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# *Phytophthora pini* Leonian resurrected to distinct species status

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***Abstract:*** *Phytophthora pini* was named by Leonian in 1925, but this species was largely ignored until 1956 and then merged with *P. citricola* by Waterhouse in 1963. This study compared the ex-type and ex-authentic cultures of these two species with isolates of *P. plurivora* and the *P. citricola* subgroups Cil I and III reported previously. Examination of these isolates revealed that the ex-type culture of *P. pini* is identical to *P. citricola* I. *Phytophthora pini* Leonian therefore is resurrected to distinct species status and redescribed here with a Latin description, replacing *P. citricola* I. Molecular, physiological and morphological descriptions of this species are presented. The molecular description includes DNA sequences of five nuclear and mitochondrial regions as well as PCR-SSCP fingerprints. The relationship among the above species and other species recently segregated from the *P. citricola* complex also is discussed.

***Key words:*** identification, Oomycete, *Phytophthora*, taxonomy

## INTRODUCTION

L.H. Leonian described *Phytophthora pini* as a new species in 1925 without a Latin description (Leonian 1925). His one culture was isolated by R.G. Pierce from the roots of red pine (*Pinus resinosa* Aiton) in Minnesota, USA. Leonian's new species essentially was ignored until 1956 when Grace Waterhouse presented the description in her compilations of original descriptions of species of *Phytophthora* (Waterhouse 1956). Two years after description of *P. pini* the species *P. citricola* was named by K. Sawada (Sawada 1927); a Latin description was given in 1935 (Ito and Tokunaga 1935). Waterhouse considered the above two species to be the same, with *P. citricola* as the preferred species name (Waterhouse 1963). She also considered *P. cactorum* var. *applanata* (Chester 1932) to be the same as *P. citricola* (Waterhouse 1957).

Subsequent to publication of Waterhouse's key, homothallic isolates with paragynous antheridia, noncaducous semipapillate sporangia and growth temperature maxima of about 30 C were identified as *P. citricola*. However the advent and use of molecular techniques in species identification have revealed that *P. citricola* is a genetically diverse species complex. For instance isozyme analysis of 125 isolates identified 10 electrophoretic types in five distinct groups (Oudemans et al. 1994). Amplified fragment length polymorphism (AFLP) analysis of 86 isolates similarly found four distinct groups (Bhat and Browne 2007). Among 10 isolates considered to be *P. citricola* single-strand conformation polymorphism (SSCP) analysis of PCR-amplified ribosomal DNA internal transcribed spacer (ITS) 1 also revealed four different fingerprints (Cil I–IV) (Kong et al. 2003). Cil IV later was found to produce papillate sporangia and was excluded from the *P. citricola* complex in a key to *Phytophthora* species (Gallegly and Hong 2008). The fingerprint group designated Cil II is described as *P. plurivora* (Jung and Burgess 2009), and Cil III still awaits description (Hong et al. unpubl data). Other new species descriptions within the citricola complex include *P. multivora* (Scott et al. 2009) and *P. mengei* (Hong et al. 2009a). This paper describes Cil I as *P. pini*. The objective of this paper was to resurrect *Phytophthora pini* to distinct species status and demonstrate its relationship to closely related taxa.

## MATERIALS AND METHODS

*Species and isolates.*—Eighty-four isolates including the ex-type cultures of *P. citricola*, *P. pini* and *P. c.* var. *applanata* were examined (TABLE I, SUPPLEMENTAL TABLES I, II). To avoid confusion in the terminology with different groups of *P. citricola* the type and authentic isolates hereafter are referred to as *P. citricola* s.s. (= sensu stricto), *P. citricola* I, II and IV to as *P. pini*, *P. plurivora* and *P. quercetorum* respectively, unless otherwise followed by a citation of original paper. In the broadest sense as defined by Waterhouse this species complex is referred to as *P. citricola* sl (= sensu lato). A dried culture of the ex-type *P. pini* is deposited at the Massey Herbarium of Virginia Polytechnic Institute and State University in Blacksburg, Virginia (VTMH: 11737).

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TABLE I. Origin and DNA regions of *Phytophthora* isolates sequenced in this study

| Species | SSCP[a] | Culture accession number[b] | | | Host | Geographic origin | Year | Sequence accession number[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VT | WVU | Alternative | | | | ITS | TUB | ELO | COX | NADH |
| *P. citricola* | Cil | 33H8 | P396 | CBS221.88$^T$ | *Citrus sinensis* | Formosa | 1927 | FJ392318 | GQ247653 | GQ247662 | GQ247647 | GQ247671 |
| | | 33J2 | P375 | CBS295.29 | *Citrus* sp. | Japan | 1929 | FJ392319 | GQ247654 | GQ247663 | GQ247648 | GQ247672 |
| *P. pini* | Pin | 45F1 | P343 | ATCC 64532$^T$ | *Pinus resinosa* | Minnesota | 1925 | FJ392322 | GQ247656 | GQ247666 | GQ247650 | GQ347675 |
| | | 3J7 | | | Irrigation water | Virginia | 2001 | GQ303146 | | | | |
| | | 22F1 | P53 | MYA-3656 | *Rhododendron* sp. | West Virginia | 1987 | FJ392320 | GQ247657 | GQ247665 | GQ247649 | GQ247674 |
| | | 22F3 | P33 | | | Ohio | | FJ392321 | | | | |
| | | 39A3 | | | Stream water | Virginia | 2006 | GQ303147 | | | | |
| | | 43H1 | | | Irrigation water | Virginia | 2007 | GQ303150 | | | | |
| | | 44B6 | | | *Rhododendron* sp. | Maryland | 2007 | GQ303151 | | | | |
| | | 45E6 | | | Stream water | Virginia | 2007 | GQ303152 | | | | |
| *P. plurivora* | Plu | 22E9 | P101 | MYA-3657 | *Kalmia latifolia* | West Virginia | 1998 | FJ392323 | GQ247658 | GQ247667 | GQ247651 | GQ247676 |
| (=*P. c.* var. *applanata*) | | 22F2 | P52 | | *Rhododendron* sp. | New York | 1987 | FJ392324 | | | | |
| | | 39A8 | | | Stream water | Virginia | 2006 | GQ303153 | GQ247659 | GQ247668 | GQ247652 | GQ247677 |
| | | 33H9 | P404 | CBS 397.61 | *Rhododendron* sp. | Germany | 1958 | FJ392325 | | | | |
| *P. citricola* III | Cil III | 1E1 | P130 | MYA-3658 | Irrigation water | Oklahoma | | FJ392326 | GU071235 | GU071242 | GU071239 | GU071246 |
| | | 15C9 | P429 | SJ (W.001) | *Acer saccharum* | Wisconsin | 1985 | FJ392327 | GU071236 | GU071243 | GU071240 | GU071247 |
| | | 39H2 | | | Irrigation water | Virginia | 2006 | GQ303148 | GU071237 | GU071244 | GU071241 | GU071248 |
| | | 42B8 | | | Irrigation water | Virginia | 2007 | GQ303149 | GU071238 | GU071245 | | GU071249 |
| *P. quercetorum* | Qct | 15C7 | P131 | SJ (AF.018) | *Hedera helix* | South Carolina | 1997 | FJ392328 | | | | |
| | | 15C8 | P303 | SJ (AF.028) | Field soil | South Carolina | 1997 | FJ392329 | | | | |
| *P. mengei* | Men | 42B2 | P340 | MYA-4554$^T$ | | California | | EU748545 | GQ247660 | GQ247669 | | GQ247678 |

[a] SSCP code (former code)

[b] VT = Virginia Polytechnic Institute and State University; WVU = West Virginia University; Alternative sources: ATCC or MYA = American Type Culture Collection; CBS = Centraal Bureau voor Schimmelcultures, SJ = Steven Jeffers at Clemson University (collection number); Superscript T denotes ex-type culture.

[c] ITS = internal transcribed spacers (Cooke et al. 2000); TUB = β-tubulin, ELO = Translation elongation factor 1α, COX = Cytochrome *c* oxidase subunit I, NADH = NADH dehydrogenase subunit I (Kroon et al. 2004)

TABLE II. Comparative summary of points of mutation and indels in the five nuclear and mitochondrial DNA regions between *P. pini* and its close relatives

| | Nuclear | | | Mitochondrial | |
|---|---|---|---|---|---|
| | β-tubulin[a] | EF-1α[a] | ITS[b] | Cox1[a] | NADH[a] |
| Fragment length (bp) | 932 | 903 | 761 | 898 | 829 |
| Total points of mutation and indels | 6 | 15 | 7 | 32 | 16 |
| *P. pini* vs. *P. citricola* s.s. | 4–5 | 6–10 | 4–5 | 16–17 | 10 |
| *P. pini* vs. *P. citricola* III | 1 | 6 | 1–2 | 3–10 | 2 |
| *P. pini* vs. *P. plurivora* | 4–5 | 6–10 | 5–6 | 13–14 | 9 |

[a] Amplified and sequenced with primer pairs of Kroon et al. (2004).
[b] Amplified and sequenced with primer pair of ITS6 and 4 according to Cooke et al. (2000)

*DNA extraction.*—Individual isolates were grown in V8 broth (Erwin and Ribeiro 1996) at room temperature (ca. 23 C) 10 d. Genomic DNA was extracted as instructed with the DNeasy® Plant Mini Kit (QIAGEN, Valencia, California).

*DNA sequencing and sequence analysis.*—One to four isolates of each species (TABLE I) were sequenced in the ribosomal DNA ITS region (Cooke et al. 2000) and genes encoding β-tubulin (*tubulin*), translation elongation factor 1α (*ef-1α*), NADH dehydrogenase subunit I (*nadh*) and cytochrome oxidase subunit I (*cox1*) (Kroon et al. 2004). Six additional isolates of *P. pini* and two additional isolates of *P. plurivora* and two isolates of *P. quercetorum* also were sequenced in the ITS regions. Each isolate and DNA region was sequenced in both directions at least twice. Consensus sequences were compared to determine intra- and interspecific variations. They also were compared with those deposited in the GenBank with BLASTn at http://ncbi.nlm.nih.gov to determine their phylogenetic position and close relatives. The phylogenic analyses were carried out with MrBayes 3 (Ronquist and Huelsenbeck 2003) in TOPALI 2.5 (Milne et al. 2009). A total of up to 23 other species of *Phytophthora* representing major clades (Cooke et al. 2000, Martin and Tooley 2003, Kroon et al. 2004, Blair et al. 2008) were selected based on the availability of type culture sequence.

*DNA fingerprinting.*—Representative isolates from 25 species (TABLE I, SUPPLEMENTAL TABLE II) were fingerprinted following a standard procedure (Gallegly and Hong 2008). This procedure is based on PCR-SSCP of rDNA ITS1 (Kong et al. 2003). The only modification was that a smaller volume (2 μL) of denatured PCR product was loaded for electrophoresis.

*Physiology.*—Two isolates each of *P. citricola* s.s., *P. pini* and *P. plurivora* were selected for temperature experiments. Agar disks (4 mm diam) were taken from actively growing areas of 5 d old cultures with a flamed cork borer and transferred to freshly prepared V8 agar in 10 cm diam Petri dishes, one disk placed with the mycelium facing down in the center of each dish. These dishes then were placed in incubators of different settings (5–35 C) in the dark. Three dishes per isolate per temperature were used. Two measurements of colony diameter were taken 2 and 6 d after onset of tests. The test was repeated once. Average daily radial growth rates of individual isolates were computed and compared by temperature with statistical analysis software V8e (SAS Institute, Cary, North Carolina).

*Morphological examination.*—Two isolates of *P. citricola* s.s. and 36 of *P. pini* were grown on clarified V8 agar at room temperature (23 C) 2 wk. Sexual structures then were examined with an Olympus IX71 inverted microscope (Olympus, Center Valley, Pennsylvania). Sporangia were induced by submerging the agar culture blocks in 10% sterile soil water extract (Erwin and Ribeiro 1996) and incubated at room temperature overnight. Morphological structures were photographed then measured with Image-Pro Plus 5.1 (Media Cybernetics Inc., Bethesda, Maryland). Mean and standard error of at least 30 measurements were calculated for each character and isolate, followed by analyses of variance to determine the difference between isolates of the same species and among the species examined. Measurements of both *P. citricola* s.s. isolates were presented, but only those of four representative isolates of *P. pini* were included in this paper.

One or two isolates from each species also were grown on lima bean and hempseed agars for comparison. These morphological examinations were performed as described by Gallegly and Hong (2008). Specifically sporangia were produced on 4 mm disks in 10% soil extract from 3 d old lima bean agar cultures for measurements and photographs, and the sexual structures were produced with hempseed agar.

## RESULTS

*Sequence analysis.*—Points of mutations and indels in the five nuclear and mitochondrial DNA regions between *P. pini* and its close relatives are summarized (TABLE II). Comparatively few variations were seen in sequences among isolates of individual species. Specifically both isolates of *P. citricola* s.s. had an identical sequence in each of the five regions as did the isolates of *P. plurivora*. Eight isolates of *P. pini* also had an identical ITS sequence except for the type culture, which has a mutation at 650 *nt*. No or little intraspecific variation was observed in other DNA regions of *P. pini* and *P. citricola* III.

TABLE III. Diagnostic morphological and physiological characters of *Phytophthora pini* compared with *P. citricola* ss, *P. citricola* III, *P. mengei, P. multivora, P. plurivora,* and *P. siskiyouensis*

| | *P. pini*[a] | *P. citricola ss*[a] | *P. citricola* III[b] | *P. mengei*[c] | *P. multivora*[d] | *P. plurivora*[e] | *P. siskiyouensis*[f] |
|---|---|---|---|---|---|---|---|
| No isolates | 4 | 2 | 2 | 2 | 6 | 7 | 9 |
| Oogonium | | | | | | | |
| Shape | globose | globose | globose | globose | globose | globose | globose |
| Diameter (μm) | 30.3 | 28.7 | 28 | 24 | 26.5 | 28.5 | 27.8 |
| Diameter range | 22.2–41.4 | 19.2–35.2 | 25–30 | 20–27 | 19–37 | 27–30 | 24–31 |
| Antheridia | | | | | | | |
| Shape | asymmetric capitate | round capitate | round | asy. capitate | obovoid | obovoid | capitate[g] |
| Diameter (μm) perpendicular | 12.5 | 9.6 | 12–17 | 10 | 13.8 | 11.1 | 9.5–13.3 |
| Diameter (μm) tangential | 11.1 | 10.1 | 10 | 10 | 8.8 | 8.4 | 8.6–11.6 |
| Oospore | plerotic | plerotic | aplerotic | plerotic | plerotic | apler. –pler. | aplerotic |
| Mean diameter (μm) | 26.0 | 25.9 | 20–26 | 21.7 | 23.6 | 25.9 | 24.6 |
| Wall thickness (μm) | 1.7 | 1.5 | 1.6 | 2.3 | 2.6 | 1.45 | — |
| Sporangia | | | | | | | |
| Shape | ovoid | ovoid | ov.-ell. | ell.-ov. | ov.-ell. | ov.-ell. | ovoid |
| l × w mean (μm) | 47.4 × 31.5 | 43.9 × 28.2 | 50.7 × 33.4 | 62.7 × 35.2 | 51.0 × 30.0 | 47.4 × 33.5 | 55 × 36 |
| l × w range | 31.5–75.3 × 21.7–49.5 | 33.2–58.8 × 22.9–38.9 | 40–60 × 30–40 | 37–95 × 27–44 | 38–97 × 24–63 | 28–81 × 16–70 | 46–70 × 30–51 |
| l/b ratio | 1.52 | 1.56 | 1.43 | 1.8 | 2.6 | 1.5 | 1.5 |
| Chlamydospore | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hyphal swelling | small | 0 | 0 | 0 | 0 | appresorial | 0 |
| Temperature-growth relation | | | | | | | |
| Maximum (°C) | 35 | 31 | 31 | 30–33 | 30–32.5 | 32 | 30 |
| Optimum (°C) | 25 | 25 | 25–28 | 25 | 25 | 25 | 25 |
| Growth rate at optimum (mm $d^{-1}$) | 6.8–7.1 | 5.4–5.6 | — | — | 5.7 | 8.1 | 7.5 |
| Growth medium | CV8A[h] | CV8A | CV8A | CV8A | V8A | V8A | V8S |

[a] This paper;
[b] from Gallegly and Hong (2008);
[c] from Hong et al. (2009);
[d] ex-type isolate from Scott et al. (2009);
[e] from Jung and Burgess (2009);
[f] from Reeser et al. (2008);
[g] 10% amphigynous;
[h] V8A = V8 juice agar and CV8A = clarified V8 juice agar and V8S = CV8A amended with 20 ppm β-sitosterol.

TABLE IV. Nomenclature relationship among studies concerning the *P. citricola* sensu lato and ex-type cultures of individual named species and taxa

| Present study | Oudemans et al. (1994) | Kong et al. (2003), Gallegly and Hong (2008) | Bhat and Browne (2007) | Original reference | Holotype or representative isolate |
|---|---|---|---|---|---|
| *P. citricola* ss | CIT1 (2) | n.a. | n.a. | Sawada (1927) | CBS221.88, IMI021173 |
| *P. mengei* | CIT5 (9) | n.a. | Avo | Hong et al. (2009) | MYA-4554 |
| *P. multivora* | CIT3 (7) | n.a. | n.a. | Scott et al. (2009) | CBS124094 |
| ***P. pini*** | CIT1 (2) | *P. citricola* I | Mix I and II | Leonian (1925) | ATCC64532, CBS181.25, IMI077970 |
| *P. plurivora* [=*P. c,* var. *applanata*] | CIT1 (1) [n.a] | *P. citricola* II [n.a.] | n.a. [n.a] | Jung and Burgess (2009) (Chester [1932]) | CBS124093 [ATCC 64532, CBS273.32] |
| *P. citricola* III | n.a. | *P. citricola* III | n.a. | Kong et al. (2003) | MYA-3658 |
| *P. citricola* E | CIT2 (4) | n.a. | n.a. | Jung and Burgess (2009) | IMI031372 |

*P. pini* and *P. citricola* III are phylogenetically close. Specifically these two taxa reside in the same terminal cluster of Bayesian inference trees constructed on the ITS (FIG. 1) and β-tubulin sequences (SUPPLEMENTAL FIG. 1). ITS sequence revealed their closest relatives are *P. plurivora*, *P. citricola* E, followed by *P. multivora* and *P. citricola* s.s., and then by *P. mengei*, *P. tropicalis*, *P. siskiyouensis* and *P. capsici* in the same ITS clade. A similar phylogenetic relationship was observed in the inference tree from the *tubulin* sequences. However isolates of *P. pini* were consistently grouped in one terminal cluster, whereas those of *P. citricola* III grouped in another cluster in the inference trees from the *ef-1α* (SUPPLEMENTAL FIG. 2) and *cox1* sequences (SUPPLEMENTAL FIG. 3). Both isolates CIT-US1 and CIT-US10 placed in *P. citricola* I (Jung and Burgess 2009) were grouped with Cil III instead of *P. pini* in the *cox1* tree. A similar phylogenetic relationship also was observed in the inference tree constructed on the *nadh* sequences (SUPPLEMENTAL FIG. 4).

*DNA fingerprints.*—All 25 species including *P. citricola* s.s., *P. pini*, *P. macrochlamydospora*, *P. multivesiculata*, *P. bisheria* and *P. siskiyouensis* each produced a distinct fingerprint (SUPPLEMENTAL FIG. 5). Both isolates of *P. citricola* s.s. produced an identical DNA fingerprint. Three isolates of *P. pini* also produced an identical fingerprint, as did all other isolates of this species from different hosts and aquatic environments (data not shown). Similarly *P. cactorum* var. *applanata* produced a fingerprint identical to that of *P. plurivora* (data not shown).

*Temperature relations.*—Among the six isolates assessed *P. plurivora* and *P. pini* reached the edge of the Petri dishes first after 135 h at the optimum temperature, 25 C. In comparison the optimum temperature was 20–25 C for *P. citricola* s.s. (SUPPLEMENTAL FIG. 6). *P. plurivora* grew 0.4 mm per d at 5 C, but *P. citricola* s.s. and *P. pini* did not grow at this temperature. *P. citricola* s.s. and *P. plurivora* grew only during the first 2 d at 30 C, indicating this is their maximum growth temperature. In contrast *P. pini* grew substantially at 30 C throughout the 6 d period (TABLE III).

## TAXONOMY

*Phytophthora pini* Leonian, 1925

Emend. Gallegly, Hong, Richardson & Kong

= *Phytophthora citricola* I Gallegly & Hong 2008

Species nova homothallica, antheridiis paragynicis. Oogonia globosa comparate uniformia, numero medio 30.3 μm diam (intra 22.2–41.4 μm). Oosporae maximam partem pleroticae, diametro numerum medium 26.0 μm advergente, parietibus cellularum 1.7 μm crassis. Antheridia relative magna, capitata vel leviter asymmetrice capitata, diclina, plerumque prope stipitem oogonialem affixa. Interdum antheridia duo per oogonium visa. Mensurae antheridiales numero medio 11.1 μm quum tangentiales atque 12.6 um quum perpendiculatae ad parietem oogonialem. Sporangia non caduca semipapillata plerumque ovoidea, sed formas monstruosas visas. Magnitudo sporangiorum numero medio 47.4 × 31.5 μm (intra 31.5–75.3 × 21.7–49.5 μm). Sympodia simplicia interdum praesentia. Chlamydosporae non visae sed interdum areae hyphales extuberantes paucae parvae atque hyphae irregulares interdum in agaro *Phaseoli lunati* L. ''Lima Bean'' dicto visae.

*Morphology.*—*Phytophthora pini* is homothallic with paragynous antheridia. Oogonia produced on clarified V8 agar are globose and relatively uniform, averaging 30.3 μm diam (range 22.2–41.4 μm diam). Oospores are mostly plerotic and average 26.0 μm diam (19.6–34.2 μm diam), their cell walls are 1.7 μm thick (1.4–1.9 μm). The antheridial characters differ from those of *P. citricola* s.s. The antheridia are larger, capitate to slightly asymmetrically capitate, diclinous,

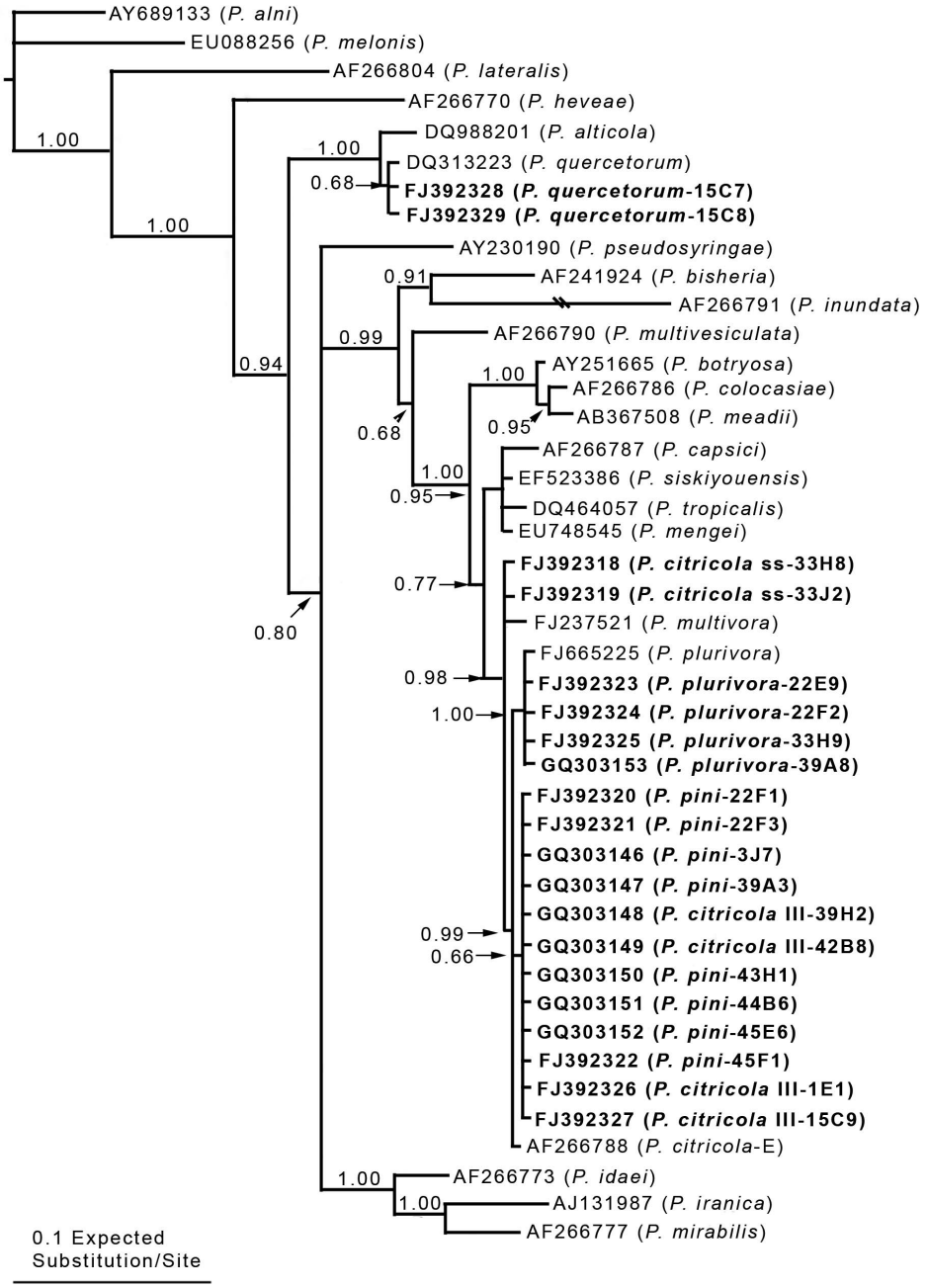


FIG. 1. Bayesian inference tree with rDNA ITS sequences showing the phylogenetic position of *Phytophthora pini* in relation to other species of *Phytophthora*. Numbers above branches represent posterior probability based on Bayesian analysis of the dataset. Sequences generated in this study are in boldface.

and attached at the base near the oogonial stalk. Occasionally two antheridia per oogonium may be seen. Antheridia are 11.1 µm tangential to the oogonial wall (range 10.2–12.9 µm) and 12.6 µm perpendicular to it (10.2–15.0 µm). The noncaducous semipapillate sporangia are mostly ovoid. However ellipsoid, bluntly ellipsoid and bizarre shapes occur in 10% soil extract. Sporangia averages 47.4 × 31.5 µm (l/b ratio is 1.52 and the range is 31.5–75.3 × 21.7–49.5 µm). Major character differences were observed among the four isolates assessed (SUPPLEMENTAL TABLE III).

Dimensions of sexual structures produced on hempseed agar average 30.1 µm for oogonia, 26.5 µm for oospores and 1.7 µm for oospore wall. These measurements are comparable to those on clarified V8 agar. Sporangia produced on lima bean agar are 56.8 µm long and 36.6 µm wide, larger than

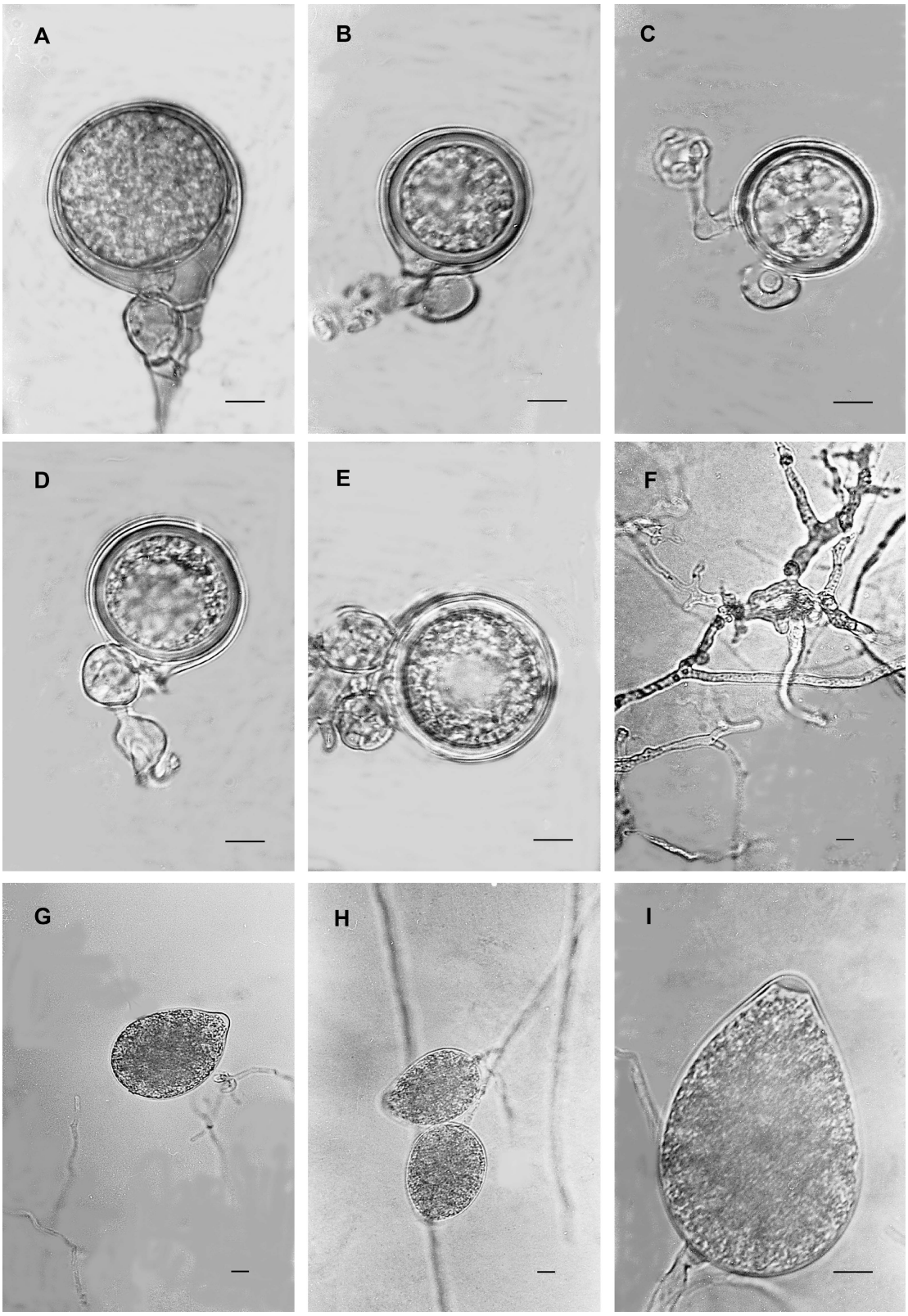


FIG. 2. *Phytophthora pini*. A. A young sexual stage with a diclinous capitate antheridium and an immature oospore. B, C, D. Sexual stages with mature oospores and the relatively large capitate or asymmetrically capitate antheridia. E. A sexual body with two antheridia. F. Irregular hyphae. G, I. Ovoid sporangia. H. A simple sympodium with two sporangia.

those on clarified V8 agar. First-flush sporangia are larger and tend toward being ellipsoid, whereas sporangia produced later are smaller and ovoid. Simple sympodia occur, sometimes close but mostly loose. Chlamydospores have not been seen, but a few small hyphal swellings and irregular hyphae sometimes occur in lima bean agar (FIG. 2).

Holotype = VTMH11737 (ATCC 664532, CBS 181.25, IMI 77970)

## DISCUSSION

The results of research (Reeser et al. 2007, Balci et al. 2008, Hong et al. 2009a, Jung and Burgess 2009, Scott et al. 2009) resolved many questions with regard to *P. citricola* s.l. but left pending issues associated with the ex-type cultures of *P. citricola* s.s., *P. pini* and *P. cactorum* var. *applanata*. This study demonstrates that *P. pini* is a distinct species and by PCR-SSCP fingerprint *P. cactorum* var. *applanata* belongs to *P. plurivora* (TABLE IV).

*Phytophthora pini* is resurrected to distinct species status. Phylogenetically *P. pini* is in a terminal cluster different from *P. citricola* s.s. and all other species recently segregated from this species complex. *P. citricola* s.s. and *P. pini* are similar morphologically. They both have globose oogonia about 30 µm diam and plerotic oospores with walls about 1.5–1.7 µm thick. Their antheridia however are different, particularly in size and shape. Those of *P. pini* are larger, 11.1 µm tangential to the oogonial wall and 12.6 µm perpendicular to the wall, whereas those of *P. citricola* s.s. are round and 10 µm or less diam. Also the antheridia of *P. pini* are capitate, usually slightly asymmetrical. In addition *P. pini* formed a few small hyphal swellings and occasional clumps of irregular hyphae, whereas *P. citricola* s.s. did not. Physiologically *P. pini* grew more quickly than *P. citricola* s.s.

*Phytophthora citricola* I (Kong et al. 2003, Gallegly and Hong 2008) is formally replaced with *P. pini* because they are phylogenetically, morphologically and physiologically identical. The holotype was deposited by Leonian, and the name *P. pini* should take precedence over other names, although a Latin description was not given and was not required before 1935. Our emended description includes a Latin description of the ex-holotype of ATCC 64532 and CBS 181.25. This isolate is listed under subgroup CIT1 (2) (Oudemans et al. 1994). It must be pointed out that isolates CIT-US1 and CIT-US10 are more closely related to *P. citricola* III than *P. pini* (= *P. citricola* I) in the *cox1* tree, although both were placed in the same group by the ITS sequences (Jung and Burgess 2009). Several differences in temperature response was observed between *P. pini* in the present study and both isolates (CIT-US1 and CIT-US10) (Jung and Burgess 2009): (i) the optimum temperature of 25 C vs. 30 C and (ii) average daily radial growth rates of 7.0 vs. 9.2 mm. These differences along with others (Hong et al. unpubl data) support *P. citricola* III as a separate taxa (Gallegly and Hong 2008).

*Phytophthora pini* is readily established in North America and Europe as a pathogen on plants in seven genera. This species also could attack a variety of other ornamental and vegetable plants (Hong et al. 2008) and European beech trees (Jung et al. 2005). It is likely that many isolates identified as *P. citricola* s.l. in major culture collections in reality are *P. pini* or *P. plurivora*. Similarly many plant species currently listed as hosts of *P. citricola* s.l. (Farr et al. 2009) might be hosts of *P. pini* instead. Much work will be needed to elucidate the host ranges of *P. pini* and other emerging entities from *P. citricola* s.l.

*Phytophthora pini* poses a growing threat to the horticulture industry for several reasons. *P. pini* favors alkaline aquatic environments with the optimum pH of 9 (Kong et al. 2009). This occurs in most agricultural runoff containment basins/irrigation reservoirs for most of the growing season (Hong et al. 2009b). This species consequently has been recovered frequently from irrigation reservoirs and natural waterways in Virginia; some isolates of which were identified as *P. citricola* s.l. or *P. citricola* I (Bush et al. 2003, Bush et al. 2006, Ghimire et al. 2009). Considering global water scarcity, the horticulture industry increasingly depends on recycled water for irrigation (Hong and Moorman 2005). Accordingly the risk of this species accumulating and being redistributed through recycling irrigation systems is expected to rise. In addition *P. pini* was the only species among the 18 isolates from 12 species tested that can establish itself in media without soil (Hong et al. 2008). Thus it is important to monitor this species closely and take it into consideration in crop health management planning. To this end this study will help put vital research on the right track (Grovers 2001) and improve the accuracy of plant disease diagnostic services.

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