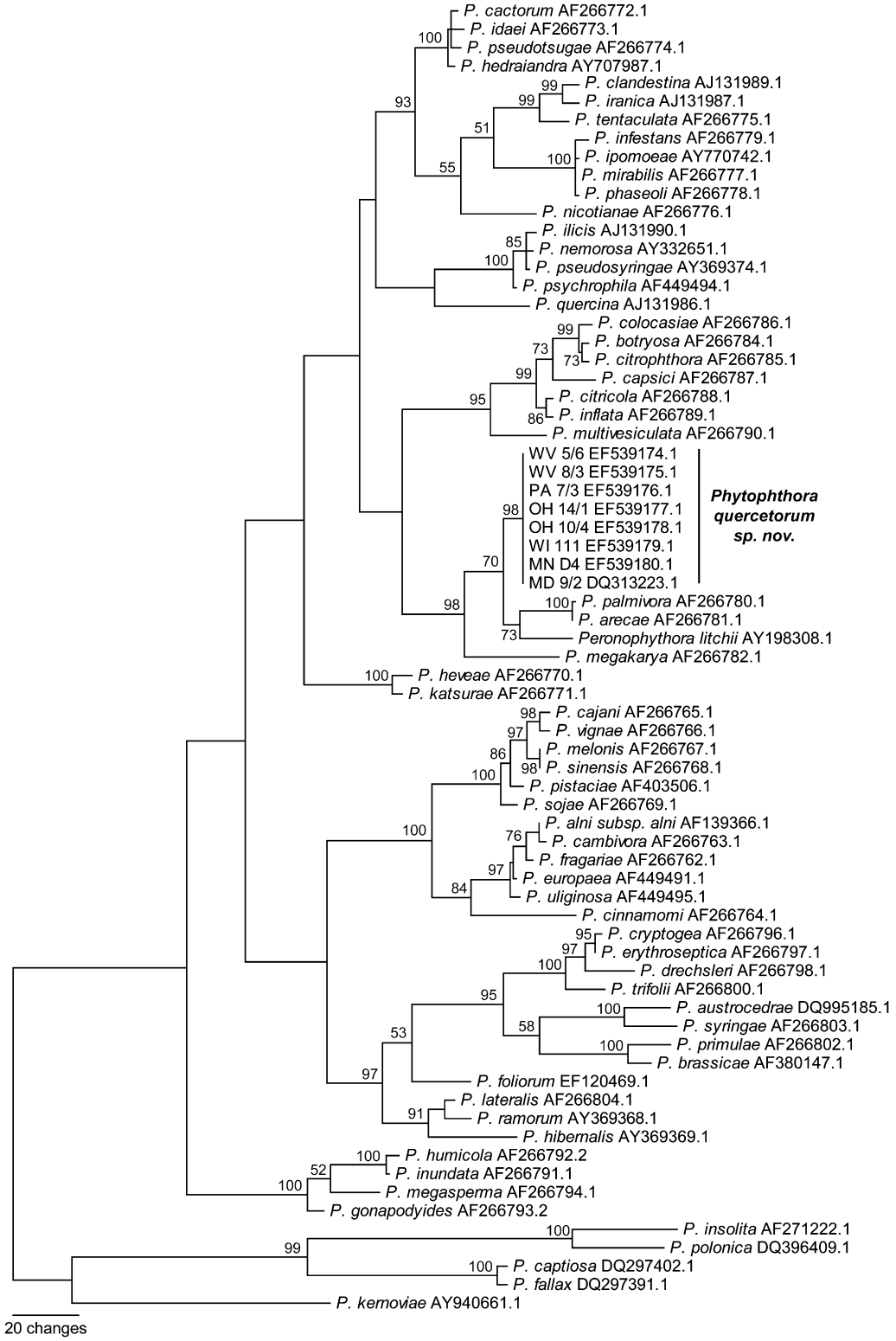

Fig 1 – MP phylogram of *Phytophthora quercetorum* sp. nov. and other *Phytophthora* spp. based on ITS sequence data. One of 27 most parsimonious trees (score 1540) is shown. Numbers above nodes represent MP BS values above 50 %. GenBank accession numbers are included to the right of species names, and are also given in Supplementary Material Table S1.

Results

Phylogenetic position of P. quercetorum *and comparison with related species*

ITS sequences generated from the eight *Phytophthora quercetorum* isolates were identical and exhibited 96 % identity to previously characterized isolates from *Peronophythora litchii* (GenBank accession AY198308; Voglmayr 2003), *P. palmivora* (GenBank accession AF266780), and *P. arecae* (GenBank accession AF266781; Cooke *et al.* 2000). The MP phylogeny of ITS accessions revealed that our *Phytophthora quercetorum* isolates were genetically distinct from previously characterized species (Fig 1). Although 27 equally parsimonious trees were found, the topologies differed only in the position of *P. nicotianae* and *P. fragariae* (as reflected by their low BS support values), and did not differ in the position of *P. quercetorum*. The position of *P. quercina* was also poorly resolved in the ITS phylogeny. We, therefore, sequenced seven additional molecular loci from the holotype isolate to verify that *P. quercetorum* should be considered a new species within the genus *Phytophthora*. Twenty-four species representing the diversity of the genus were included in the phylogenetic analyses, and an isolate of *Pythium vexans* was used as the outgroup. Although the relationships among the *Phytophthora* clades were not fully resolved, our results from the multilocus phylogeny do support the classification of *P. quercetorum* as a new species, and place the holotype within clade 4 with high BS support (Fig 2). Other members of clade 4 include *P. arecae*, *P. palmivora*, and *P. megakarya*, species that have traditionally been placed in Waterhouse group IV, and *P. quercina*, which, together with *P. quercetorum*, can be classified in Waterhouse group I. The results presented here are consistent with those of a separate study where phylogenetic relationships across the genus were examined using multilocus sequence data (Blair *et al.* 2008).

P. quercetorum can be distinguished from *P. aracae*, *P. palmivora*, *P. megakarya* (Erwin & Ribeiro 1996) by being 'homothallic' in culture and forming paragynous antheridia.

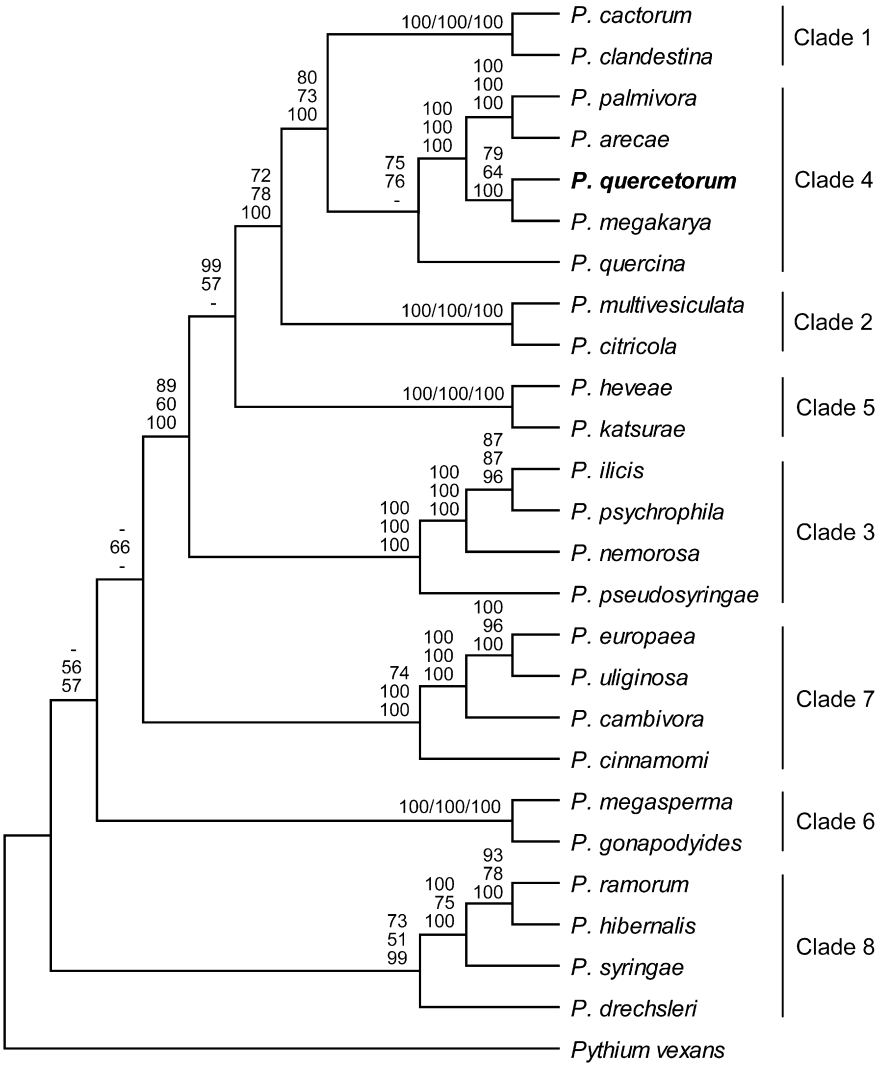

Fig 2 – Multilocus phylogeny based on seven nuclear loci, showing the 50 % MP BS consensus tree and rooted with *Pythium vexans*. Numbers above nodes represent BS values for NJ (top) and MP (middle), and Bayesian PPs presented as percentages (bottom). Support values less than 50 % are not shown.

It can be differentiated morphologically from *P. quercina* (Jung *et al.* 1999) by its spherical, non-elongated oogonial shapes and common coiled oogonial stalk, twice faster growth rate at the optimum temperature, and different colony types on V8A, MEA, PDA, and CMA. The sporangia of *P. quercetorum* are similar to those of *P. quercina*; however, they are slightly shorter. *P. quercetorum* can be also separated from *P. quercina* by its coralloid branched hyphae and absence of protuberances.

Distribution and ecology

Phytophthora quercetorum was associated with oak roots and never isolated from aboveground portions of oak. It was isolated from soils collected at 12 oak forest sites located in Maryland, Minnesota, Ohio, Pennsylvania, Wisconsin, and West Virginia (Fig 3). Its geographic origin remains unknown, but it appears to be a common inhabitant of oak forest soils in the eastern and north central USA. *P. quercetorum* was isolated along with *P. citricola* (one site), *P. cinnamomi* (one site), and *P. europaea* (two sites) in four of the soil samples collected from different sites. In addition, *P. quercetorum* also was isolated from sites where *P. cinnamomi* and *P. cambivora* had been isolated from different trees.

Taxonomy

Phytophthora quercetorum Y. Balci & S. Balci, **sp. nov.** (Figs 4–5)
MycoBank no.: MB 510683

Etym.: '*quercetorum*' refers to oak forests

Coloniae submersae striatae in agaris 'V8 juice agar (V8A)' et 'cormeal agar'. In agaro 'V8A' 5–35 °C, optime 22.5 °C crescent. Sporangia pauca in agaris solidis, sed abundantia in cultura liquida; papillata, forma variabilia: ovoidea, fabiformia vel irregularia, interdum appendicibus brevibus praedita, in medio 40.5 × 30 μm (25–57.5 × 20–42.5 μm), ratio longitudinis: latitudinis in medio 1.4. Oogonia numerosa in agaro 'V8A'; terminalia, globosa vel modice elongata 31.4 μm (17–40 μm) diam. Oosporae apleroticae, globosae, 25.2 μm (14.5–32.5 μm) diam. Antheridia semper paragyna, singularia, hyalina, globosa vel clavata.

Typus: **USA**: *Maryland*: Townhill, isol. ex solo rhizophaerae arboris *Quercus rubra*, July 2004, *S. Balci* (BPI 878357 – holotypus; *culturae vivae* CBS 121119 et ATCC MYA-4186).

Homothallic and producing gametangia abundantly on V8A within 5 d. *Oogonia* terminal at the main hyphae, globose, and predominantly turning yellow when mature. Mean oogonial diameter (eight isolates) 31.5 ± 3 μm (holotype 31.5 ± 3 μm; Table 3) ranging from 17–40 μm on V8A. The oogonial stalk predominantly bent or coiled, occasionally forming a curved tapering base (Fig 4A–J). The tapering, elongated and coiled stalk as well as the markedly aplerotic oospores were more apparent in liquid culture (Fig 4I, J). *Oospores* always spherical, markedly aplerotic (Fig 4A–J), average diameter (eight isolates) 25 ± 2.5 μm (holotype 25.5 ± 2.5 μm) (Table 3), ranging from 14.5–32.5 μm. Average oospore wall thickness 1.9 ± 0.7 μm. Average size of oogonia and oospores of the holotype strain on CMA 31 μm and 24.5 μm, respectively. *Antheridia* mostly lateral and sessile with short stalk, one per oogonium, attached near the stalk and rarely displaced, always paragynous, cylindrical (Fig 4A–D, G–H, J) or club-shaped, averaging 11 ± 2.5 × 9 ± 1.5 μm. *Sporangia* occasionally produced on solid agar substrate (V8A) and abundantly on agar plugs immersed in soil extract water; with sympodial sporangiophores and no internal proliferation (Fig 5H). Sporangia non-caducous, papillate (Fig 5A–F) and occasionally bipapillate (Fig 5C). Sporangia usually ovoid-elongated (Fig 5D–F) with common hyphal projections (Fig 5F) and are frequently laterally attached (Fig 5B, D, F–G). Displacement of papilla, globose and peanut-like distorted shapes infrequently produced. On empty sporangia a conspicuous basal plug was occasionally observed (Fig 5 G).

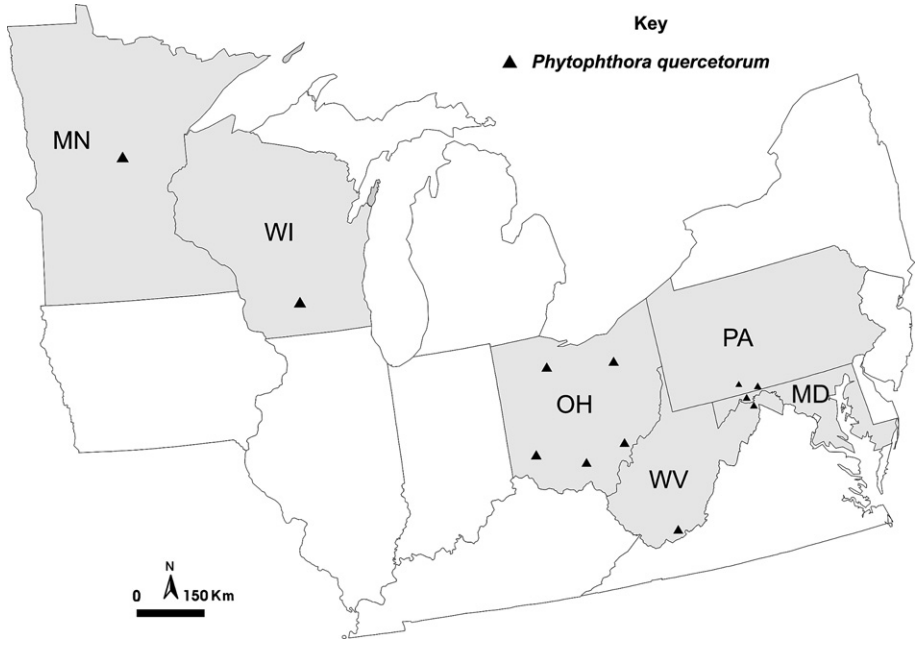

Fig 3 – Map showing the locations from where *Phytophthora quercetorum* sp. nov. was isolated.

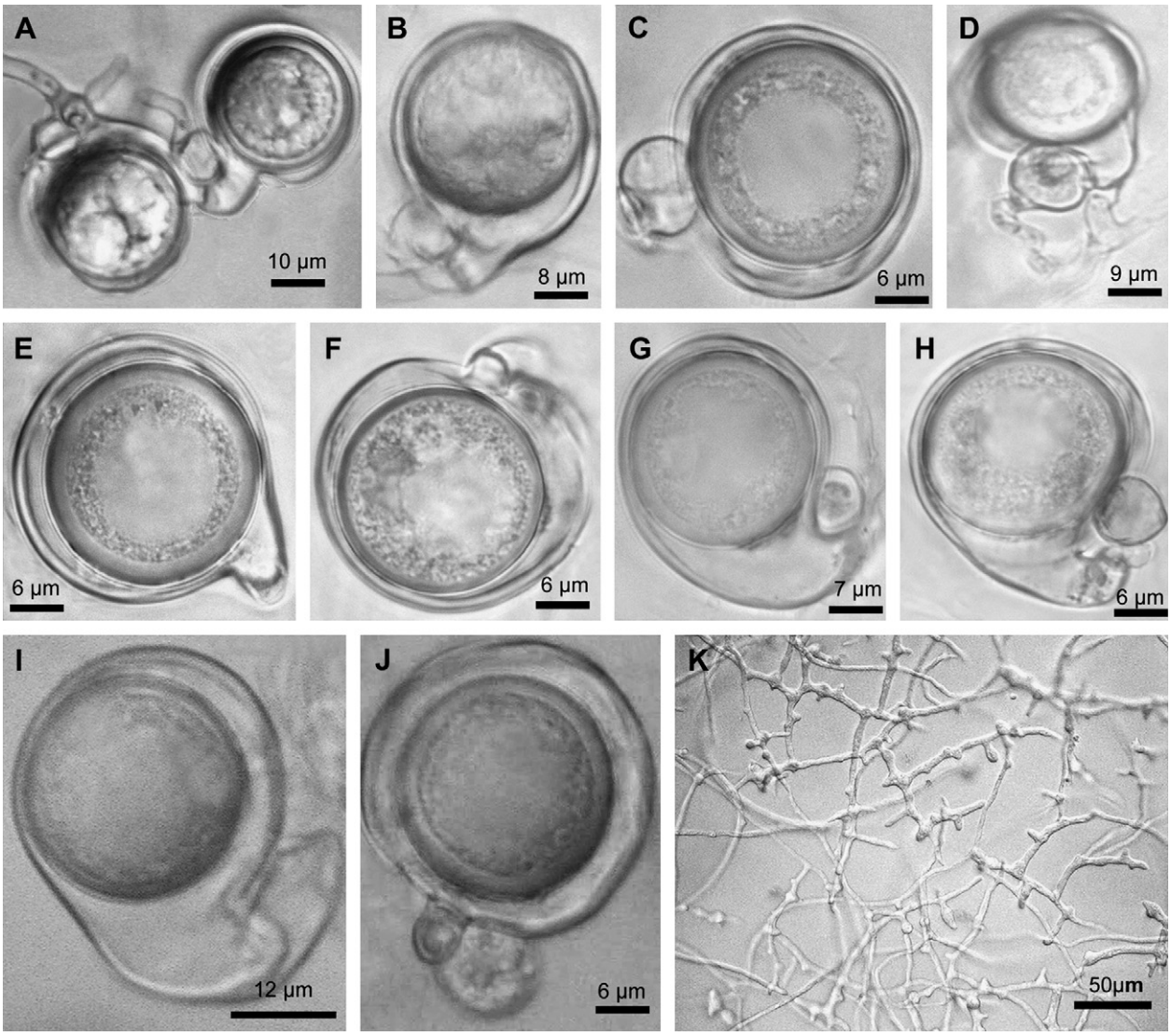

Fig 4 – Representative oogonia, aplerotic oospores, and antheridia of *Phytophthora quercetorum* sp. nov. (A–C, E, F) Common type of oogonia with aplerotic oospores. (G–I) Oogonia with markedly bented or coiled stalk and tapering base. (A–D, G, H, J) Oogonia with paragynous globose antheridia. (I) Markedly elongated oogonial stalk in liquid culture. (J) Markedly aplerotic oospore with globose antheridia in liquid culture. (K) Coralloid hyphae in V8A after 3 d.

Sporangial sizes (length × width) in eight isolates ranging from 17–40 × 14.5–32.5 µm (holotype 40.5 ± 6 × 30 ± 3.5 µm) with a length:width ratio of 1.4 (Table 3). *Colonies* on CMA with a striate pattern, largely submerged, with no aerial mycelia; on V8A faintly stellate or radiate with restricted aerial mycelia, which disappear on older cultures (Fig 6); on MEA faintly petaloid; on PDA stoloniferous with a waxy appearance. Optimum temperature for growth on V8A (eight isolates) *ca* 22.5 °C, with upper temperature limit of *ca* 32.5 °C (Fig 7). Radial growth rate at 22.5 °C in darkness ranging from 5.7–9.8 mm d^{-1}, average 7.5 mm d^{-1} (holotype 7.2 mm d^{-1}). *Main hyphae* in average 5 µm thick (4–10 µm), with a coralloid branching pattern on V8A (Fig 4 K) and markedly inflated in liquid culture (Fig 5I). *Chlamydospores* rarely produced, in average 30 ± 3 µm.

Habitat: In the rhizosphere soil of *Quercus* spp.

Notes: Being homothallic with paragynous antheridia and papillate sporangia, *Phytophthora quercetorum* falls in Group I of the Waterhouse classification based on morphological characteristics (Waterhouse 1963), which includes the following species: *P. cactorum*, *P. clandestina*, *P. idaei*, *P. iranica*, *P. italica*, *P. pseudotsugae*, and *P. tentaculata*. Morphologically, *P. quercetorum* differs from *P. cactorum* by the absence of caducous and persistent ovoid or globose sporangia; from *P. clandestina* and *P. iranica* by absence of amphigynous antheridia and by sporangial features; from *P. pseudotsugae* and *P. idaei* by its markedly aplerotic, smaller oospores and production of distorted sporangia with hyphal projections; from *P. tentaculata* by the absence of arachnoid antheridia and abundant chlamydospores. It differs from *P. italica* by its average larger oogonia, oospores, antheridial sizes and its different sporangial features (Erwin & Ribeiro 1996). *P. quercetorum* can also be distinguished from *P. hedraiandra* (De Cock & Levesque 2004) by the absence of predominantly sessile antheridia and caducous sporangia and by different colony patterns on

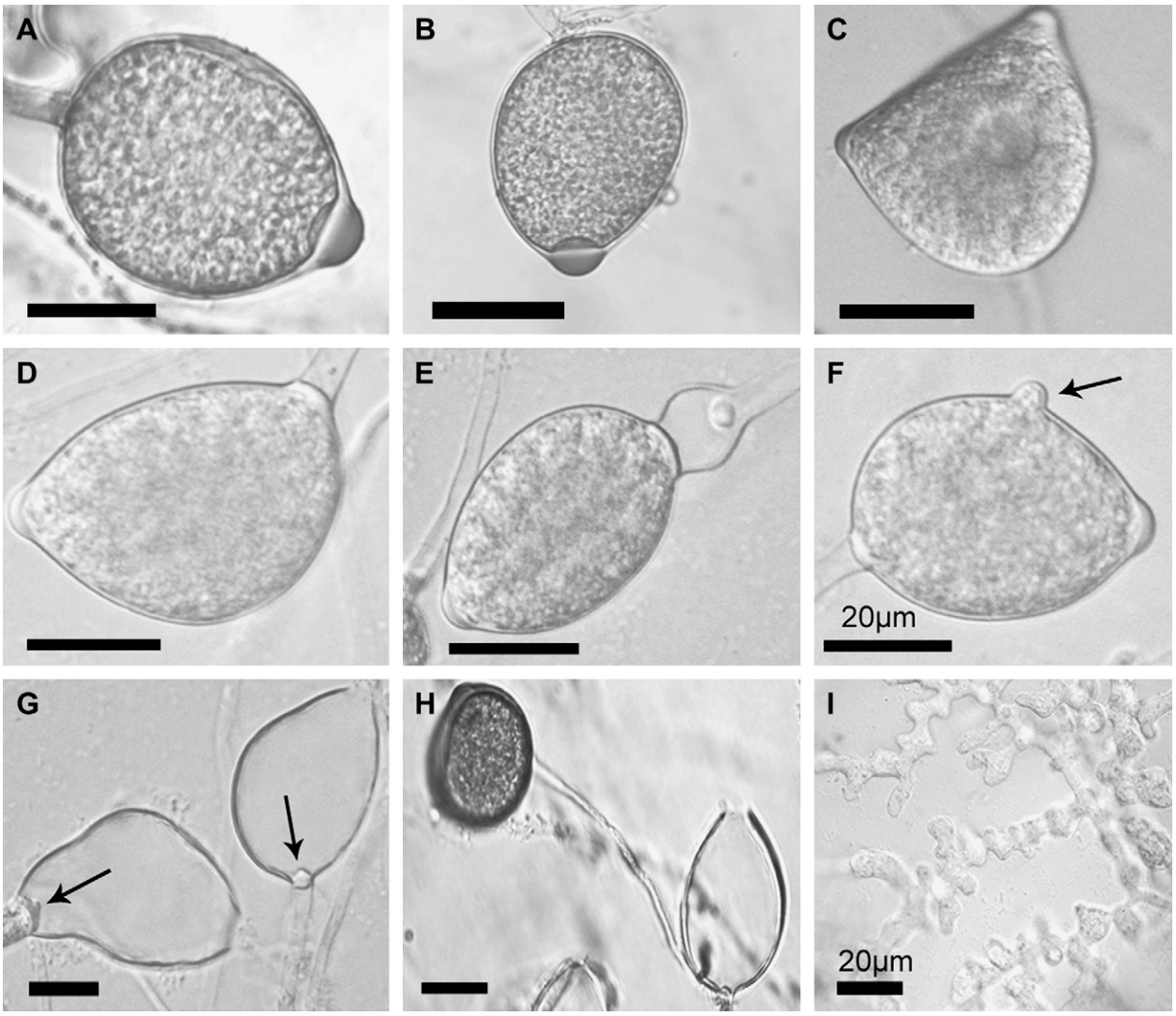

Fig 5 – Representative sporangia of *Phytophthora quercetorum* sp. nov. (A–B, D–F) Ovoid, ovoid-elongated papillate sporangia, terminal or laterally attached. (C) Bipapillate sporangium. (E) Ovoid-elongated sporangium with intercalary swelling on the sporangiophore. (F) Hyphal projection on sporangium (arrow). (G) Conspicuous basal plugs on empty sporangia (arrows). (H) Sympodial sporangiophore. (I) Inflated, coralloid hyphae in liquid culture.

CMA and PDA. However, molecular analyses suggest that this species is more closely related to *P. arecae*, *P. palmivora*, *P. megakarya*, and *P. quercina* in clade 4.

The ex-holotype cultures of *P. quercetorum* have been deposited at the American Type Culture Collection (MYA-4186), Centraalbureau voor Schimmelcultures (CBS 121119) and the World *Phytophthora* Genetic Resources Collection at UC-Riverside (P15555). Subcultures also are held in the Plant Pathology Culture Collection at West Virginia University. A dried culture of MD 9/2 (ex-type culture) on V8A was deposited at the US National Fungus Collection (BPI 878357).

Table 3 – Mean diam (μm) and standard deviation of oogonia, oospores, and sporangial sizes of eight isolates of *Phytophthora quercetorum*

Isolate	Oogonium	Oospore	Sporangia		
			Length	Width	L:W ratio
WV 5/6	31.9 ± 3	25.7 ± 2.5	43.4 ± 5.6	32.6 ± 3.6	1.3
WV 8/3	28.2 ± 2	23.4 ± 1.4	42 ± 5.1	30 ± 3.8	1.4
PA 7/3	32.4 ± 2.1	26.1 ± 1.8	40.4 ± 5.6	28.6 ± 3.1	1.4
OH 14/1	31.8 ± 2.6	25.3 ± 1.5	41.7 ± 6.5	29.2 ± 3	1.4
OH 10/4	32.5 ± 3.9	26.6 ± 3.2	36.6 ± 5.6	28.9 ± 3.9	1.3
WI 111	31.1 ± 2.4	24.6 ± 2	39.1 ± 4.2	26.8 ± 3	1.5
MN D4	33.1 ± 2.9	25.9 ± 1.7	44.1 ± 4	32.3 ± 3.1	1.4
MD 9/2 (holotype)	31.8 ± 3	25.9 ± 2.2	40.8 ± 5.8	30 ± 3.5	1.4
Average	31.4 ± 3	25.2 ± 2.3	40.5 ± 5.7	29.7 ± 4	1.4

Discussion

Phytophthora quercetorum is a newly recognized oomycete species, which occurs in diverse oak ecosystems in the eastern

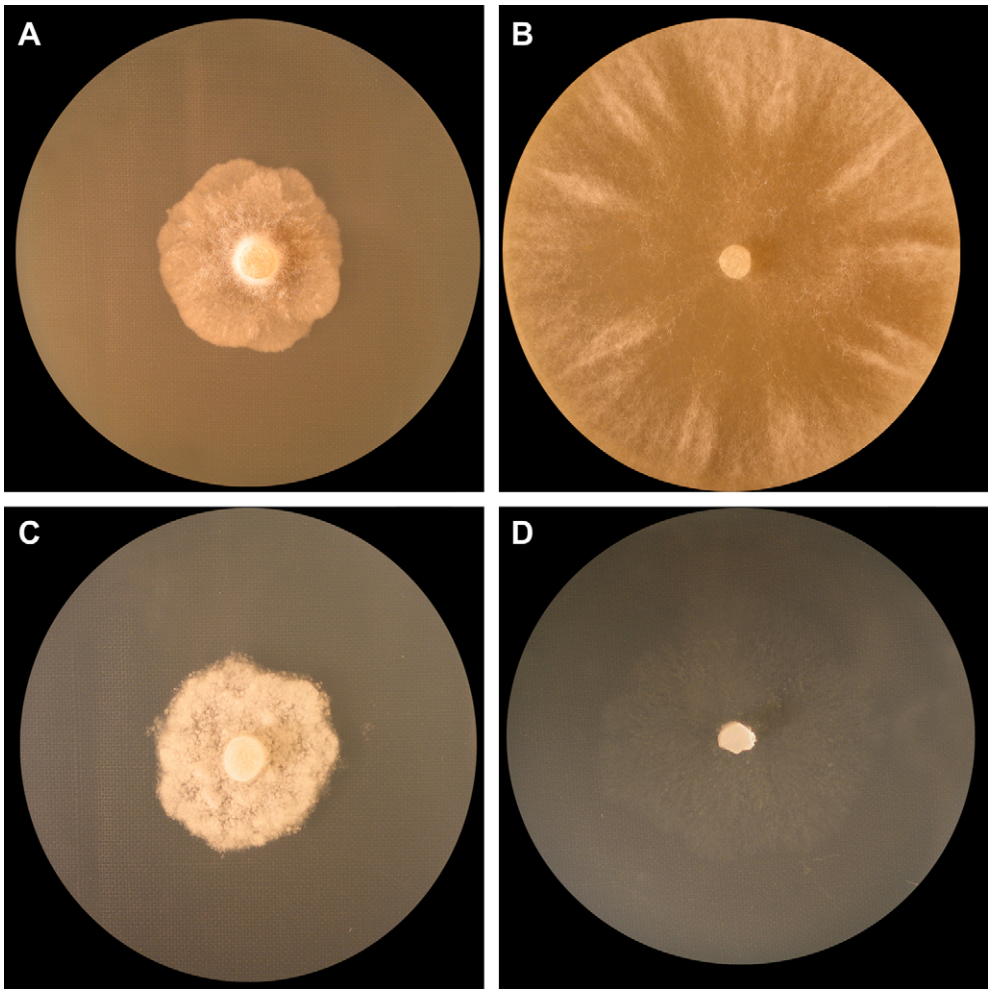

Fig 6 – Colony morphologies of *Phytophthora quercetorum* sp. nov. Cultures were grown at 20 °C on (A) MEA, (B) V8A, (C) PDA, and (D) CMA. Photographed 7 d after inoculation.

USA. Whereas *P. cinnamomi* was shown to be restricted below the 40° N latitude in the eastern USA (Balci *et al.* 2007), *P. quercetorum* appears to be distributed throughout the area studied. Species that were isolated from the same soil sample or at the same site together with *P. quercetorum* are known to cause disease on a broad range of hosts (Erwin & Ribeiro 1996). It remains to be determined whether *P. quercetorum* exhibits a similarly broad host range or is associated with any diseases. As the sampled oak forest sites comprise other forest tree species as well, sampling of soils around other plant species is required to determine the natural host preference of *P. quercetorum*.

In sites where *P. quercetorum* was isolated, no significant association was found between the presence of *P. quercetorum* and the crown status of oak trees (Balci *et al.* 2007). Similarly, in European oak forests the presence of various *Phytophthora* species was not recognized because of the absence of aboveground symptoms, such as dark lesions with exudates on the collar or stem of the host (Jung *et al.* 2000; Vettraino *et al.* 2002; Balci & Halmschlager 2003a,b; Delatour 2003; Jönsson 2004; Jönsson *et al.* 2005). The involvement of *P. quercetorum* in root dieback and decline of oak trees remains unknown; however, data obtained from preliminary pathogenicity tests (Y. Balci, unpubl. data) suggest that it has the potential to affect the root system and thus may play a role in oak decline.

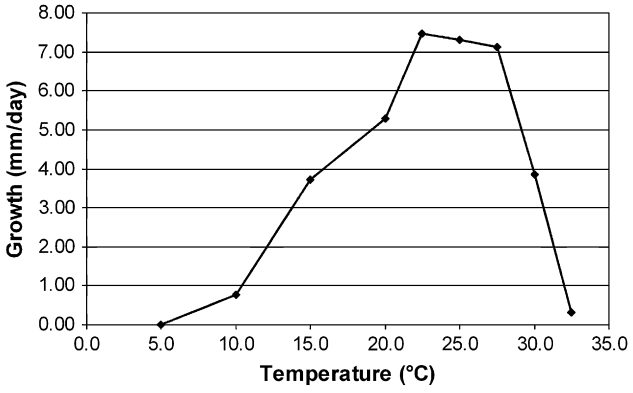

Fig 7 – Growth–temperature relationship of *Phytophthora quercetorum* sp. nov. on V8A. Averages of eight isolates were provided.

The origin of *P. quercetorum* is not known. Because of rapidly increasing international plant trade, *Phytophthora* species have been introduced into new environments and have invaded large areas within a short time as has been demonstrated by *P. ramorum* (Werres *et al.* 2001; Rizzo *et al.* 2002). An exotic origin was suggested for the oak fine-root pathogen *P. quercina* (Cooke *et al.* 2005) despite its common presence throughout European oak forests from Sweden to Turkey (Jung *et al.* 1996, 1999, 2000; Vettraino *et al.* 2002; Balci & Halmschlager 2003a,b; Delatour 2003; Jönsson *et al.* 2003). This species appears to be closely related to *P. quercetorum*, and both reside in similar ecosystems. Recently two new species of *Phytophthora* were described from South African *Eucalyptus* plantations, among them *P. alticola*, which is phylogenetically closely related to *P. quercetorum* (Maseko *et al.* 2007). Other species in clade 4 were originally described from tropical plants with similar temperature optima and maxima, which raise questions about whether *P. quercetorum* is of exotic origin.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.mycres.2008.02.008.

REFERENCES

Balci Y, Balci S, Eggers J, MacDonald WL, Juzwik J, Long RP, Gottschalk KW, 2007. *Phytophthora* species associated with forest soils in Eastern and North-central U.S. oak ecosystems. *Plant Disease* **91**: 705–710.

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

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

Brasier CM, Beales PA, Kirk SA, Denman S, Rose J, 2005. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesion on forest trees and foliar necrosis of ornamentals in the UK. *Mycological Research* **109**: 853–859.

Brasier CM, Robredo F, Ferraz JFP, 1993. Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. *Plant Pathology* **42**: 140–145.

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

Cooke DEL, Jung T, Williams NA, Schubert R, Oßwald W, Duncan JM, 2005. Genetic diversity of European populations of the oak fine-root pathogen *Phytophthora quercina*. *Forest Pathology* **35**: 57–70.

De Cock AWAM, Levesque CA, 2004. New species of *Pythium* and *Phytophthora*. *Studies in Mycology* **50**: 481–487.

Delatour C, 2003. *Phytophthora* in the European oak forest-results of a European Union research project. In: McComb JA, Hardy GE, Tommerup IC (eds), *Phytophthora in Forests & Natural Ecosystems. 2nd International IUFRO Working Party 7.02.09 Meeting, Albany, W. Australia 30 Sep – 5 Oct 2001*. Murdoch University Print, Perth, pp. 78–88.

Erwin CD, Ribeiro OK, 1996. *Phytophthora Diseases Worldwide*. The American Phytopathological Society Press, St Paul, MN.

Hansen EM, Reeser PW, Davidson JM, Garbelotto M, Ivors K, Douhan L, Rizzo DM, 2003. *Phytophthora nemarosa*, a new species causing cankers and leaf blight of forest trees in California and Oregon, USA. *Mycotaxon* **88**: 129–138.

Jönsson U, 2004. *Phytophthora* species and oak decline – can a weak competitor cause significant root damage in a nonsterilized acidic forest soil. *New Phytologist* **162**: 211–222.

Jönsson U, Jung T, Sonesson K, Rosengren U, 2005. Relationships between health of *Quercus robur*, occurrence of *Phytophthora* species and site conditions in southern Sweden. *Plant Pathology* **54**: 502–511.

Jönsson U, Lundberg L, Sonesson K, Jung T, 2003. First records of soilborne *Phytophthora* species in Swedish oak forests. *Forest Pathology* **33**: 175–179.

Jung T, Blaschke H, Neumann P, 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology* **26**: 253–272.

Jung T, Blaschke H, Oßwald 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, Oßwald W, 1999. *Phytophthora quercina* sp. nov., causing root rot of European oaks. *Mycological Research* **103**: 785–798.

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

Jung T, Nechwatal J, Cooke DEL, Hartmann G, Blaschke M, Oßwald W, Duncan JM, Delatour C, 2003. *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. *Mycological Research* **107**: 772–789.

Maseko B, Burgess TI, Caoutinho TA, Wingfield MJ, 2007. Two new species of *Phytophthora* from South African *Eucalyptus* plantations. *Mycological Research* **111**: 1321–1338.

Posada D, Crandall KA, 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Kike T, 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* sp. and *Lithocarpus densiflorus* in California. *Plant Disease* **86**: 205–214.

Robin C, Desprez-Loustau ML, Capron G, Delatour C, 1998. First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. *Annales des Sciences Forestiére* **55**: 869–883.

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

Swofford DL, 1998. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sinauer Associates, Sunderland, MA.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The CLUSTALX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876–4882.

Vettraino AM, Barzanti GP, Bianco MC, Ragazzi A, Capretti P, Paoletti E, Luisi N, Anselmi N, Vannini A, 2002. Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. *Forest Pathology* **32**: 19–28.

Voglmayr H, 2003. Phylogenetic relationship of *Peronospora* and related genera based on nuclear ribosomal ITS sequences. *Mycological Research* **107**: 1132–1142.

Waterhouse GM, 1963. Key to the species of *Phytophthora* de Bary. *Mycological Papers* **92**: 1–22.

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

Werres S, Marwitz R, Man in't Veld WA, de Cock AWAM, Bonants PJM, deWeerdt M, Themann K, Illieva E, Baayen RP, 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycological Research* **105**: 1155–1165.