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Phytophthora aquimorbida sp. nov. and *Phytophthora* taxon 'aquatilis' recovered from irrigation reservoirs and a stream in Virginia, USA

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Abstract: Two distinct subgroups (L2 and A^{-2}) were recovered from irrigation reservoirs and a stream in Virginia, USA. After molecular, morphological and physiological examinations, the L2 subgroup was named *Phytophthora aquimorbida* and the A^{-2} designated as *Phytophthora* taxon 'aquatilis'. Both taxa are homothallic. *P. aquimorbida* is characterized by its noncaducous and nonpapillate sporangia, catenulate and radiating hyphal swellings and thick-walled plerotic oospores formed in globose oogonia mostly in the absence of an antheridium. *P.* taxon 'aquatilis' produces plerotic oospores in globose oogonia mostly with a paragynous antheridium. It has semi-papillate, caducous sporangia with variable pedicels, but it does not have hyphal swelling. Analyses of ITS, CO1, β-tubulin and NADH1 sequences revealed that *P. aquimorbida* is closely related to *P. hydropathica*, *P. irrigata* and *P. parsiana*, and *P.* taxon 'aquatilis' is related to *P. multivesiculata*. The optimum temperature for culture growth is 30 and 20 C for *P. aquimorbida* and *P.* taxon 'aquatilis' respectively. Both taxa were pathogenic to rhododendron plants and caused root discoloration, pale leaves, wilting, tip necrosis and dieback. Their plant biosecurity risk also is discussed.

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Key words: aquatic environment, biosecurity, *Phytophthora aquimorbida*, *P.* taxon 'aquatilis'

Introduction

The genus *Phytophthora* was added to the list of water molds by Blackwell (1944), but only a few of its members originally were isolated from aquatic environments. Notably the type culture of *P. gonapodyides* was isolated from a decaying apple in a reservoir (Petersen 1910) and that of *P. siskiyouensis* from streams (Reeser et al. 2007, Reeser et al. 2011). Similarly *P. irrigata* and *P. hydropathica* first were isolated from nursery irrigation reservoirs (Hong et al. 2008a, b). *P. irrigata* also has been recovered from streams (Hong et al. 2008a), while *P. hydropathica* was recovered from diseased rhododendron and kalmia plants (Hong et al. 2008b). Likewise, *P. siskiyouensis* has been recovered from soil and diseased myrtle wood and tanoak (Reeser et al. 2007).

During investigations into pathogen ecology in irrigation reservoirs and a statewide survey of natural waterways for *P. ramorum* (Werres et al. 2001, Rizzo et al. 2002), a number of isolates with DNA fingerprints distinct from those of existing species (Kong et al. 2003, 2004; Gallegly and Hong 2008) were recovered. This study examines two of these new subgroups designated A^{-2} and L2. Specifically, four DNA regions were sequenced for each of four representative isolates of the L2 subgroup and the only isolate of the A^{-2}. Additional cultures also were examined with respect to their physiological, pathological and morphological characteristics. Accordingly, the L2 subgroup is formally described as *Phytophthora aquimorbida* and the A^{-2} is designated *Phytophthora* taxon 'aquatilis'.

Materials and methods

Isolation and isolate storage.—Seven L2 isolates and one A^{-2} isolate (Table I) were examined. L2 isolates were representatives of cultures recovered from three runoff containment basins at three ornamental nurseries in eastern and central Virginia Jun–Sep. These isolates were recovered by baiting with rhododendron leaves (Ghimire et al. 2011). The only A^{-2} isolate also was recovered from rhododendron leaf bait, which was deployed and retrieved from Fishpond Creek in central Virginia by the Virginia Department of Forestry. Single zoospore isolates were obtained by spreading a 100 μL suspension containing about 20 zoospores on water agar in

TABLE I. Isolate origin, ITS sequence and type culture deposits of *Phytophthora aquimorbida* and *P.* taxon 'aquatilis'

Species	Isolate	Substrate	Origin	Year	ITS sequence[a]	Culture deposit[b]
P. aquimorbida	40A6	Irrigation water	Virginia (A-6)[c]	2006	FJ666127	MYA-4578
	40C9	Irrigation water	Virginia (B-7)	2006		
	40E3	Irrigation water	Virginia (C-8)	2006	FJ666128	
	40F9	Irrigation water	Virginia (C-9)	2006	FJ666129	
	40G6	Irrigation water	Virginia (A-9)	2006		
	44G9	Irrigation water	Virginia (C-6)	2007	FJ666130	
	44H1	Irrigation water	Virginia (C-6)	2007		
P. taxon 'aquatilis'	38J5	Stream water	Virginia	2006	FJ666126	MYA-4577
P. bisheria	31E6[d]	*Fragaria* × *ananassa*	North Carolina	1999		
P. frigida	47G8[d]	*Eucalyptus smithii*	South Africa	2001		
P. hydropathica	5D1	Irrigation water	Virginia	2000		
P. irrigata	23J7	Irrigation water	Virginia	2000		
P. multivesiculata	29E3[§]	*Cymbidium* sp.	the Netherlands	1995		

[a] ITS = rDNA internal transcribed spacer regions (Cooke et al. 2000).
[b] Deposited at the American Type Culture Collection in Manassas, Virginia, USA.
[c] In parenthesis are nursery (A, B, C) and month codes (6 = June 7 = Jul, 8 = Aug, 9 = Sep).
[d] All ex-type cultures: 31E6 received from G. Abad (Cg2.3.3), 47G8 from M. Wingfield (CMW20311), and 29E3 from W. Man in't Veld (95/8679).

a 10 cm Petri dish. Colonies from single zoospores were subcultured on PARP-V8 agar (Ferguson and Jeffers 1999) with minor modifications (Hong et al. 2002). Clean, monosporic isolates were grown in clarified V8 agar (CV8A) (Erwin and Ribeiro 1996) and blocks of fresh agar cultures were transferred into microtubes with sterile distilled water and kept at 15 C for long-term storage. The dry cultures were deposited at the Massey Herbarium of Virginia Polytechnic Institute and State University, Blacksburg, Virginia, and ex-types deposited at the American Type Culture Collection in Manassas, Virginia. One isolate each of *P. bisheria*, *P. frigida*, *P. hydropathica*, *P. irrigata* and *P. multivesiculata* also were included in temperature assays (TABLE I).

DNA extraction.—Isolates were grown in V8 broth (Erwin and Ribeiro 1996) at room temperature 10 d. Culture DNA was extracted with the DNeasy® Plant Mini Kit (QIAGEN, Valencia, California) following manufacturer's instructions.

Sequence analysis.—The ITS region of all isolates was amplified with forward primer ITS6 (Cooke et al. 2000) and reverse primer ITS4 (White et al. 1990). Genes encoding β-tubulin (β-TUB), cytochrome c oxidase (CO1) and NADH dehydrogenase subunit I (NADH1) also were amplified for one isolate of each subgroup as described by Kroon et al. (2004). Amplification products were sequenced in both directions and at least twice (Hong et al. 2008a).

The sequences of both directions from repeated runs were compared with Clustal W multiple sequence alignment at http://workbench.sdsc.edu/. The consensus sequences of the four L2 isolates then were aligned to determine intraspecific variations. Basic local alignment query tool (BLASTn) at http://ncbi.nlm.nih.gov was used to identify the closest sequences of each subgroup. Subsequently phylogenetic analyses (Ronquist and Huelsenbeck 2003) were performed in TOPALi 2.5 (Milne et al. 2009), resulting in Bayesian inference trees that include these two new subgroups, close relatives and representatives of other clades within the genus *Phytophthora*.

Physiology.—Eight isolates of the two subgroups and five of their close relatives were assessed for optimum growth temperature requirements. Agar blocks (4 mm diam) were taken from actively growing areas of 5–6 d old cultures and transferred to 10 cm Petri dishes with CV8A, one agar block placed with mycelium facing down in the center of each dish. These dishes were incubated at 5–40 C in the dark in triplicate. Two perpendicular measurements of colony diameter were taken per dish at day 6 for the A^{-2} isolate and its close relatives, or day 7 for the L2 isolates and related species. Daily radial growth rate was calculated for each isolate but for simplicity only the data from four of the seven L2 isolates are presented.

Pathogenicity test.—Test plants *Rhododendron catawbiense* cv. 'Boursault' were tissue culture-propagated liners with a 6 × 6 cm root ball. Half of the plants were wounded at the roots by cutting four corners of the root ball; both wounded and nonwounded plants then were potted in Metro mix 360 infested with vermiculite cultures at 5 : 1 by volume (Roiger and Jeffers 1991). The same soilless medium mixed with noninfested vermiculite at the same ratio was used as controls. All potted plants, three replicates per treatment, were grown under shade cloth in a greenhouse and watered as needed. Foliar symptom development was evaluated 0–5 (0 = no symptom, 1 = pale, 2 = leaf curl or wilting, 3 = tip necrosis, 4 = total wilt, 5 = dieback). Five root portions randomly taken from each root ball were washed with tap water then plated into 10 cm a Petri dish with selective medium to determine pathogen colonization; soilless medium infestation also was assessed on the day of inoculation and at the termination of each test as described by Hong et al. (2008c).

Isolates of the A^{-2} and L2 subgroups were assessed in two separate tests. Plants used for testing pathogenicity of the

A^{-2} isolate were potted 22 Jan 2009 with the first and last disease ratings 27 and 89 d post inoculation (DPI). Three additional ratings were performed 35, 55 and 67 DPI. Root infection and soilless medium infestation were determined immediately after the last disease rating. For test of the L2 isolates, liners were potted 31 Mar 2009 with the first rating of foliar symptom 30 DPI. The second and last ratings were done 45 and 77 DPI, followed by root isolation and soilless medium plating. Only the last disease rating and root infection data were compared between isolates and control, using Statistical Analysis System 9.2 (SAS Institute, Cary, North Carolina). Similarly recovery of individual isolates from soilless medium also was compared between the beginning and termination of tests: 0 versus 89 DPI for the A^{-2} isolate or 0 versus 77 DPI for the L2 isolates.

Morphology.—Selected isolates (TABLE I) were examined as described by Waterhouse (1963), Erwin and Ribeiro (1996) and Gallegly and Hong (2008). Briefly, monosporic cultures were grown on CV8A and carrot agar at room temperature (ca. 23 C) 2–4 wk. Subcultures were examined with an Olympus IX71 inverted microscope (Olympus, Center Valley, Pennsylvania). Agar blocks of individual isolates were submerged in sterile soil-water extract (Erwin and Ribeiro 1996) and incubated overnight at room temperature to induce sporangium production. Oospores, zoospores, sporangia and other structures were photographed and measured withImage-Pro Plus 5.1 (Media Cybernetics Inc., Bethesda, Maryland). A minimum of 20 measurements were taken per isolate and type of structure. Wall index (Dick 1990) was calculated for oospores by isolate.

Observations on a given isolate first were summarized to qualify descriptions of major morphological characters. Analysis of variance was performed to determine the differences in the diameters of oogonia and oospores as well as oospore wall index within and among the L2 isolates. Similar analyses were performed for the length and width as well as the length to width ratio of sporangia.

RESULTS

Phylogenetic analysis.—All four isolates of the L2 subgroup, designated *P. aquimorbida* sp. nov. below, had identical 786 bp ITS sequences, while the A^{-2} isolate we designated *P.* taxon 'aquatilis' below produced an 804 bp sequence (TABLE I). Sequence alignments of the ITS regions against the collection in the NCBI database revealed that both *P. aquimorbida* and *P.* taxon 'aquatilis' are phylogenetically distinct from all described species. The closest relatives of *P. aquimorbida* are *P. irrigata* (Hong et al. 2008a), *P. hydropathica* (Hong et al. 2010) and *P. parsiana* (Mostowfizadeh-Ghalamfarsa et al. 2008). The four isolates of *P. aquimorbida* and the three close relatives of this species occur in two well supported terminal clusters in ITS Clade 9 (FIG. 1). The closest relative of *P.* taxon 'aquatilis', *P. multivesiculata* (Ilieva et al. 1998), occurs in a sister relationship to *P.* taxon 'aquatilis' within ITS Clade 2 (FIG. 1).

The 5.8S sequences of *P. aquimorbida* and *P. irrigata* were identical, but ITS1 and ITS2 sequences differed for the two species. Overall, the ITS sequence of *P. aquimorbida* was 37 bp longer than that of *P. irrigata*. There were a total of 39 indels and 60 point mutations between these two species (TABLE II). More indels occurred in the ITS1 region than in ITS2 (21 vs. 18), but there were fewer points of mutation in the ITS1 than ITS2 (12 vs. 48). Although the ITS sequence of *P.* taxon 'aquatilis' was only 1 bp longer than that of *P. multivesiculata*, the sequences differed by 13 point mutations and one indel (TABLE II). The majority of the mutations occurred in the ITS2, and the only indel occurred in the ITS1 region. Both species also had identical 5.8S sequences.

DNA sequences encoding β-TUB, CO1 and NADH1 of *P.* taxon 'aquatilis' and *P. aquimorbida* were submitted to GeneBank under accession numbers GQ294533, GQ294534, GQ294535, GQ294536, GQ294537 and GQ294538 respectively. Bayesian analyses of these sequences supported the distinct phylogenetic positions of *P. aquimorbida* and *P.* taxon 'aquatilis' within the genus *Phytophthora* as illustrated with β-TUB (FIG. 2) and CO1 sequences (FIG. 3). Similar results were obtained from analyses of NADH1 sequences (data not shown). These results are in agreement with the phylogenetic analyses of the ITS sequences (FIG. 1).

Temperature-growth relations.—The four isolates of *P. aquimorbida* grew well 25–35 C with the minimal, optimal and maximal around 5, 30 and 40 C respectively (FIG. 4). An identical growth pattern and cardinal temperatures were observed in isolates 40G6, 44G9 and 44H1 of the same species (data not shown). This growth pattern and cardinal temperatures were similar to those of *P. hydropathica* (5D1) and *P. irrigata* (23J7) (FIG. 4). *P.* taxon 'aquatilis' had a relatively narrow range of growth temperature with the optimum at 20 C (FIG. 5). This optimal temperature is the same as that of *P. multivesiculata*, but 5 and 10 C lower than those of *P. bisheria* and *P. frigida* respectively. This taxon grew at 5 C but did not grow at 35 C (FIG. 5).

Pathogenicity.—Foliar symptom or root infection of rhododendron plants were not observed in any controls at the termination of tests (TABLE III). Pale (off-color) leaves and total wilt symptoms first were observed on plants with wounded roots grown in soilless medium infested with *P.* taxon 'aquatilis' 27 and 55 d post inoculation respectively. Similar symptoms were observed ca. 30 d later on plants with unwounded roots, and severe foliar blight was observed 89 d post inoculation when the test was terminated ($P \leq 0.0451$). This taxon was re-isolated from 73.3% of wounded ($P = 0.0004$) and 53.3% of

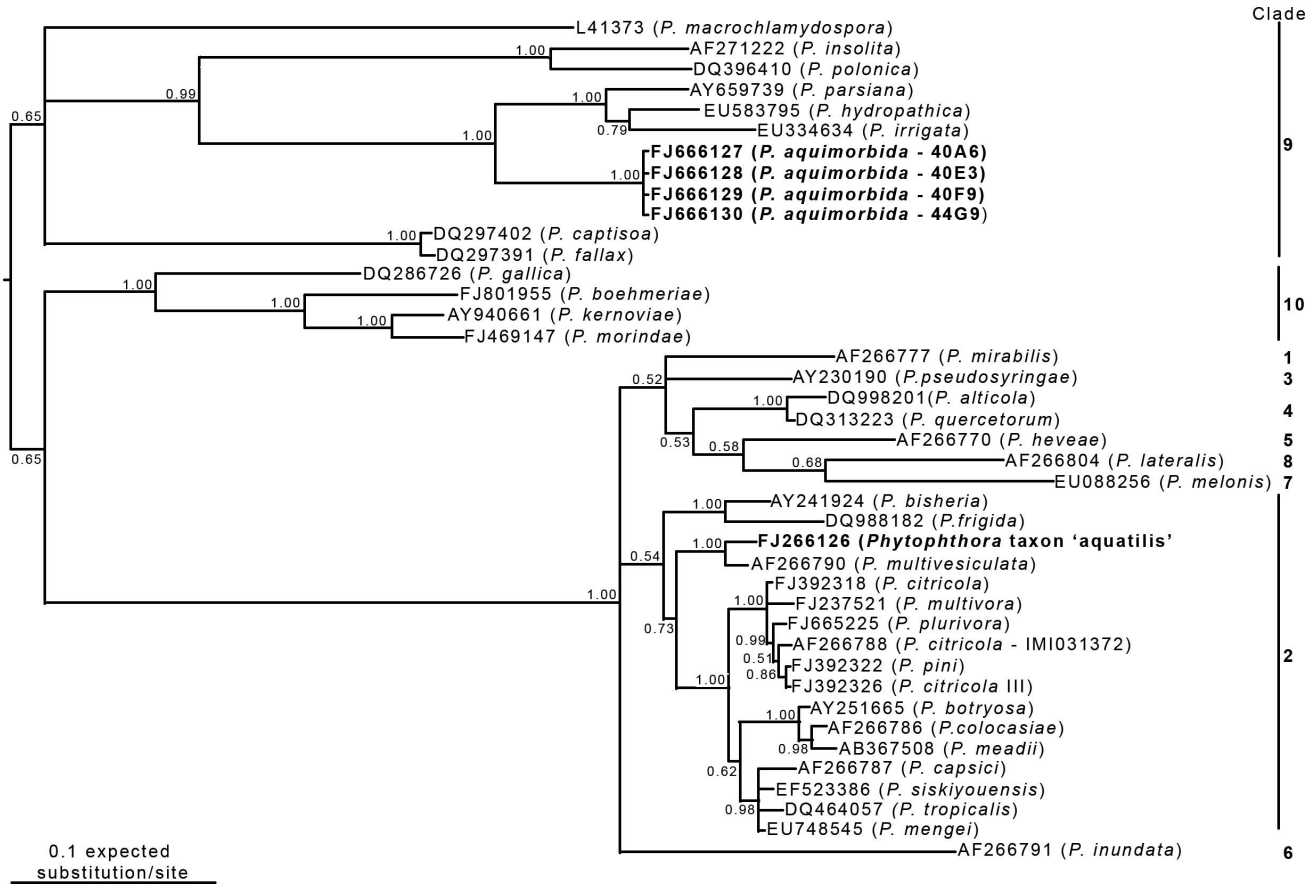

FIG. 1. Bayesian inference tree using rDNA ITS sequences showing the phylogenetic position of *Phytophthora aquimorbida* and *P.* taxon 'aquatilis' in relation to closely related species and representatives of other clades within the genus *Phytophthora*. Numbers above branches represent posterior probability based on MrBayes 3 analysis of the dataset. Sequences generated in this study are in boldface.

nonwounded root portions ($P = 0.0390$). Infestation of soilless medium as measured by the colony-forming unit (cfu) of this pathogen declined by more than 50% during an 89 d test period ($P < 0.0001$, TABLE IV).

Similar foliar symptoms developed on plants growing in medium infested with *P. aquimorbida* (TABLE III). Four of the six test plants with wounded roots were dead within 45 d, whereas the uncut plants only had chlorosis ($P = 0.0520$). There was no difference in disease rating between plants with unwounded roots grown in infested medium with *P. aquimorbida* and those grown in control medium ($P = 0.6297$). *P. aquimorbida* was re-isolated from 100% of the root portions no matter whether their roots were wounded ($P < 0.0001$). However, this pathogen did not survive well in the soilless medium with the initial per-gram cfu counts of more than 2765 and final counts of less than 55 within 77 d of inoculation (TABLE IV).

TABLE II. Comparative analyses of rDNA internal transcribed spacers (ITS) sequences of *Phytophthora aquimorbida* and *P.* taxon 'aquatilis' with respective closest relatives

	P. aquimorbida vs. *P. irrigata*			*P.* taxon 'aquatilis' vs. *P. multivesiculata*		
Region	Indels	Points of mutation	Σ	Indels	Points of mutation	Σ
ITS1	21	12	33	1	4	5
5.8S	0	0	0	0	0	0
ITS2	18	48	66	0	9	9
Σ	39	60	99	1	13	14

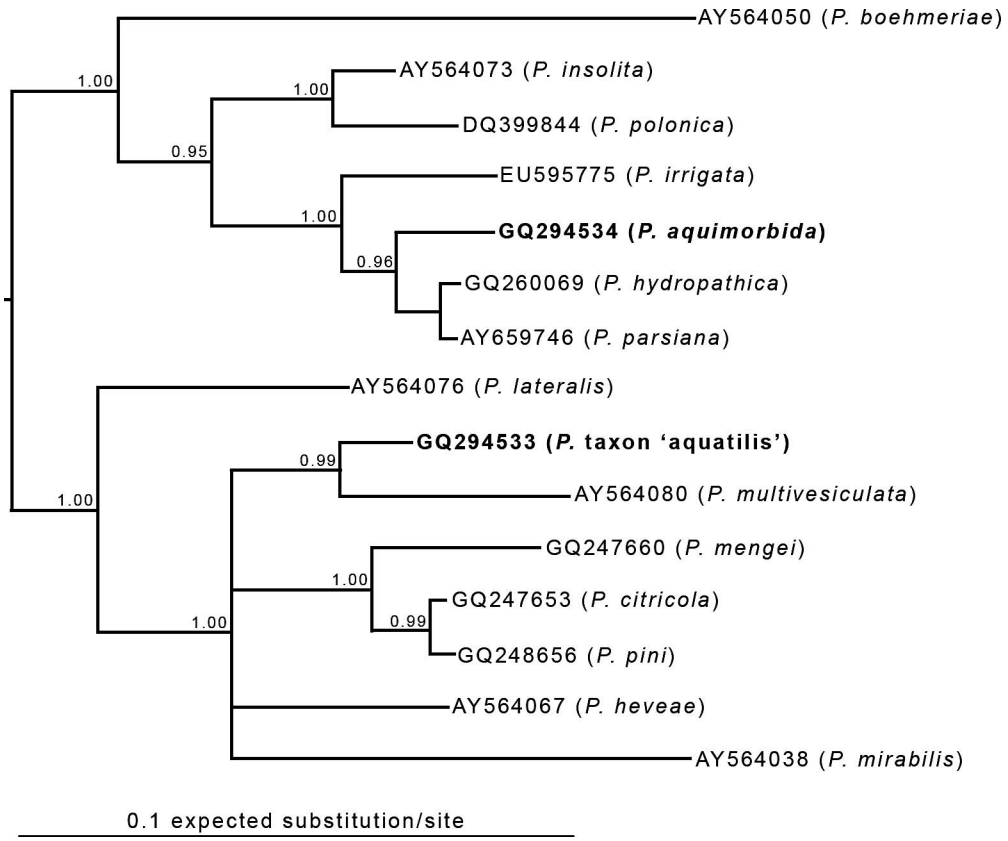

FIG. 2. Bayesian inference tree derived from partial sequences of β-tubulin gene showing the phylogenetic position of *Phytophthora aquimorbida* and *P.* taxon 'aquatilis' in relation to closely related species and representatives of other clades within the genus *Phytophthora*. Numbers above branches represent posterior probability based on MrBayes 3 analysis of the dataset. Sequences generated in this study are in boldface.

TAXONOMY

Phytophthora aquimorbida C.X. Hong, sp. nov. FIG. 6A–P

MycoBank MB513049

Phytophthora aquimorbida is homothallic, producing terminal, intercalary or lateral oogonia with a plerotic oospore. Oospores average 29.1–36.4 μm diam. Antheridia are seldom present and if so are amphigynous. Sporangia are noncaducous, nonpapillate and predominantly ovoid to ellipsoidal with an average length to width ratio of 1.3 : 1.4. Most sporangia grow on unbranched sporangiophores. Some have nested or extended proliferation. Catenulate and radiating hyphal swellings occur in some isolates.

Holotype: VTMH11739, dried culture of isolate from a nursery irrigation reservoir, central Virginia, USA, 2006. Ex-holotype: 40A6 (MYA-4578) [Virginia Tech Massey Herbarium, Blacksburg, Virginia, USA].

Morphology.—Monosporic cultures of *P. aquimorbida* produced abundant sexual structures and some hyphal swellings in both CV8 and carrot agar, but sporangia were produced only in sterile soil water extract (TABLE V). Some oogonia had a tapered base (FIG. 6A). Many oogonia were intercalary and lateral (FIG. 6B–E). Oogonia from all isolates were globose with a smooth surface (FIG. 6A–F) and varied in size among the isolates ($P < 0.0001$). Oogonia of isolate 40A6 were 23.9–40.1(33.2) μm diam; those of 44G9 were 30.8–48.5(40.7) μm. Plerotic oospores were formed in oogonia mostly in the absence of an antheridium (FIG. 6A–E). Average size of oospores was 29.1–36.4 μm. Oospore wall indexes varied among isolates, 45–66%, with average of 52%. Antheridia, seldom observed, were amphigynous (FIG. 6F, G). Sporangia were nonpapillate (FIG. 6H) but appeared ''semi-papillate'' at maturity (right before releasing zoospores) (FIG. 6I, J) and noncaducous. Sporangia were mostly ovoid (FIG. 6I–O), and their sizes differed by isolate. Sporangia of isolate 40E3 were 27.6–58.8(41.0) μm long and 21.3–44.8(32.7) μm wide, while 44G9 sporangia were 39.0–68.0(54.2) μm long and 26.4–47.5(37.9) μm wide. The average length to width ratio was 1.3–1.4. Most sporangia grew on unbranched sporangiophores. Nested and extended proliferation (FIG. 6L–O) also occurred. Hyphal swellings were catenulate and radiating (FIG. 6P), common in 40A6 and 40E3 but less so in 40F9 and 44G9.

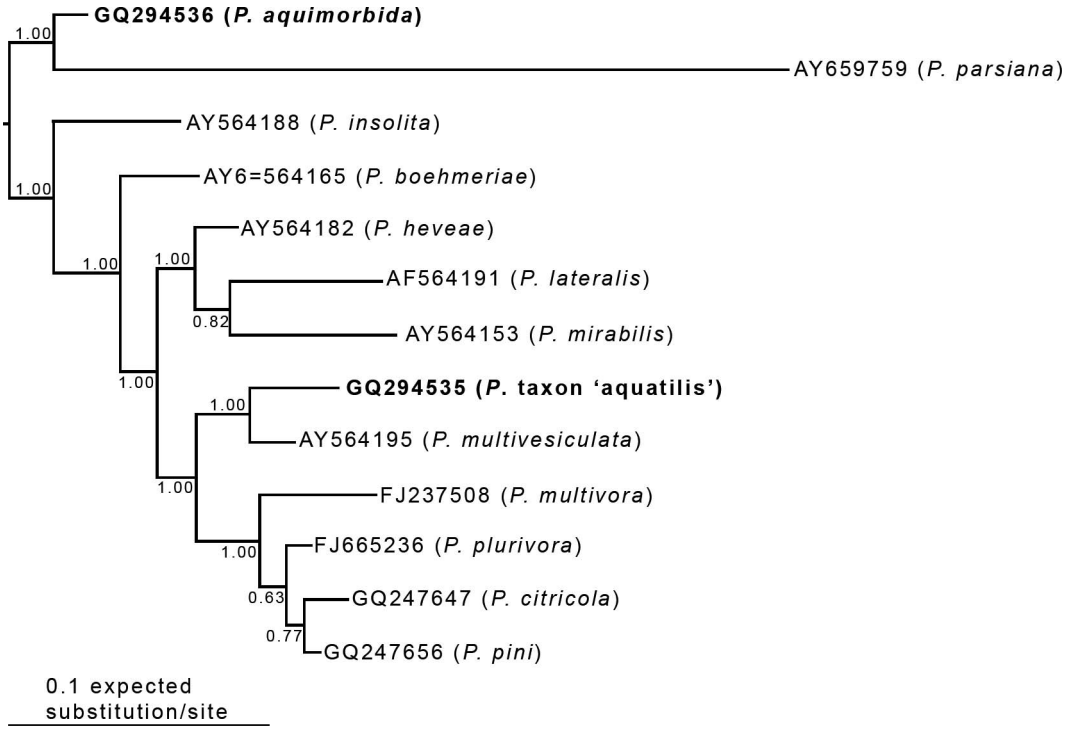

FIG. 3. Bayesian inference tree derived from partial sequences encoding cytochrome c oxidase subunit 1 showing the phylogenetic position of *Phytophthora aquimorbida* and *P.* taxon 'aquatilis' in relation to closely related species and representatives of other clades within the genus *Phytophthora*. Numbers above branches represent posterior probability based on MrBayes 3 analysis of the dataset. Sequences generated in this study are in boldface.

Etymology: ''aqui'' refers to the aquatic habitat from which it was isolated and ''morbida'' refers to its potential to cause diseases.

Habitat: Agricultural irrigation reservoirs, Virginia, USA.

Phytophthora taxon 'aquatilis'*:* The monosporic culture of *P.* taxon 'aquatilis' produced abundant sexual structures on carrot agar and sporangia on CV8A. Oogonia were globose (FIG. 7A–K). Their diameters were 27.4–42.5(38.2) µm. Most antheridia

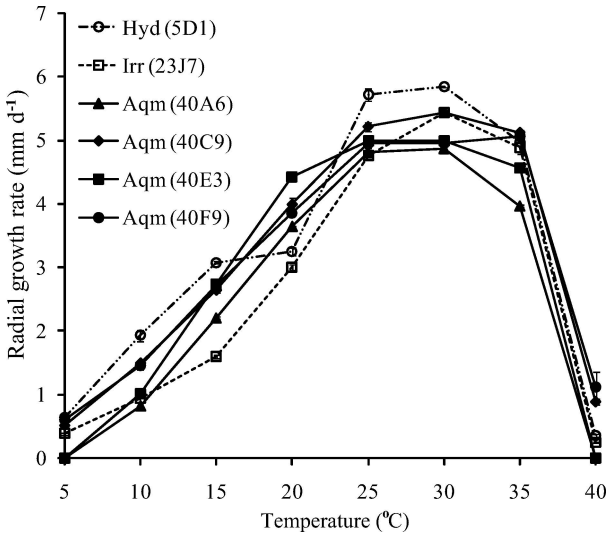

FIG. 4. Radial growth of *Phytophthora aquimorbida* (Aqm, four isolates), *P. hydropathica* (Hyd) and *P. irrigata* (Irr) on clarified V8 agar at different temperatures over a 7 d period.

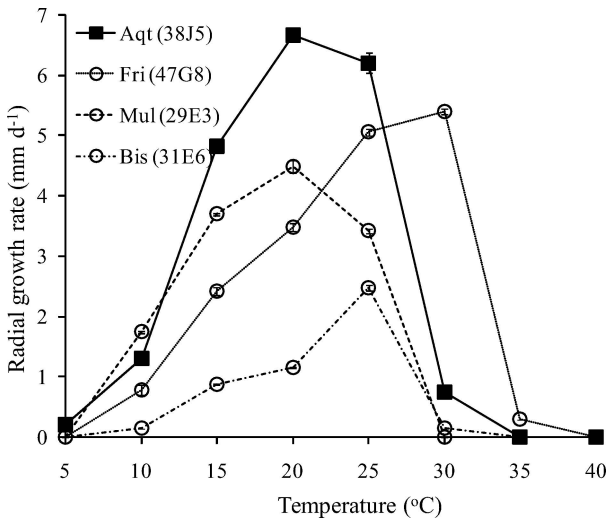

FIG. 5. *Phytophthora* taxon 'aquatilis' (Aqt), *P. bisheria* (Bis), *P. frigida* (Fri) and *P. multivesiculata* (Mul) on clarified V8 agar at different temperatures over a 6 d period.

TABLE III. Infection of *Rhododendron catawbiense* cv. 'Boursault' by *Phytophthora aquimorbida* and *P.* taxon 'aquatilis' in greenhouse tests by potting tissue culture-propagated liners in soilless medium infested with vermiculite culture of individual isolates

	Foliar disease rating (0–5)[b]		% root pieces infected	
Test[a]	NC	CR	NC	CR
P. aquimorbida				
40A6	0.3 a[c]	3.3 a	100 a	100 a
44G9	0.3 a	3.7 a	100 a	100 a
None	0 a	0 b	0 b	0 b
P	0.6297	0.0452	< 0.0001	< 0.0001
P. taxon 'aquatilis'				
38J5	3.7 a	5.0 a	53.3 a	73.3 a
None	0 b	0 b	0 b	0 b
P	0.0451	< 0.0001	0.0390	0.0004

[a] Test plants were potted 31 Mar 2009 and disease assessment and root isolation performed 17 Jun 2009 for *P. aquimorbida*; potting and disease assessment dates for *P.* taxon 'aquatilis' were 22 Jan and 21 Apr 2009 respectively.

[b] CR = test plants were wounded by cutting four corners of the root ball and NC = no cutting.

[c] Numbers followed with a different letter within each column differed according to the least square difference test at $P = 0.05$.

were paragynous (FIG. 7A–G, I) with variable attachments to oogonium. Amphigynous attachments occurred infrequently (approximately 6%) (FIG. 7H, J, K). Oospores were plerotic, 23.6–38.5(34.1) μm (FIG. 7A–K). Oospore wall index was 18–60%, averaging 33%. Sporangia were semi-papillate and caducous with variable pedicels 0–75.4 μm (FIG. 7O–Q). Most sporangia were ovoid (FIG. 7L, O, P), 26.2–66.1(45.9) μm long and 19.1–39.6(29.7) μm wide. The sporangial length to width ratio was approximately 1 : 6. Irregular sporangia shapes occasionally occurred (FIG. 7M, N). A few sporangia had more than one papilla (FIG. 7M) or appeared curved or even sickle-shaped (FIG. 7N). Most sporangia grew primarily on unbranched sporangiophores and some were in a simple sympodium. Lateral attachment to sporangiophore occurred (FIG. 7Q–T) and direct germination of sporangia was common (FIG. 7T)

DISCUSSION

This study demonstrated that both L2 and A^{-2} subgroups are phylogenetically and morphologically distinct from all known species of *Phytophthora* and from each other. Consequently, the L2 subgroup is formally named *P. aquimorbida*. A^{-2} is designated *P.* taxon 'aquatilis' without a formal name at present because only one strain has been isolated to date. The present study expanded the baseline information on *Phytophthora* biodversity to identify potential emerging pathogens. The descriptions of these two taxa along with recent reports of multiple new species (Rea et al. 2010, Jung et al. 2011) will facilitate identification of *Phytophthora* species from aquatic environments as well as terrestrial habitats. They also will help assess the overall plant biosecurity risk posed by *Phytophthora* species.

TABLE IV. Survival of *Phytophthora aquimorbida* and *P.* taxon 'aquatilis' in soilless medium Metro Mix 360 as measured by colony-forming units (cfu) at the beginning and termination of tests

	P. aquimorbida		*P.* taxon 'aquatilis'
CFU count[a, b]	40A6	44G9	(38J5)
Initial count	2765 a[c]	5315 a	950 a
Final count - CR	15 b	0 b	435 b
Final count - NC	55 b	5 b	390 b
P	< 0.0001	< 0.0001	< 0.0001

[a] Initial and final cfu counts of *P. aquimorbida* were determined 31 Mar and 17 Jun 2009 and those of *P.* taxon 'aquatilis' 22 Jan and 21 Apr 2009 respectively.

[b] CR = test plants were wounded by cutting four corners of the root ball and NC = no cutting.

[c] Numbers followed with a different letter within each column differed according to the least square difference test at $P = 0.05$.

Phytophthora aquimorbida can be distinguished easily from *P. hydropathica*, *P. irrigata* and *P. parsiana* by its homothallism, intercalary and lateral oogonia. It also can be distinguished from closely related homothallic species by morphology and cardinal temperatures. Specifically, both *P. gallica* and *P. macrochlamydospora* are sterile (Erwin and Ribeiro 1996, Jung and Nechwatal 2008). Neither of these species grew at 35 C at which *P. aquimorbida* grew well. Both *P. polonica* and *P. insolita* have optimal and maximal growth temperatures close to those of *P. aquimorbida*, but unlike *P. aquimorbida*, *P. polonica* does not produce sporangia in soil water extract (Belbahri et al. 2006) and *P. insolita* does not produce antheridia (Ann and Ko 1980). *P. aquimorbida* can be separated easily from the rest of the clade by sequence analysis and DNA fingerprint. It has substantial differences in the ITS sequence from its closest relatives. It also has a distinct fingerprint from other species of *Phytophthora* found in water to date (data not shown) and those in terrestrial habitats (Kong et al. 2003, 2004; Gallegly and Hong 2008).

Phytophthora taxon 'aquatilis' was designated based on a single available culture, and formal description of this taxon is deferred until more isolates become

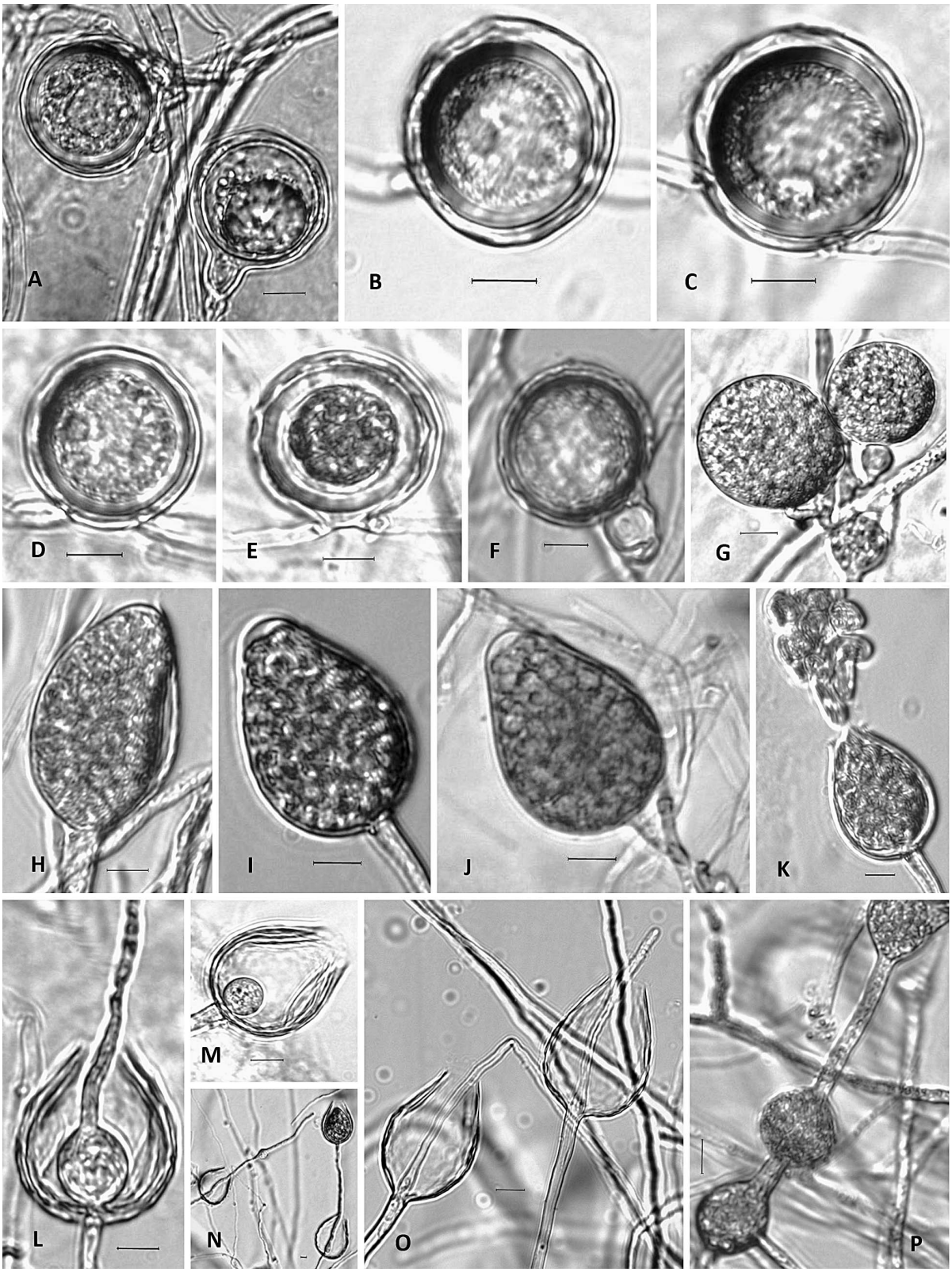

FIG. 6. Morphological characters of *Phytophthora aquimorbida*. A–E. Oogonia each with a thick-walled plerotic oospore in the absence of an antheridium. A. Two oogonia with one having a tapered base. B–E. Intercalary or lateral oogonia. F. An oogonium with an amphigynous antheridium. G. Two immature oogonia. H. An slightly curved nonpapillate sporangium. I, J. Sporangia ready to release zoospores. K. A sporangium releasing zoospores. L, M. Nested proliferation. N, O. Extended internal proliferation. P. Catenulate hyphal swellings. Bars = 10 μm.

TABLE V. Comparison of *Phytophthora aquimorbida* with selected close relatives[a]

	P. aquimorbida (This study)	*P. insolita* (Ann and Ko 1980)	*P. irrigata* (Hong et al. 2008)	*P. polonica* (Belbahri et al. 2006)
Sexual type	Homothallic	Homothallic	Heterothallic	Homothallic
Oogonium	Intercalary or lateral	Terminal	Terminal	Terminal
Shape	Globose	Globose	Globose	Globose
Diameter (μm)	33.2–40.7	29.0–36.0	42.4(30.6–51.2)	41.8
Surface	Smooth	Smooth	Smooth	Smooth
Antheridium	− (mostly)	−	+	+
Form of attachment	amphigynous	−	Amphigynous	Mostly paragynous
Shape	Variable	−	Variable	Variable
Oospore	Plerotic	Plerotic	Plerotic	Plerotic
Diameter (μm)	29.1–36.4	27–31	39.5	38.1
Wall thickness (μm)	3.0–3.7	2	2.8–3.0	2.9
Sporangium	Nonpapillate	Nonpapillate	Nonpapillate	Nonpapillate
Shape	Ovoid to ellipsoidal	Ovoid	Spheroid to obpyriform	Ovoid
Length (μm)	41.0–54.2	39–68	43.5(34.0–51.0)	52.0–67.0
Width (μm)	31.8–37.9	29–39	33.2(27.2–41.5)	32.0–44.0
L/W ratio	1.3–1.4	1.3–1.7	1.3	1.6
Sporangiophore				
Unbranched	+	+	+	+
Simple sympodium	+	+	−	−
Internal proliferation	+	+	+	+
Nesting	+	+	+	+
Chlamydospore				
Lateral	−	−	−	+
Terminal	−	+	−	+
Intercalary	−	−	−	+
Hyphal swellings				
Irregular	+	+	−	+
Catenulate	+	+	−	+
Radiating	+	…[b]	−	−
Cardinal temperature				
Minimum (C)	5–10	< 9	5–10	5
Optimum (C)	30	32	30	30
Maximum (C)	Near 40	38–40	40	38
Maximal growth rate (mm d^{-1})	5.0	8.8	5–6	6.9
Agar medium used[c]	CV8A/CA	V8A	CV8A/HSA	CA

[a] Qualitative observation: + = present and − = absent.
[b] Not available.
[c] CA = carrot agar, HAS = hemp seed agar (for production of sexual structures), V8A = V8 juice agar, CV8A = clarified V8A.

available. However this taxon can be differentiated easily from other *Phytophthora* species in Clade 2 by molecular analyses and morphology. Specifically, *P.* taxon 'aquatilis' can be separated from *P. multivesiculata* by its paragynous antheridia, caducous sporangia and the absence of hyphal swellings. Caducous sporangia also separate this taxon from *P. bisheria* (Abad et al. 2008), *P. citricola*, *P. multivora* (Scott et al. 2009), *P. elongata* and *P.* taxon 'elongata'-like (Rea et al. 2010), *P. pini* (Leonian 1925, Hong et al. 2011), *P. plurivora* (Jung and Burgess 2009) and *P. citricola* III (Gallegly and Hong 2008). In addition, *P.* taxon 'aquatilis' readily can be distinguished from other species producing caducous sporangia. For example, *P. siskyouensis* produces smaller oogonia/oospores and larger sporangia and has a lower maximal growth temperature (Reeser et al. 2007). *P. botryosa*, *P. capsici*, *P. colocasiae*, *P. meadii*, *P. tropicalis* (Gallegly and Hong 2008) and *P. frigida* (Maseko et al. 2007) are heterothallic and they all

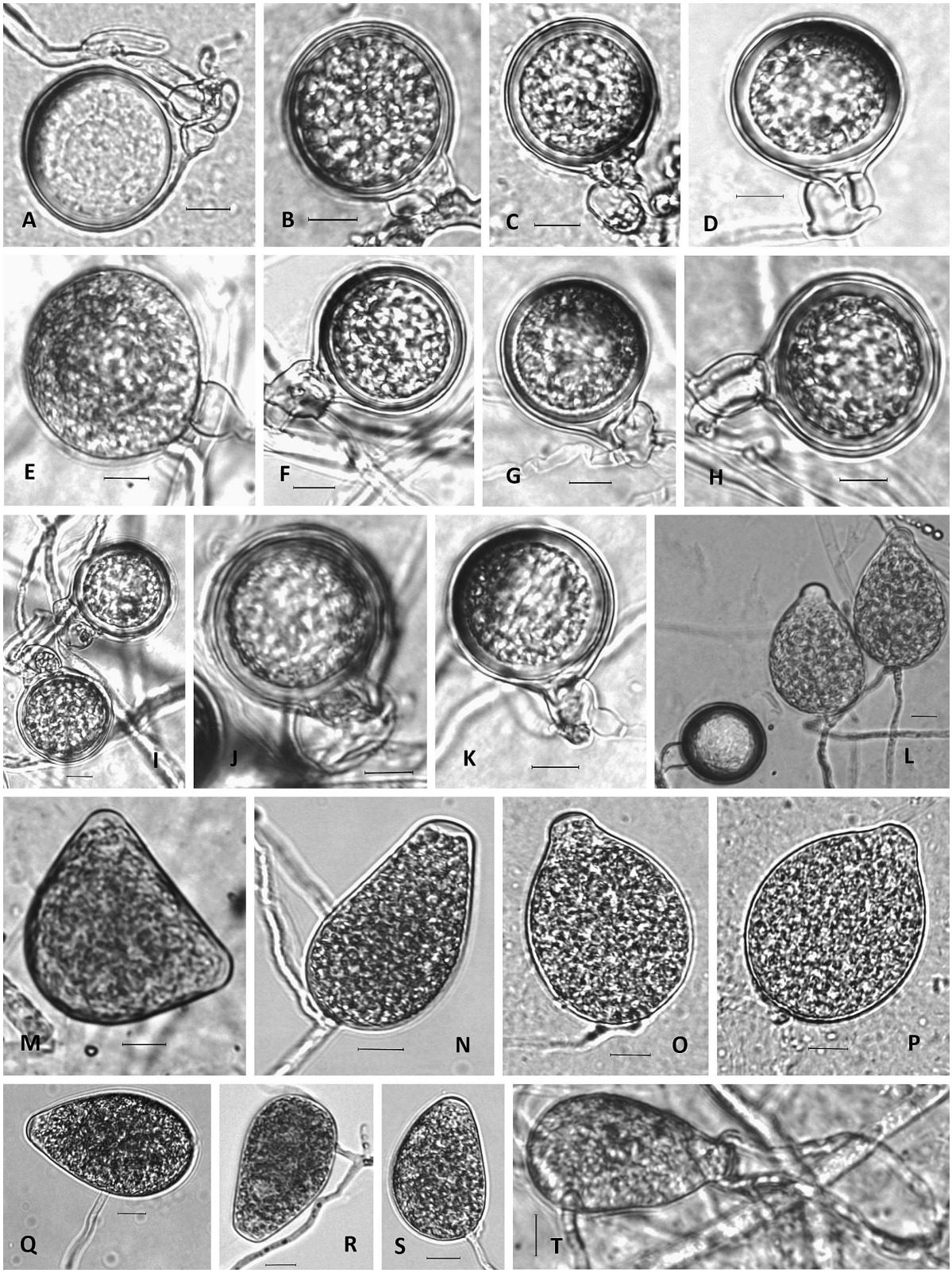

FIG. 7. Morphological characters of *Phytophthora* taxon 'aquatilis. A–G, I. Variable paragynous antheridia and spherical oogonia each with a thin-walled plerotic oospore. H, J, K. Oogonia and amphigynous antheridia. L. Two semi-papillate ovoid sporangia and one oogonium with a paragynous antheridium. M. A sporangium with two papillae. N. A semi-papillate sporangium. O–Q. Caducous sporangia with variable pedicels. R–T. Lateral attachment to sporangiophore. T. Direct germination of a sporangium. Bars = 10 μm.

produce papillate sporangia, with the exception of *P. colocasiae*, which produces semipapillate sporangia.

Phytophthora aquimorbida appears to be widespread, but *P.* taxon 'aquatilis' is known only from Virginia. *P. aquimorbida* commonly was recovered from runoff containment basins at several nurseries in eastern and central Virginia. Because the optimum growth temperature for *P. aquimorbida* is around 30 C and it also grows well at 35 C with some growing at 40 C, it was encountered more frequently during the summer months. *P.* taxon 'aquatilis' was recovered only once from Fishpond Creek in central Virginia during a statewide survey of six streams sampled in Apr, May, Jun, Jul and Sep 2006. It should be noted that the optimum and maximum temperatures we found for *P. frigida* (FIG. 5) were higher than those reported by Maseko et al. (2007). It is unclear what caused this difference between the previous and present studies.

Both *P. aquimorbida* and *P.* taxon 'aquatilis' were pathogenic to Rhododendron, but their potential effects on local horticulture industry and natural ecosystems are not known at this time. Comparatively *P. aquimorbida* caused more severe disease of wounded (100 vs. 73) and nonwounded roots (100 vs. 53) than *P.* taxon 'aquatilis'. The latter, however, resulted in more severe foliar symptoms and survived better in the potting medium than *P. aquimorbida*. To assess their plant biosecurity risk, understanding whether these two taxa are endemic or introduced is essential. It is worth noting that *P. aquimorbida* was not recovered from any reservoirs that did not directly receive irrigation runoff water from production areas on the same properties. It is possible that infestation in the runoff containment basins originated from production areas, although the species has not been isolated from any diseased plants.

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