

A high-temperature tolerant species in clade 9 of the genus *Phytophthora*: *P. hydrogena* sp. nov.

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Abstract: A previously unknown *Phytophthora* species was isolated from irrigation water in Virginia, USA. This novel species produces abundant noncaducous and nonpapillate sporangia in soil water extract solution. It sometimes produces chlamydospores and hyphal swellings in aged cultures and in Petri's solution. This species has optimum vegetative growth at 30 C and grows well at 35 C. The lowest and highest temperatures for growth are 5 and 40 C. All isolates examined in this study are compatibility type A1 and produce mostly plerotic oospores when paired with an A2 mating-type tester of *P. cinnamomi*. Sequence analyses of the rDNA internal transcribed spacer (ITS) regions and the mitochondrially encoded cytochrome *c* oxidase 1 (*cox 1*) gene placed this species in clade 9 of the genus *Phytophthora*. These characteristics support the description of this taxon as a new species for which we propose the name *P. hydrogena* sp. nov. Further phylogenetic and physiological investigations of clade 9 species revealed a high-temperature tolerant cluster including *P. hydrogena*, *P. aquimorbida*, *P. hydrophatica*, *P. irrigata*, *P. chrysanthemi*, *P. insolita*, *P. polonica* and *P. parsiana*. These species all grow well at 35 C. The monophyly of the species in this heat-tolerant cluster except *P. insolita* and *P. polonica* is highly supported by the maximum-likelihood analyses of the ITS and *cox 1* sequences.

Key words: clade 9, high-temperature tolerance, irrigation water, *Phytophthora hydrogena*

INTRODUCTION

Many species in the genus *Phytophthora* have been considered destructive plant pathogens. Two notorious examples are that *P. infestans* caused Irish potato famine 1845–1849 (Erwin and Ribeiro 1996) and *P. ramorum* caused sudden oak death (SOD), which has killed millions of trees in the forests of Oregon and California (Goheen et al. 2002, Rizzo et al. 2002). The SOD disaster reminded the science community and the public of the constant plant biosecurity threat posed by *Phytophthora* species and invigorated research in these pathogens (Grünenwald et al. 2012). To date the total number of formally described *Phytophthora* species has reached 120, which is double the number 17 y ago (Erwin and Ribeiro 1996).

Traditional taxonomy of *Phytophthora* species was based on morphological characteristics, as exemplified by the classic morphological key by Waterhouse (1963), which separated the genus into six groups and is still widely used today. However, due to the plasticity and overlapping of morphological characteristics among species, taxonomy based on morphology cannot be used to reliably distinguish some species within the genus (Erwin and Ribeiro 1996, Naher et al. 2011).

Phylogenetic analyses of various DNA regions have greatly advanced the taxonomy of this important genus. Ten clades were proposed for genus *Phytophthora* after phylogenetic analyses of mitochondrial and nuclear DNA sequences (Cooke et al. 2000, Martin and Tooley 2003, Kroon et al. 2004, Villa et al. 2006, Blair et al. 2008, Robideau et al. 2011). By taking advantage of the phylogenetic analyses, many new species have been separated from some well known species complexes. For instance, *P. brassicae* (Man in 't Veld et al. 2002) was separated from *P. porri*. *P. obscura* (Grünenwald et al. 2012) was separated from *P. syringae*. Similarly *P. hydrophatica* (Hong et al. 2010) and *P. irrigata* (Hong et al. 2008) were separated from *P. drechsleri*. Likewise *P. plurivora* (Jung and Burgess 2009) and *P. pini* (Hong et al. 2011) were separated from *P. citricola*. In addition, many new species, such as *P. aquimorbida* (Hong et al. 2012), *P. bilorbang* (Aghighi et al. 2012), *P. borealis* and *P. riparia* (Hansen et al. 2012), were discovered.

Among the 10 *Phytophthora* clades, clade 9 contains many relatively new members. The only exceptions are *P. insolita* (Ann and Ko 1980), *P. macrochlamydospora* (Irwin 1991) and *P. quininea* (Crandall 1947). All other species in this clade were described after 2005. Many

members in this clade, such as three recently named species *P. irigata* (Hong et al. 2008), *P. hydrophatica* (Hong et al. 2010) and *P. aquimorbida* (Hong et al. 2012), are well adapted to the irrigation reservoir environment. The objective of this study was to examine another group of water isolates with distinct features and formally describe it as *Phytophthora hydrogena* sp. nov.

MATERIALS AND METHODS

Isolation and isolate maintenance.—Three isolates examined in this study (TABLE I) were recovered by baiting with fresh rhododendron or camellia leaves (Hong et al. 2002, Bush et al. 2003, Ghimire et al. 2011) from irrigation reservoirs in an ornamental plant nursery in Virginia, USA, in May and Oct 2007. Cultures were grown on 20% clarified V8 juice agar (CV8A) (Erwin and Ribeiro 1996), and blocks of fresh agar cultures were transferred into microtubes with sterile distilled water for long-term storage at 15 C. The ex-type was deposited at the American Type Culture Collection (MYA-4919) in Manassas, Virginia. The dry culture as holotype was deposited at the Massey Herbarium of Virginia Polytechnic Institute and State University (VTMH 14882) in Blacksburg, Virginia.

Colony morphology.—To examine colony morphology, cultures were grown on CV8A, carrot agar (CA), malt extract agar (MEA) and potato dextrose agar (PDA). Each 10 cm Petri dish with 12 mL medium was seeded upside down with an agar block (5 mm diam) taken from 10 d cultures with the aid of a flamed cork borer. The dishes were placed in the dark at 20 C. Colony morphology was noted after 10 d.

DNA extraction, amplification and sequencing.—A 5 × 5 mm agar plug from an actively growing culture was placed in 20% V8 juice broth (Erwin and Ribeiro 1996) at room temperature (ca. 23 C) for 7 d to produce mycelial mass. The mycelial samples were harvested, dried and lysed with a FastPrep[®]-24 system (MP Biomedicals, Santa Ana, California). Purified DNA was extracted as instructed with the DNeasy[®] Plant Mini Kit (Qiagen, Valencia, California). Amplifications were performed with forward primer ITS6 and reverse primer ITS4 (Cooke et al. 2000) for the internal transcribed spacer (ITS) region covering ITS1, 5.8S rRNA gene and ITS2, following previously described reaction mix recipe and PCR program (Kong et al. 2003). Amplifications also were performed with primer pair COX4FR (Kroon et al. 2004) for sequencing the mitochondrially encoded cytochrome *c* oxidase 1 gene (*cox 1*) following the PCR protocol with a 45 C annealing temperature. Excess primer and dNTPs were removed from PCR products with shrimp alkaline phosphatase and exonuclease I (USB 70092Y and 70073Z). One U each enzyme was added to 15 mL PCR product, incubated at 37 C for 30 min, followed by heat inactivation at 65 C for 15 min. Sequencing was performed in both directions for all regions with the same primers as for PCR by the University of Kentucky Advanced Genetic Technologies Center (Lexington, Kentucky). Sequences of both directions were viewed with Finch TV 1.4.0. and aligned with Clustal W.

Phylogenetic analysis.—Sequences generated in this study were compared with those of all other species in the same

clade and selected species in other clades (TABLE I). Sequences were aligned with Clustal W. Phylogeny reconstruction was conducted in MEGA 5 (Tamura et al. 2011) with the maximum-likelihood method based on the Tamura-Nei model (Tamura and Nei 1993) with 1000 bootstrap replications. GenBank accession numbers of the sequences derived in this study are provided (TABLE I). The alignments and trees are available in TreeBASE (S14412).

Growth temperature studies.—Three isolates were assessed for cardinal temperatures on CV8A and CA. Agar blocks (5 mm diam) taken from actively growing areas of 10 d cultures were placed at the center of Petri dishes with fresh media. These dishes were placed in the dark at 5, 10, 15, 20, 25, 30, 35, 36, 37, 38, 39 or 40 C, triplicate dishes per isolate per temperature. Two perpendicular diameter measurements of each colony were taken when the margin of the fastest growing colony was about 1 cm from the dish edge. The test was repeated. Means of radial growth along with standard errors were plotted against temperature with the gplots package 2.11.0 (Warnes et al. 2012) in R statistical software 2.15.0 (R Core Team 2012).

Sporangial morphology.—Sporangia were produced by transferring 10 × 10 mm square agar plugs of 1–2 wk old colonies on 10% CV8A to Petri dishes containing non-sterile 1.5% soil water extract solution (SWE, 15 g non-*Phytophthora* containing nature soil/1 L distilled water) and incubating at room temperature approximately 10 h until mature sporangia formed. Sporangia were photographed with a Nikon Fujix Digital Camera HC-300Zi connected to a Nikon Labophot-2 microscope. At least 50 randomly selected mature sporangia were measured for length and width with Image-Pro[®] Plus 5.1.2.53.

Breeding system of gametangia.—Because gametangia were not observed in single cultures on any media, several methods were employed to determine the sexuality and mating type of three isolates of *P. hydrogena*. They were tested in dual culture with either A1 or A2 mating type tester of *P. cinnamomi* on hemp seed agar (HSA) or CV8A. Also, the polycarbonate technique (Ko 1978, Gallegly and Hong 2008) was used to induce selfing and prevent mixing the gametangia of target isolates and testers. Specifically, each isolate was paired with either mating type of *P. cinnamomi* on CA or HSA amended with 30 mg β-sitosterol L⁻¹ then incubated at 20 and 25 C as well as room temperature. For isolates that did not produce oospores, additional tests were conducted with the mating type testers of *P. meadii* and *P. cambivora* at the same temperatures.

Statistical analysis.—Analyses of variances were carried out with R (R Core Team 2012) to determine the differences in the measurements of morphological and physiological characteristics among repeating experiments and isolates.

RESULTS

Colony morphology.—After growing at 20 C in dark for 10 d, three isolates of *P. hydrogena* showed a similar growth pattern (FIG. 1). Overall, colonies expanded

TABLE I. Origins and sequence accession numbers of *Phytophthora hydrogena* and reference species

Species	Clade	Isolate	Substrate/host	Origin	Year	Sequence accession no. ^a	
						ITS	cox 1
<i>P. hydrogena</i>	9	44C8	Irrigation water	Virginia	2007	KC249958	KC249961
		46A3 ^b	Irrigation water	Virginia	2007	KC249959	KC249962
		46A4	Irrigation water	Virginia	2007	KC249960	KC249963
<i>P. andina</i>	1	EC3421	<i>Solanum muricatum</i>	Ecuador	n/a ^c	AY770738	AY564160
<i>P. hedraiaandra</i>	1	CBS111725	<i>Viburnum</i> sp.	The Netherlands	2004	AY707987	AY769115
<i>P. infestans</i>	1	2C	<i>Solanum tuberosum</i>	Mexico	1992	KC733443	KC733447
<i>P. meadii</i>	2	CBS21988	<i>Hevea brasiliensis</i>	India	1987	HQ643268	AY564192
<i>P. ilicis</i>	3	CBS114348	<i>Ilex aquifolium</i>	The Netherlands	1957	JX524158	JX524159
<i>P. heveae</i>	5	CBS29629	<i>Hevea brasiliensis</i>	Malaysia	1929	AF266770	AY564182
<i>P. gonapodyides</i>	6	CBS55467	Fruit bait from water	UK	1967	HQ643236	KC733448
<i>P. humicola</i>	6	CBS20081	Soil slurries	Taiwan	1977	HQ643243	AY564184
<i>P. pinifolia</i>	6	CMW26668	<i>Pinus radiata</i>	Chile	2007	EU725806	JN935961
<i>P. cinnamomi</i>	7	CBS14422	<i>Cinnamomum burmannii</i>	Indonesia	1922	HQ643189	n/a
		10A6	<i>Persea americana</i>	n/a	n/a	n/a	AY564169
<i>P. rubi</i>	7	CBS96795	Base of raspberry cane	Scotland	1985	HQ643340	DQ674736
<i>P. foliorum</i>	8	LT192	<i>Rhododendron</i> sp.	Tennessee	2004	HQ261560	EU124918
<i>P. aquimorbida</i>	9	40A6	Irrigation water	Virginia	2006	FJ666127	G-Q294536
<i>P. capitosa</i>	9	310C	<i>Eucalyptus saligna</i>	New Zealand	1992	DQ297402	KC733449
<i>P. chrysanthemi</i>	9	GF749	<i>Chrysanthemum</i> sp.	Japan	2000	AB437135	n/a
<i>P. constricta</i>	9	CBS125801	Soil	Australia	2006	HQ013225	KC733450
<i>P. fallax</i>	9	310L	<i>Eucalypti delegatensis</i>	New Zealand	1997	DQ297391	KC733451
<i>P. hydrophatica</i>	9	5D1	Irrigation water	Virginia	2000	EU583793	KC733452
<i>P. insolita</i>	9	PMC5-1	Soil slurries	Taiwan	1980	GU111612	AY564188
<i>P. irrigata</i>	9	23J7	Irrigation water	Virginia	2000	EU334634	KC733453
<i>P. macrochla-</i> <i>mydospora</i>	9	UQ778	<i>Glycines max</i>	Australia	1974	KC733445	KC733454
<i>P. parsiana</i>	9	SUC25	<i>Ficus carica</i>	Iran	1991	KC733446	KC733455
<i>P. polonica</i>	9	UASWS0198	<i>Alni glutinosae</i>	Poland	2004	DQ396410	n/a
		PI5005	n/a	US	n/a	n/a	KC733456
<i>P. quininea</i>	9	CBS40648	<i>Cinchona officinalis</i>	Peru	1947	DQ275189	n/a
		CBS40748	<i>Cinchona officinalis</i>	Peru	1947	n/a	AY564200
<i>Pythium apha-</i> <i>nidermatum</i>	<i>Pythium</i>	PI779	n/a	n/a	n/a	GU983641	n/a
		P2	n/a	n/a	n/a	n/a	AY564163

^aNumbers in boldface are those generated in this study.

^bExo-type isolate.

^cn/a = not available.

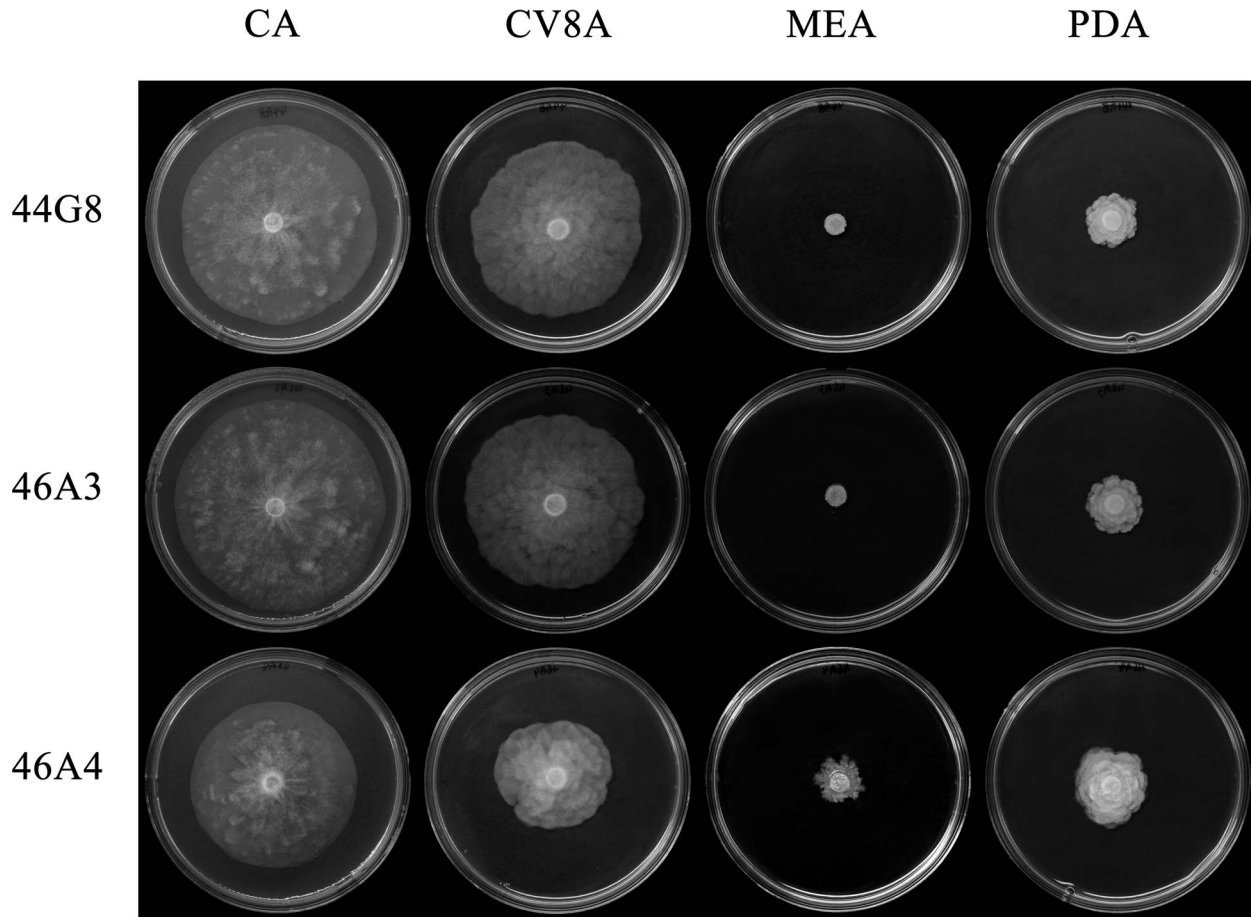


FIG. 1. Colony morphology of *Phytophthora hydrogena* isolates on various media incubated at 20 C for 10 d in the dark. CA = carrot agar, CV8A = 20% clarified V8 juice agar, MEA = malt extract agar, PDA = potato dextrose agar.

the fastest on CA among four tested media. Colonies on CA had a relatively smooth edge and produced radiate to slightly chrysanthemum-like patterns. On CV8A and PDA, all isolates formed typical chrysanthemum-like colonies with irregular edges, although colonies expanded more slowly and were denser on PDA than on CV8A. Only isolate 46A4 had limited but discernible growth with irregular colony and radiate mycelia on MEA (FIG. 1).

Sequence analysis and phylogenetic position.—Three isolates of *P. hydrogena* have an identical 744 bp ITS sequence and an identical 867 bp partial *cox 1* sequence. *P. hydrogena* is placed within *Phytophthora* clade 9, according to the maximum-likelihood phylogenetic trees based on ITS and *cox 1* sequences (Blair et al. 2008). It forms a distinct new group with its closest relatives *P. hydropathica*, *P. irrigata* and *P. parsiana*, with strong bootstrap support (FIG. 2). *P. hydrogena* differs in ITS sequence from *P. hydropathica*, *P. irrigata* and *P. parsiana* by 31 bp, 53 bp and 43 bp respectively. In *cox 1* sequence, *P. hydrogena* is different

from *P. hydropathica*, *P. irrigata* and *P. parsiana* by 25 bp, 28 bp and 26 bp respectively.

Cardinal temperatures for vegetative growth.—Vegetative growth rates were statistically identical among three isolates ($P = 0.98$) and between two experiments ($P = 0.26$). The average daily radial growth of three isolates were plotted against temperature (FIG. 3). Generally, *P. hydrogena* grew discernibly faster on CA than on CV8A. The optimum temperature for growth was 30 C and it grew well at 35 C (FIG. 3). Only notable growth was observed at 38 and 39 C and no growth at 5 and 40 C after 5 d (FIG. 3).

TAXONOMY

Phytophthora hydrogena X. Yang and C. X. Hong.,
sp. nov. FIG. 4

Mycobank MB803274

Phytophthora hydrogena produced few sporangia in aged CA and CV8A. When mycelial plugs were submerged in 1.5% SWE under light, they produced abundant sporangia within 10 h. Sporangia varied

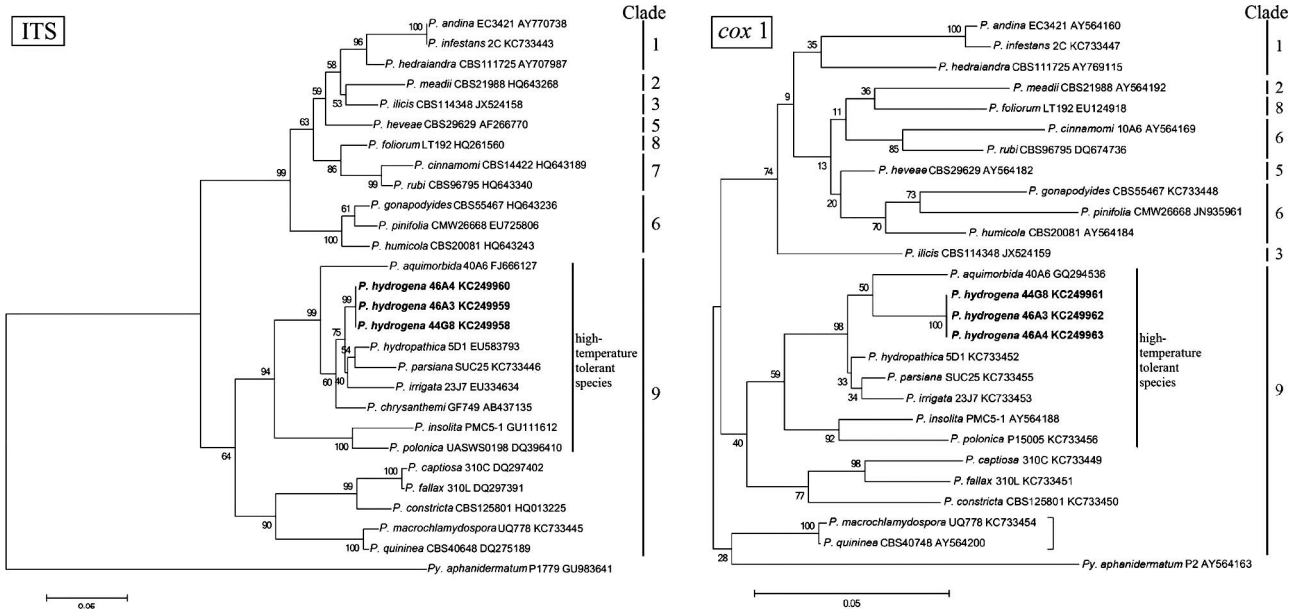


FIG. 2. Maximum likelihood phylogenetic trees based on ITS and *cox 1* sequences. Alignment was conducted with Clustal W, and the phylogenetic tree was generated in MEGA 5.

from ovoid to obpyriform, limoniform and ellipsoid (FIG. 4A–G). Sporangia were terminal, nonpapillate and noncaducous; they averaged 44.58 μm long and 29.27 μm wide. Most sporangia had a conspicuous cell wall at the joint point between sporangial base and pedicel tip. Approximate half of the sporangia observed in this study had characteristic widening at the pedicel tip of sporangiophores or tapered sporangial base toward the point of attachment (FIG. 4K–M). Sporangia also commonly had a conspicuous basal plug (FIG. 4H, K). Nested and extended internal proliferation (FIG. 4I, J) was common. Knobby and angular mycelia were observed especially in aged cultures (FIG. 4S, T). Hyphal swelling of sporangiophores (FIG. 4P, R) occasionally was observed when submerged in modified Petri’s solution. Chlamydospores were not observed in fresh culture on any agar media or in SWE. However, a limited

number of chlamydospores, average 34.5 μm diam, were observed in aged cultures (> 50 d) in CA and CV8A or cultures incubated in modified Petri’s solution for more than 12 h under light (FIG. 4N, O).

Phytophthora hydrogena is heterothallic and all examined isolates are A1. None of these isolates produced any sexual structure in single culture. Polycarbonate membrane tests on CA and HSA at 20, 25 C and room temperature induced the formation of gametangia in A2 tester isolates of *P. cambivora* and *P. cinnamomi*. Gametangia were not observed in any isolates of *P. hydrogena* or testers of *P. meadii*. Gametangia also were observed when each *P. hydrogena* isolate was paired with an A2 tester of *P. cambivora* or *P. cinnamomi* in the dual culture without separation by a polycarbonate membrane. Most of the oogonia produced in the dual cultures were typical of *P. cinnamomi*, which had a funnel-shaped base or broad, tapering stalk through an antheridium (Gallegly and Hong 2008; FIG. 5I). A small number of oogonia were distinct from those typical oogonia produced by *P. cinnamomi*. They most likely were produced by *P. hydrogena* (FIG. 5A–H). These oogonia were globose with golden to brown pigment, averaged 41.1 μm diam. Oospores, average 37.6 μm , were mostly plerotic (FIG. 5A–C, E, F), sometimes aplerotic (FIG. 5D, G) and eccentric (FIG. 5G). These oogonia were attached by antheridia that were mostly amphigynous and averaged about 15 μm wide and deep (FIG. 5A–H). Multiple paragynous antheridia occasionally were associated with an oogonium (FIG. 5H).

Holotype: VTMH 14882 (Virginia Tech Massey Herbarium, Blacksburg, Virginia, USA), dried culture

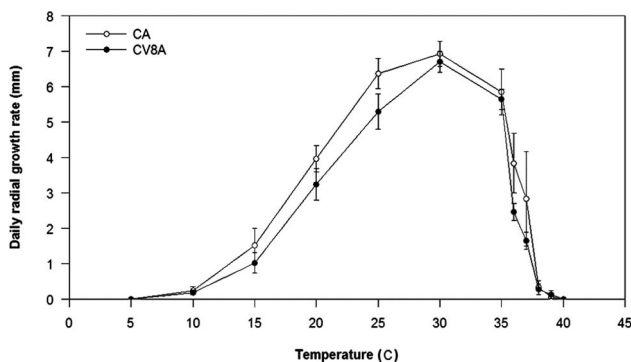


FIG. 3. Radial growth of *Phytophthora hydrogena* isolates in carrot agar (CA) and 20% clarified V8 juice agar (CV8A) over a 5 d period.

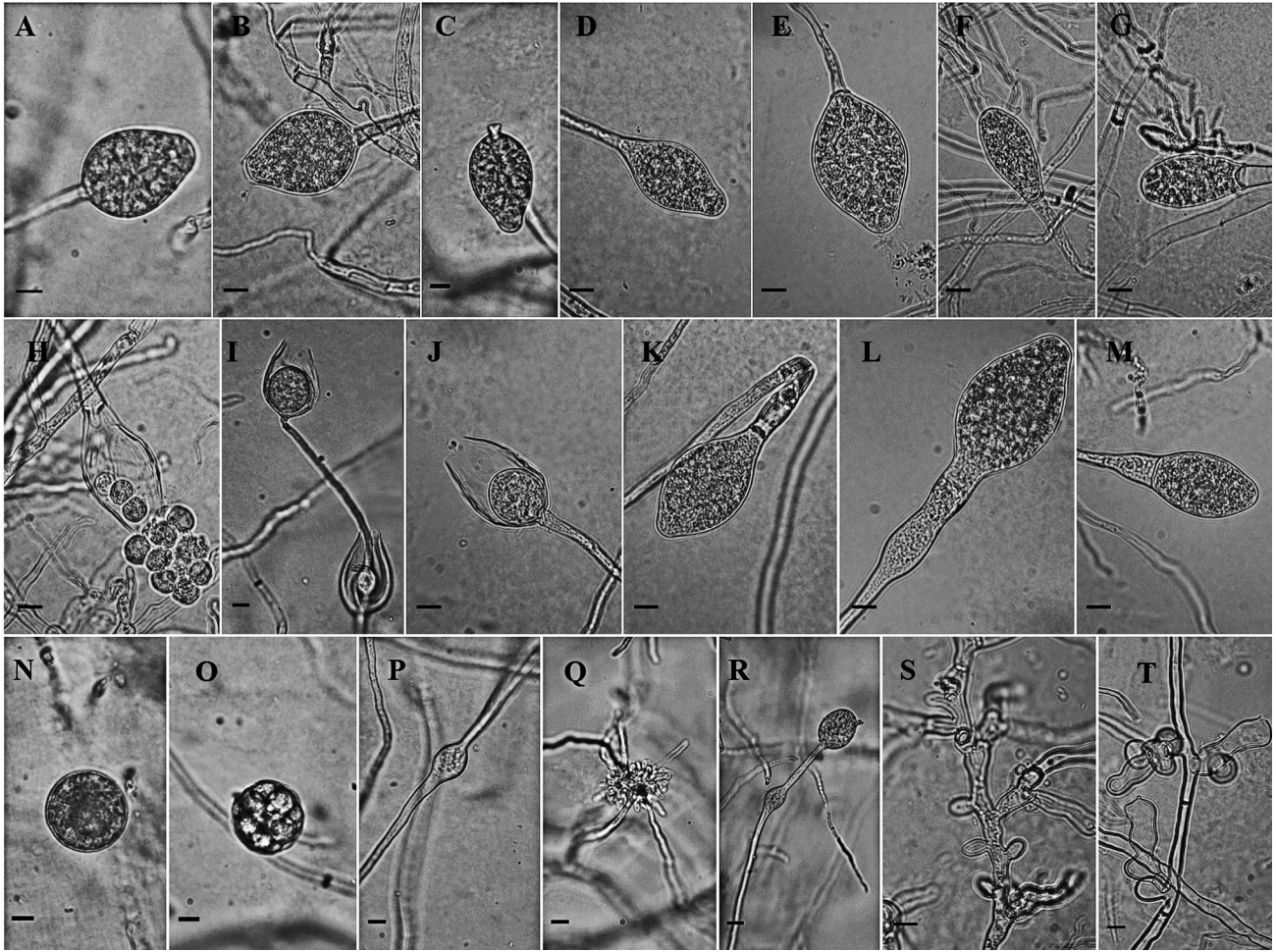


FIG. 4. Morphology of asexual structures of *Phytophthora hydrogena*. A–G. Nonpapillate sporangia in various shapes. A, B. Ovoid sporangia. C. An obpyriform to ovoid sporangium. D, E. Limoniform sporangia with a tapered base. F, G. Ellipsoid sporangia. H. A sporangium with a conspicuous basal plug releasing zoospores. I, J. Nested proliferation. K. A sporangium with a conspicuous basal plug, a tapered base and a widening sporangiophore tip. L, M. Sporangia with a tapered base and a widening sporangiophore tip. N, O. Chlamydozoospores infrequently produced in modified Petri's solution. P, R. Intercalary swelling on sporangiophore occasionally produced in modified Petri's solution. Q. Hyphal aggregation rarely formed in modified Petri's solution. S, T. Knobby and angular coralloid-like mycelia. Bars = 10 μ m.

of an isolate from irrigation water of a nursery reservoir, Virginia, USA, Oct 2007. Ex-holotype: 46A3 (ATCC MYA-4919).

Etymology: “*hydrogena*” refers to the aquatic habitat where it was isolated.

Habitat: Irrigation water of a perennial plant nursery, Virginia, USA.

DISCUSSION

This study described a novel species, *Phytophthora hydrogena*, based on its distinct phylogenetic position, morphology and physiology, and revealed a cluster of high-temperature tolerant species within *Phytophthora* clade 9. These findings have several ramifications.

Description of *P. hydrogena* is the first and critical step to assess the potential plant biosecurity threat posed by this species and also will help reduce the risk in misidentification of high-impact pathogens within this genus. *P. hydrogena* is morphologically, physiologically and phylogenetically distinct from any other known *Phytophthora* species. As a heterothallic species, *P. hydrogena* can be easily distinguished from homothallic species. Among heterothallic species, *P. drechsleri*, *P. hydrophatica*, *P. inundata*, *P. irrigata*, *P. melonis* and *P. parsiana* as well as this new species, *P. hydrogena*, produce nonpapillate sporangia and grow well at 35 C (Gallegly and Hong 2008, Hong et al. 2010). *P. hydrogena* differs from *P. drechsleri*, *P. inundata*, *P. melonis*, and *P. parsiana* by producing

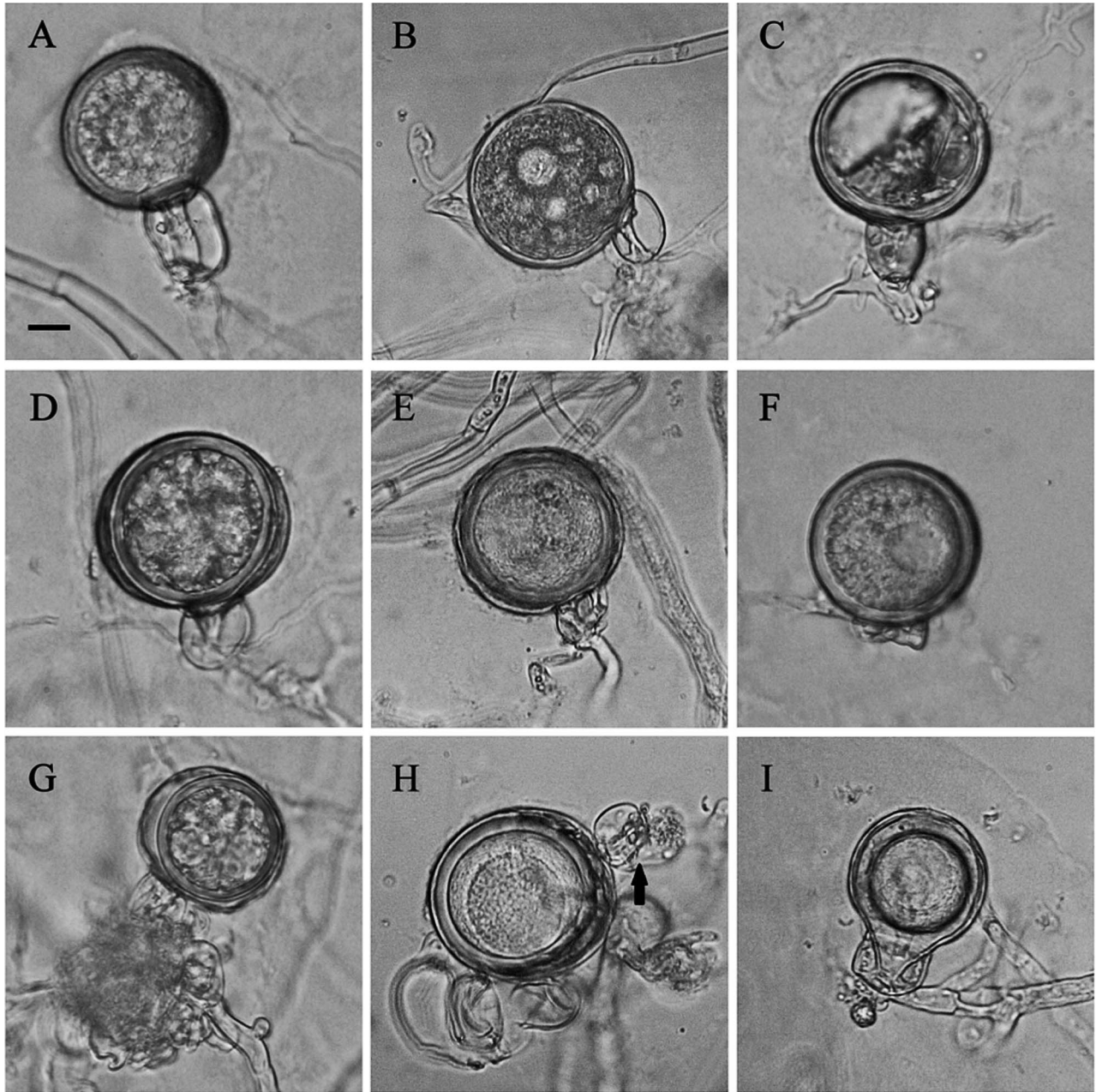


FIG. 5. Morphology of sexual organs of *Phytophthora hydrogena*. A. A plerotic oospore with a long, cylindroid, amphigynous antheridium. B. A plerotic oospore with a globose antheridium. C. A plerotic oospore with a golden coniform antheridium. D. A slightly aplerotic oospore with a globose antheridium. E, F. Plerotic oospores with a short antheridium. G. Excentric aplerotic oospore. H. A slight aplerotic oospore with an amphigynous antheridium (arrow) and multiple paragynous antheridia. I. A typical sexual organ produced by *Phytophthora cinnamomi* with a characteristic broad tapering stalk in the antheridium and a funnel-shaped base of the oogonium within the antheridium. Bar = 10 μ m.

much smaller sporangia. It also differs from *P. drechsleri*, *P. hydropathica*, *P. inundata*, *P. melonis* and *P. parsiana* by not producing chlamydospores in CV8A, CA, MEA or PDA. Morphologically *P. hydrogena* is very similar to *P. irrigata*, but the former can be distinguished by its characteristic widening of sporangio-phore tips and tapered sporangial bases.

Many species in *Phytophthora* clade 9 share a common physiological character of high-temperature tolerance. Analyses of ITS and *cox 1* sequences grouped *P. hydrogena* with *P. aquimorbida* (Hong et al. 2012), *P. hydropathica* (Hong et al. 2010), *P. irrigata* (Hong et al. 2008), *P. chrysanthemi* (Naher et al. 2011) (only supported by ITS sequence), *P. insolita* (Ann and Ko

1980), *P. polonica* (Belbahri et al. 2006) and *P. parsiana* (Mostowfizadeh-Ghalamfarsa et al. 2008) in a distinct cluster (FIG. 2). Species in this cluster have an optimum temperature around 30 C, and they all grow well at 35 C. This unique characteristic of high-temperature tolerance has not been found from other species in clade 9 or in members of any other *Phytophthora* clade. The monophyly of species in this high temperature-tolerant cluster, except *P. insolita* and *P. polonica*, is highly supported by the maximum-likelihood analyses of the ITS (99%) and *cox 1* (98%) sequences (FIG. 2). Many species in this cluster including *P. aquimorbida*, *P. hydropathica*, *P. irrigata* and *P. hydrogena* initially were isolated from irrigation reservoirs. The host ranges of these aquatic species are unknown with the only exception of *P. hydropathica*, which is known to attack *Kalmia latifolia* and other horticultural crops (Hong et al. 2010). These findings might imply that the ancestors of these high temperature-tolerant species may originate from tropical or subtropical environments.

It is intriguing that all isolates of this novel species are A1. Similar observations were made in two other heterothallic species, *P. hydropathica* (Hong et al. 2010) and *P. irrigata* (Hong et al. 2008), that also were recovered initially from irrigation reservoirs in Virginia. The implication of this phenomenon remains unknown. One possibility is that two mating types of these heterothallic species were separated according to geographic distributions after their global migrations. This geographical separation of mating-type isolates has been reported in other *Phytophthora* species. For example, the A2 mating type of *P. infestans* was not found outside Mexico until 1984 (Hohl and Iselin 1984). Another example is all European isolates of *P. ramorum* before 2003 were A1 mating type while all North American isolates were A2 (Hansen et al. 2003). Similarly, it is possible that only A1 mating-type isolates of *P. hydrogena*, *P. hydropathica* and *P. irrigata* migrated from their origins to Virginia.

The economic and ecological significance of this new species is not clear at this point. Symptomatic plant samples infected by this species have never been recorded from the Disease Clinic at Hampton Road AREC, Virginia Tech, in Virginia Beach (C. Hong unpubl). *P. hydrogena* caused asymptomatic infection on roots of *Rhododendron catawbiense*, but it was a poor survivor in potting mix in a preliminary pathogenicity test (data not shown). Further studies are warranted on its host range, ecological role in aquatic and terrestrial ecosystems, as well as its origin.

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