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Phytophthora borealis and *Phytophthora riparia*, new species in *Phytophthora* ITS Clade 6

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Abstract: *Phytophthora borealis* and *Phytophthora riparia*, identified in recent *Phytophthora* surveys of forest streams in Oregon, California and Alaska, are described as new species in *Phytophthora* ITS Clade 6. They are similar in growth form and morphology to *P. gonapodyides* and are predominately sterile. They present unique DNA sequences, however, and differ in temperature/growth relations and geographic distribution.

Key words: aquatic fungi, β-tubulin, cytochrome oxidase, forest streams

Introduction

Phytophthora species are best known as pathogens of agricultural crops or forest trees. Our concept of the genus is expanding, however, as new habitats are explored and new species are discovered. Recent epidemics of the invasive *Phytophthora* species *P. alni*, *P. ramorum* and *P. kernoviae* in forests and woodlands of Europe and North America (Hansen 2007) have triggered systematic sampling in forests and streams for early detection of the pathogens and renewed interest in the assemblage of *Phytophthora* species already resident in natural habitats. Here we describe two new species identified in recent *Phytophthora* surveys of forest streams in Oregon, California and Alaska (Reeser et al. 2011) and riparian alder stands in western Oregon (Sims et al. unpubl).

Recent interest in characterizing the *Phytophthora* populations in forest streams, coupled with new molecular techniques to ease species identification, have led to recognition of the common occurrence of *P. gonapodyides* and other representatives of *Phytophthora* ITS Clade 6 (Cooke et al. 2000) in waterways and adjacent riparian vegetation (Brasier et al. 2003). Similarly, water sampling as well as re-examination of culture collections has led to recognition of new Clade 6 taxa in Australia (Jung et al. 2011). In addition to phylogenetic relatedness, these taxa are morphologically similar, homothallic or sexually ambiguous, and usually associated with water or wet soils. Brasier and colleagues (Brasier et al. 2003) recognized at least 12 distinct taxa within the clade, most lacking formal nomenclatural designation. Only the homothallic pathogen *P. megasperma* and the most abundant *P. gonapodyides* were recognized before molecular techniques enabled differentiation of taxa within this group. We found five of these previously reported Clade 6 taxa in western North American streams and two undescribed taxa (Reeser et al. 2011). Here we formally describe the latter as new *Phytophthora* species, *P. borealis* and *P. riparia*.

Materials and methods

Isolations from streams were made with bait leaves and filtration. Various leaf baits, always including rhododendron (*R. catawbiense* or *R. macrophyllum*), were placed in open-weave nylon mesh bags and floated in relatively slow-moving water in streams. Leaf baits were exchanged at 2 wk intervals (Oregon samples) or collected after a single 1 wk exposure (Alaska samples, see Reeser et al. 2011). Baits were rinsed in tap water, then petioles and symptomatic areas of exposed stream leaf baits were excised and plated in CARP+ (cornmeal agar amended with 20 ppm Delvocid [50% natamycin], 200 ppm Na-ampicillin and 10 ppm rifamycin SV with 25 ppm hymexazol [99%] and 30 ppm Benlate [benomyl 50WP]). At some western Oregon sites 1 L samples of stream water were filtered onto 5 μm nitrocellulose filters (Millipore SMWP04700) by vacuum filtration, and the filters cultured on CARP+.

Isolation plates were incubated at 20 C in the dark and examined at approximately 3 and 7 d. When *Phytophthora* species grew on isolation plates, hyphal tips were transferred to fresh CARP (cornmeal agar amended with 20 ppm Delvocid [50% natamycin], 200 ppm Na-ampicillin, 10 ppm rifamycin SV) for confirmation of purity, then to CMAβ (cornmeal agar amended with 20 ppm β-sitosterol) for characterization, DNA extraction and storage. Colonized agar plugs were stored at room temperature in sterile deionized water with and without hemp seed pieces.

Characterization of isolates.—Carrot agar (CA), (200 g chopped fresh carrots simmered 45 min in 1 L deionized H_2O, filtered and water added to 1 L, and 15 g Bacto™ agar) (after Brasier 1969) was the standard growth medium for morphology and growth rate. Sporangia were produced on 5 mm disks cut from the edge of colonies grown on V8S agar (15% clarified V8 neutralized with 14.3 mg/mL $CaCO_3$, 1.5% Bacto™ agar and 20 ppm β-sitosterol) and incubated in soil extract water or stream water.

Mating tests.—Six isolates of an undescribed taxon from Alaska stream samples, described below as *P. borealis* sp.

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TABLE I. Gene regions and primer pairs used for amplification and subsequent sequencing of new species *P. borealis* and *P. riparia*

Region	Amplicon length analyzed		Primer	Citation
	P. borealis	*P. riparia*		
β-tubulin	1124	1124	BTUBF1	Blair J.E. et al. 2008
			BTUBF2[a]	Kroon L.P.N.M. et al. 2004
			BTUBR1	Kroon L.P.N.M. et al. 2004
			BTUBR2[a]	Blair J.E. et al. 2008
cox I	1197	636	FM50[a]	Martin F.N., Tooley P.W. 2003
			FM83	Martin F.N., Tooley P.W. 2003
			FM84	Martin F.N., Tooley P.W. 2003
			FM85[b]	Martin F.N., Tooley P.W. 2003
cox spacer	337	332	FMPH8	http://www.ars.usda.gov/Research/docs.htm?docid=8737
			FMPH10	http://www.ars.usda.gov/Research/docs.htm?docid=8737
ITS	1196	1195	DC6	Cooke D.E.L. et al. 2000
			ITS2[a]	White T.J. et al. 1990
			ITS3[a]	White T.J. et al. 1990
			ITS4	White T.J. et al. 1990

[a] Used for sequencing only.
[b] Used for sequencing only for *P. borealis* and amplification and sequencing for *P. riparia*.

nov., were paired on CMAβ incubated in the dark at 20–22 C for 20 d. These same isolates were paired with A1 and A2 tester strains of *P. cinnamomi* and *P. cryptogea* on CA and incubated in the dark at 20–22 C for 20 d. An isolate producing oogonia in this test was re-paired with the same tester strains with the cultures separated by a 0.4 μm polycarbonate membrane to elucidate the origin of oogonia and to obtain pure culture specimens for measurement and illustration.

Eleven isolates of an undescribed taxon from Oregon streams, described as *P. riparia* sp. nov. below, were paired on CMAβ incubated in the dark at 20–22 C for 40 d. These same isolates were paired with A1 and A2 tester strains of *P. cambivora*, *P. cinnamomi* and *P. cryptogea* on CA incubated in the dark at 20–22 C for 27 d.

Phylogenetic analysis.—DNA was extracted from *Phytophthora* isolates growing on CMAβ with a CTAB buffer with ethanol precipitation protocol (Winton and Hansen 2001). ITS, β-tubulin, cytochrome oxidase unit I (*cox* I) and the *cox* spacer regions of DNA were amplified with appropriate primers (TABLE I) and sequenced, as described by Reeser et al. (2011). Sequences were aligned with Clustal X (Thompson et al. 1997). Sequences were compared to closely related reference isolates in our collection, those in the validated database Phytophthora ID (http://phytophthora-id.org/) and those available at GenBank. Species were delimited based on morphological similarity and position in the same well supported terminal ITS clade in phylogenetic analysis (see Reeser et al. 2011).

Bayesian inference of phylogeny of ITS sequences was carried out with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The analysis was set to use a general time reversible (GTR) model with gamma distributed rate variation and a proportion of invariable sites (invgamma). Two independent runs of Metropolis coupled Markov chain Monte Carlo (MC^3) were run 1 000 000 generations, with three heated and one cold chain. Sampling every 100th generation ensured at least 10 000 sample trees. Burn-in was set at 2500, resulting in 7501 samples. Using these samples, convergence diagnostics were done in two ways. The first method analyzed the resulting average standard deviation of split frequencies (ideal values approach zero), and the second checked the potential scale reduction factor (PSRF) where values should be reasonably close to one before assuming convergence (Gelman 1992). Actual resulting average standard deviation of split frequencies was approximately 0.0051, and the resulting PSRF value for a 95% credibility interval was 1.00. The consensus tree was built from the most selective set, consisting of a 50% credible set of 7499 trees. The phylogenetic tree was generated with TreeVIEW (Page 1996). Posterior probabilities of key branching points were determined by the MC^3 algorithm and set priors.

RESULTS

The final ITS alignment comprised 26 sequences, including six of *Phytophthora borealis* sp. nov., below, and six of *P. riparia* sp. nov., below, with 827 characters. The consensus tree with posterior probabilities is illustrated (FIG. 1), with *P. borealis* and *P. riparia* forming well supported terminal clades.

ITS and β-tubulin sequences from six isolates designated *Phytophthora borealis* were identical except for double peaks at 3 and 2 positions respectively. *Cox* spacer sequences were identical among the isolates, and *cox* I sequences were variable at a single position. The *P. borealis* ITS sequence was closest to unnamed GenBank ITS accessions from soils in Christmas tree plantations in Michigan (Fulbright et al. 2006, GenBank AY995371) but differed from them at five

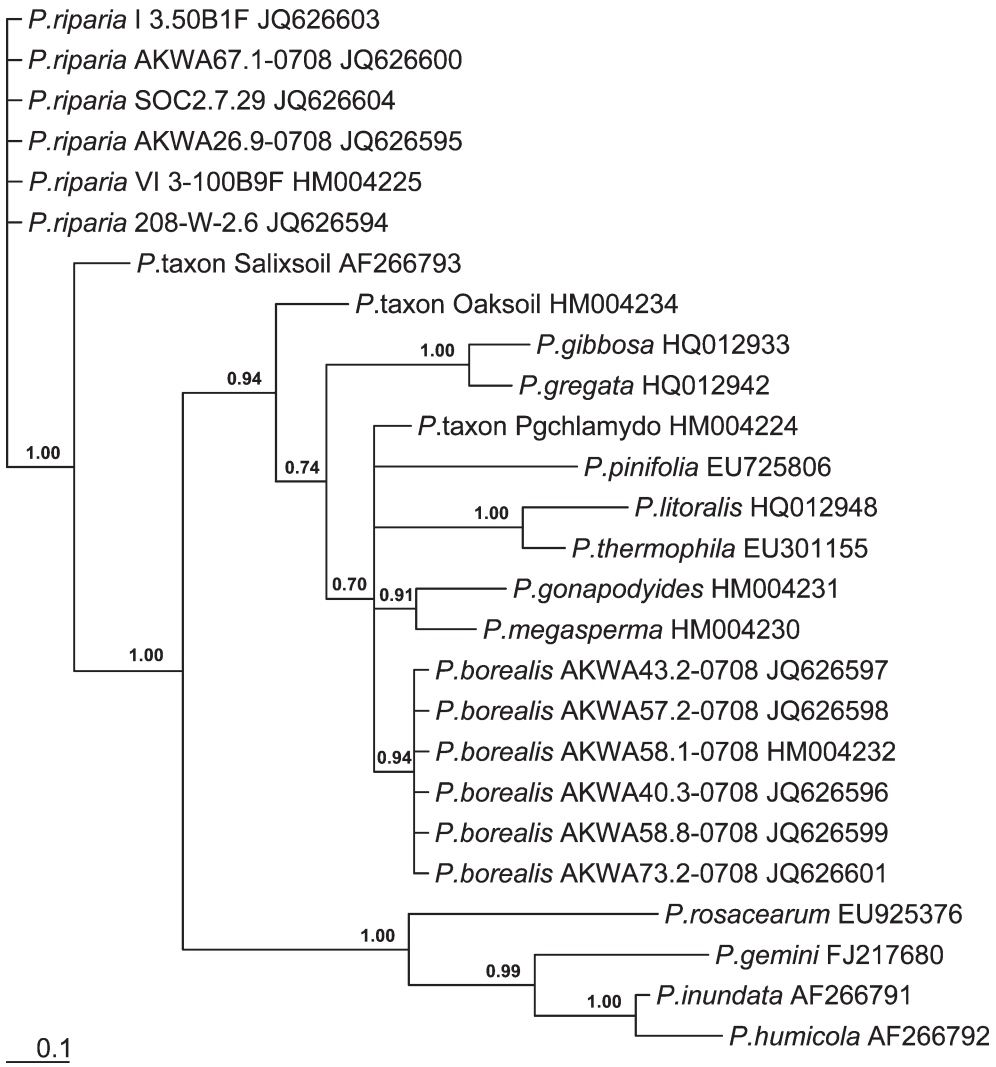

FIG. 1. Phylogenetic relationships of *Phytophthora borealis* and *Phytophthora riparia* based on Bayesian analysis of ITS sequences. Numbers at the nodes are posterior probabilities determined by the MC^3 algorithm and set priors.

or more nucleotide positions. The recognized species (not yet formally described) with the most similar ITS sequence is *P.* taxon Pgchlamydo (FIG. 1) from which it differs at seven nucleotide positions.

ITS sequences of six isolates designated *P. riparia* all were identical except for double peaks at three nucleotide positions. *Cox* spacer sequences included small differences at three nucleotide positions (one single nucleotide polymorphism and single nucleotide indels at two positions). Isolates of *P. riparia* were readily distinguished from related species by ITS DNA sequences (FIG. 1). The six isolates differed from *P.* taxon Salixsoil isolate (P245) (Brasier et al. 2003), the closest sequence among taxa compared, at six nucleotide positions plus one indel position with an additional six nucleotide positions that produced double peaks in some isolates.

Full length *cox* I sequences could not be obtained for *P. riparia* isolates, but a shorter sequence (FM 84 and FM 85, 600 + base pairs) was identical for all six isolates, except one that was polymorphic at one nucleotide position. β-tubulin sequences were identical for all isolates except for one nucleotide position. *Cox* spacer sequences of *P. riparia* differed from *P.* taxon Salixsoil at 10 nucleotide positions.

One additional isolate (EBE1.8.27) with colony growth and morphological features similar to *Phytophthora* taxon Salixsoil had DNA sequences consistent with a hybrid origin between *P. riparia* and *P.* taxon Salixsoil (TABLE II). This isolate was identical to *P. riparia* in the ITS region (except for double peak at 462), but the nuclear β-tubulin sequence was marked by double peaks combining nucleotides from *P. riparia* and *P.* taxon Salixsoil (13 nucleotide positions). This

isolate was identical to *P.* taxon Salixsoil in mitochondrial *cox* I (TABLE II) and *cox* spacer regions (data not shown).

Isolates of *P. borealis* and *P. riparia* differed in both optimal growth temperatures and temperature range. Growth of *P. borealis* on CA was poor above 25 C, with optimum growth at 15–20 C. Isolates of *P. riparia* grew up to 35 C, with optimum growth at 30 C (FIG. 2).

TAXONOMY

Phytophthora borealis E Hansen, Sutton and Reeser., sp. nov. FIGS. 3, 4

MycoBank MB 564286

Phytophthora borealis was sexually self-sterile. It formed no oogonia in single-strain culture and formed oogonia only rarely when paired with mating type testers of heterothallic species (TABLE III). Sporangia were persistent, ovoid or obpyriform and non-papillate, with a slight apical thickening. Sporangial length was 69 ± 10 μm (one standard deviation). Isolate mean sporangial length of six isolates was 64–75 μm. Sporangial breadth averaged 39 ± 7 μm. Length to breadth ratio of the six isolates averaged 1.8 with isolate mean ratios of 1.7–1.9 (FIG. 3). Sporangia formed in water. Sporangiophores were unbranched, exhibiting internal proliferation, both nested and extended. Subsporangial elongation was rarely observed. Chlamydospores were not formed in agar. The colony pattern on CA was angular and petaloid, with hyphae appressed. On V8S agar growth was fluffy aerial with an underlying petaloid pattern (FIG. 4). Radial growth on CA at 25 C was about 2 mm/d, with a growth optimum (3.1 mm/d) 15–20 C. Slow growth was evident at 30 C, and no growth was observed at 35 C (FIG. 2).

Phytophthora borealis resides in ITS Clade 6 (Cooke et al. 2000) with *P. megasperma* s.s., *P. gonapodyides* and a number of provisionally identified taxa (Brasier et al. 2003). It is readily distinguished by ITS DNA sequences from related species in Clade 6 (Cooke et al. 2000) (FIG. 1). Phylogenetic analysis of β-tubulin and *cox* I sequences (Blair et al. 2008), as well as cox spacer sequences, supported this placement (data not shown).

Holotype. OSC 144,115, dried culture from OSU AKWA58.1-0708, isolated Jun 2008 from Bear Creek, Alaska, N63.61533, W-143.98484.

Additional specimens examined. Subcultures of AKWA58.1-0708 deposited at American Type Culture Collection MYA-4881 and CBS 132023. Key DNA sequences of isolates examined in detail (TABLE III) are in GenBank.

Etymology. ''Borealis'' references the northern latitudes from which the species was identified.

Commentary. Although most *P. borealis* isolates were sexually sterile, one isolate formed oogonia inconsistently when paired with an A2 tester isolate of *P. cryptogea* (TABLE III). It did not induce oogonia in the tester isolate. In this and other morphological features it was similar to *P. gonapodyides* and some other sterile Clade 6 species, although colony growth pattern and optimum temperature were distinctive.

Phytophthora borealis was recovered 31 times from six of 49 sampled Alaskan streams, scattered from the Kenai Peninsula to Fairbanks. It was the third most frequently identified species in Alaska waters, after *P. gonapodyides* and *P. riparia.* It was not identified in extensive sampling in Oregon streams (Reeser et al. 2011). *Phytophthora borealis* previously was cited as *P.* New species 1 (Reeser et al. 2011).

Phytophthora riparia Reeser, Sutton and E Hansen., sp. nov. FIGS. 5, 6

MycoBank MB 564287

Phytophthora riparia was sexually sterile, with no oogonia formed in single-strain culture or when paired with mating type testers of heterothallic species. Sporangia were ovoid or obpyriform, non-papillate, with a slight apical thickening, and not caducous (FIG. 5). Mean sporangial length (six isolates) was 55 ± 10 μm (one standard deviation), with isolate means of 47–62 μm. Sporangial breadth averaged 31 ± 4 μm. Length to breadth ratio of the six isolates averaged 1.8 with isolate mean ratios of 1.6–2.1. Sporangia formed in water. Sporangiophores were unbranched, exhibiting internal proliferation, both nested and extended. Subsporangial elongation was observed rarely. Chlamydospores were not formed in agar.

Radial growth on CA at 25 C was about 2.4 mm/d, with a growth optimum (2.6 mm/d) near 30 C. Slow growth was measured at 35 C, but no growth occurred at 40 C (FIG. 2). The colony pattern on V8S and on CA was angular and petaloid, with hyphae appressed (FIG. 6).

Phytophthora riparia is in ITS Clade 6 (Cooke et al. 2000, Brasier et al. 2003) and is differentiated from related species on the basis of ITS DNA sequences (FIG. 1) including its closest relative, *P.* taxon Salixsoil (Brasier et al. 2003). Six isolates differed from the reference *P.* taxon Salixsoil isolate (P245) at six nucleotide positions plus one indel position and six nucleotide positions with double peaks in some isolates.

Holotype. OSC 144,116, dried culture from OSU Isolate VI 3-100B9F, collected Apr 2006 from Oak Creek near Corvallis, Oregon. N44.566593, W-123.300984.

Additional specimens examined. Subcultures of VI 3-100B9F at American Type Culture Collection MYA-4882 and CBS 132024. Key DNA sequences of isolates examined in detail (TABLE III) were deposited in GenBank.

Etymology. ''Riparia'' refers to the streams and adjacent soils of riparian forests where this species is found.

Commentary. Phytophthora riparia was recovered 64 times from 10 of 49 sampled Alaska streams, from the Kenai Peninsula to Fairbanks. It was the second most frequently identified species in Alaska waters, after *P.*

TABLE II. Base alignment for ITS, ß-tubulin and *cox* I sequences for isolates representing *P. riparia*, *P.* taxon Salixsoil and a possible hybrid of the two

		ITS											
Species	Isolate	425	434	462	536	537	834	896	954	1116	1159	1171	
P. riparia	VI 3-100B9F	T	G	T	C	–	C	C	T	G	G	G	
P. riparia/Salixsoil	EBE1.8.27	T	G	Y[a,b]	C	–	C	C	T	G	G	G	
P. taxon Salixsoil	P245[c]	C	R	T	T	T	Y	G	C	A	C	R	
		β-tubulin											
		60	81	108	114	315	336	357	381	417	483	513	541	558
P. riparia	VI 3-100B9F	A	C	C	A	T	T	C	C	C	C	A	C	C
P. riparia/Salixsoil	EBE1.8.27	C	Y	Y	M	C	C	Y	Y	Y	C	R	Y	M
P. taxon Salixsoil	P245	C	C	T	C	C	C	C	T	T	Y	G	C	A
		591	594	783	822	837	882	912	990	1032	1033	1038	1044	
P. riparia	VI 3-100B9F	G	A	T	C	T	G	T	T	C	A	C	C	
P. riparia/Salixsoil	EBE1.8.27	S	C	Y	M	C	R	K	Y	Y	A	Y	Y	
P. taxon Salixsoil	P245	C	C	C	A	C	A	G	C	C	R	T	C	
		cox I												
		87	111	129	192	249	264	282	291	321	354	364	378	483
P. riparia	VI 3-100B9F	A	T	C	T	A	T	C	G	G	C	T	A	A
P. riparia/Salixsoil	EBE1.8.27	T	C	T	C	T	A	T	T	A	T	C	G	T
P. taxon Salixsoil	P245	T	C	T	C	T	A	T	T	A	T	C	G	T
		486	493	525	561	573	594	609	610					
P. riparia	VI 3-100B9F	T	T	C	T	C	C	C	T					
P. riparia/Salixsoil	EBE1.8.27	A	C	T	C	T	G	T	C					
P. taxon Salixsoil	P245	A	C	T	C	T	G	T	C					

[a] Double peaks are coded by R = G+A, Y = T+C, M = A+C, S = C+G, K = T+G.
[b] Shading indicates nucleotides different from *P. riparia*.
[c] P245 GenBank numbers: ITS JQ626605, β-tubulin JQ626619, COI JQ626633.

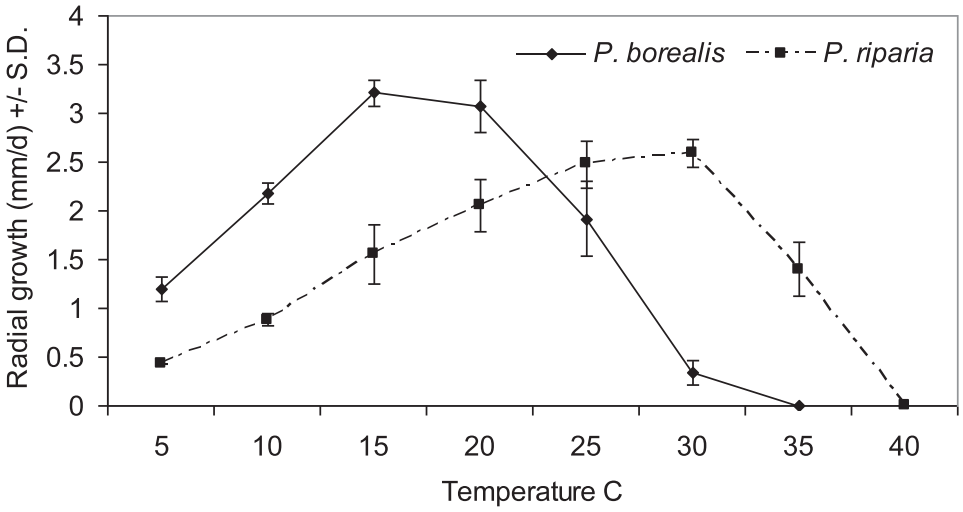

FIG. 2. Radial growth of *Phytophthora borealis* and *P. riparia* on CA at various temperatures.

TABLE III. Isolates of *Phytophthora borealis* and *P. riparia* examined in detail

Isolate	Site	State	Location		Date Recovered	GenBank accession number				Mating Behavior
			Latitude	Longitude		β -Tubulin	*cox* 1	*cox* spacer	ITS	
P. borealis										
AKWA58.1-0708[a]	058_BearCreek	Alaska	N63.61533	W-143.98484	2008	JQ626615	JQ626625	JQ626586	HM004232	sterile
AKWA40.3-0708	040_LittleNelchinaRiver	Alaska	N61.98993	W-146.94340	2008	JQ626612	JQ626622	JQ626583	JQ626596	sterile
AKWA43.2-0708	043_PultnaCreek	Alaska	N61.81154	W-148.13398	2008	JQ626613	JQ626623	JQ626584	JQ626597	sterile
AKWA58.8-0708	058_BearCreek	Alaska	N63.61533	W-143.98484	2008	JQ626616	JQ626626	JQ626587	JQ626599	sterile
AKWA73.2-0708	073_DonnellyCreek	Alaska	N63.67634	W-145.88079	2008	JQ626617	JQ626628	JQ626589	JQ626601	Partially heterothallic[b]
AKWA57.2-0708	057_TananaRiver	Alaska	N63.38738	W-143.74307	2008	JQ626614	JQ626624	JQ626585	JQ626598	sterile
P. riparia										
AKWA26.9-0708	026_Mendeltna	Alaska	N62.04842	W-146.53833	2008	JQ626607	JQ626621	JQ626582	JQ626595	Sterile
AKWA29.1-0708	029_PippinLake	Alaska	N61.71368	W-145.16108	2008	—[e]	—	—	—	Sterile
AKWA41.4-0708	041_TesoroStation	Alaska	N61.93944	W-147.16706	2008	—	—	—	—	Sterile
AKWA45.2-0708	045_TulsonaCreek	Alaska	N62.43081	W-144.96327	2008	—	—	—	—	Sterile
AKWA56.1-0708	056_MoonLakeStaRecArea	Alaska	N63.37601	W-143.54098	2008	—	—	—	—	Sterile
AKWA61.1-0708	066_BirchLakeRecArea	Alaska	N64.31252	W-146.64297	2008	—	—	—	—	Sterile
AKWA67.1-0708	067_SalchaRiver	Alaska	N64.46992	W-146.93108	2008	JQ626608	JQ626627	JQ626588	JQ626600	Sterile
I 3.50B1F	Oak Creek	Oregon	N44.566593	W-123.300984	2008	JQ626610	JQ626630	JQ626591	JQ626603	Sterile
I 4-100A1F	Marys River	Oregon	N44.555922	W-123.265328	2008	—	—	—	—	Sterile
IV 3-B3L	Oak Creek	Oregon	N44.566593	W-123.300984	2008	—	—	—	—	Sterile
IV 5-200B3F	Willamette River	Oregon	N44.567113	W-123.25563	2008	—	—	—	—	Sterile
VI 3-100B9F[c]	Oak Creek	Oregon	N44.566593	W-123.300984	2008	JQ626618	JQ626632	JQ626580	HM004225	Sterile
208-W-2.6	Little Butte Creek	Oregon	N42.45435	W-122.86445	2010	JQ626606	JQ626620	JQ626581	JQ626594	Sterile
SOC2.7.29	Soctish Creek	California	N41.08650	W-123.709188	2004	JQ626611	JQ626631	JQ626592	JQ626604	Sterile
EBE1.8.27[d]	S Fork east branch Eel	California	N40.071996	W-123.788066	2004	JQ626609	JQ626629	JQ626590	JQ626602	Sterile

[a] *P. borealis* type.

[b] Oogonia occasionally formed in pairings with *P. cryptogea* A2 tester.

[c] *P. riparia* type.

[d] *P.* taxon Salixsoil hybrid.

[e] Sequence not submitted to GenBank.

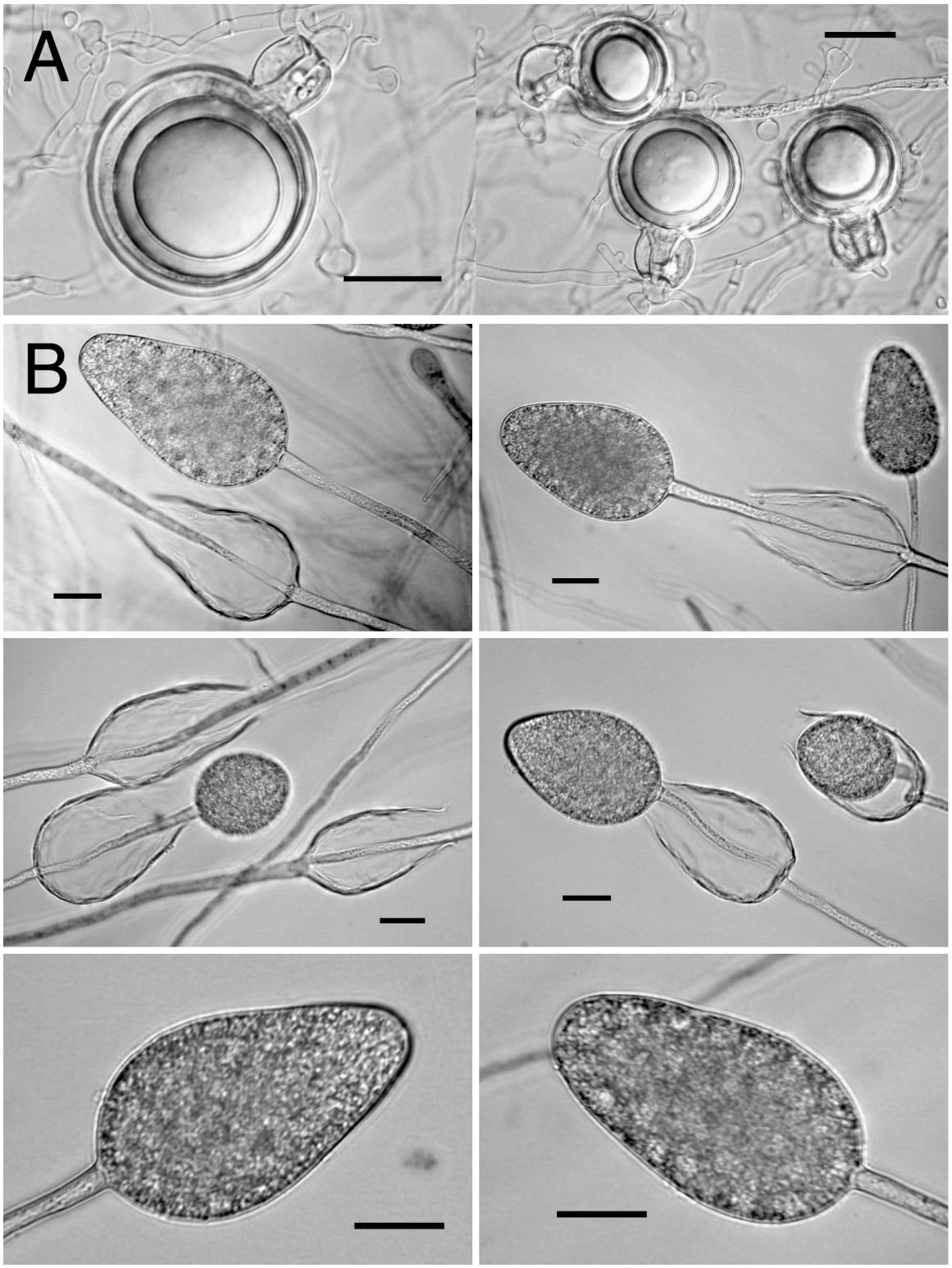

FIG. 3. Morphology of *Phytophthora borealis* (bar = 20μm). A. Oogonia, amphigynous antheridia and oospores formed by isolate AKWA 72.3-0708 when paired with A2 tester of *P. cryptogea*. B. Non-papillate sporangia with internal proliferation formed by isolate AKWA 58.1-0708.

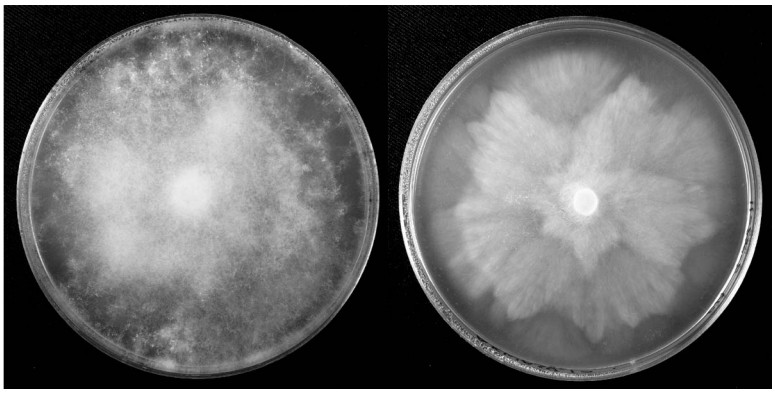

FIG. 4. Colony development by *P. borealis* isolate AKWA58.1-0708 on V8S (left) and CA (right) at 15 d.

gonapodyides. It also was identified in multiple water samples and a single riparian soil sample from western Oregon (Reeser et al. 2011, Sims et al. unpubl) and California streams. It did not appear in the extensive sampling from SW Oregon. *Phytophthora riparia* was referred to as *P.* New species 2 in Reeser et al (2011).

DISCUSSION

Phytophthora borealis and *P. riparia* are similar in appearance to other Clade 6 species, although they are phylogenetically distinct. The relatively low optimum growth temperature of *P. borealis* is distinctive, and sporangia of this species are larger than others on average.

Description of *Phytophthora borealis* and *P. riparia* brings the total number of described species in Clade 6 in Oregon and Alaska streams to five. Descriptions for two species (*P.* taxon Oaksoil and *P.* taxon Pgchlamydo), also abundant in our collections, are in process. Collectively these Clade 6 taxa account for 83% of all *Phytophthora* isolates from Oregon and Alaska streams (Reeser et al. 2011). These species are diverse, and they are abundant in stream water, but they are not overtly pathogenic; at least they have not been associated with disease in adjacent riparian ecosystems. The ecology of these new species and the other species in the clade remains to be elucidated. The low temperature optimum for growth of *P. borealis*, especially unusual in Clade 6, might be an adaptation for aquatic life in northern, snow-fed streams. Such speculation must be tempered, however, because *P. riparia* with a growth optimum near 30 C, more typical for Clade 6, was still more abundant in the same Alaska streams. It will be interesting to compare the Alaskan *Phytophthora* assemblage to those found in other stream collections from similar northern or southern latitudes.

Many species in *Phytophthora* Clade 6, including *Phytophthora borealis* and *P. riparia*, present very similar morphological and behavioral profiles. They are ''good'' phylogenetic species and often can be distinguished by subtle differences in growth pattern, but they are difficult to identify reliably without DNA sequencing tools. They undoubtedly have been misidentified, as *P. drechsleri* or *P. cryptogea* (Hansen et al. 1988), or lumped with *P. gonapodyides* (Brasier et al. 1993). Speciation processes remain puzzling in these clonal lineages as do the selective processes that maintain species identities. Our limited understanding is highlighted by the single ''hybrid'' isolate described here.

Isolate EBE1.8.27 is plausibly the product of a somatic fusion between individuals of *P. riparia* and *P.* taxon Salixsoil. It clusters with *P. riparia* in the ITS phylogeny, but β-tubulin sequence mixes nucleotides from both parents, and *cox* I sequence is identical to *P.* taxon Salixsoil. This isolate is perhaps the product of the sorting out of organelles after the chance meeting and fusion of two zoospores. Given the opportunity for such meetings between these two common species in forest streams, and the demonstrated possibility for subsequent hybridizations between individuals, the larger mystery is probably the nature of the forces that counter the homogenizing influence of hybridization, maintaining genetically discrete populations.

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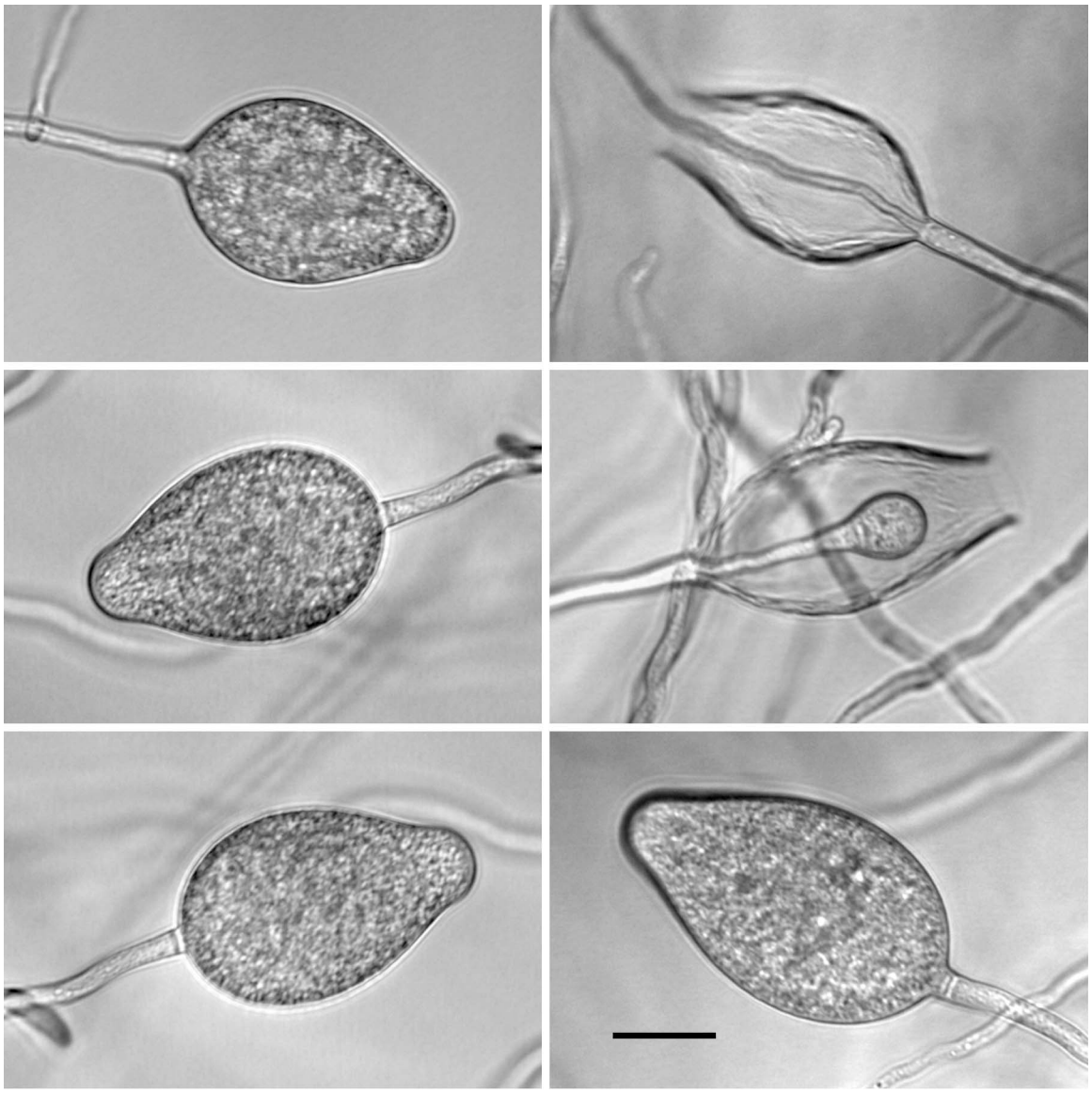

FIG. 5. Sporangia of *P. riparia* isolate VI 3-100B9F with internal proliferation. Bar = 20 μm.

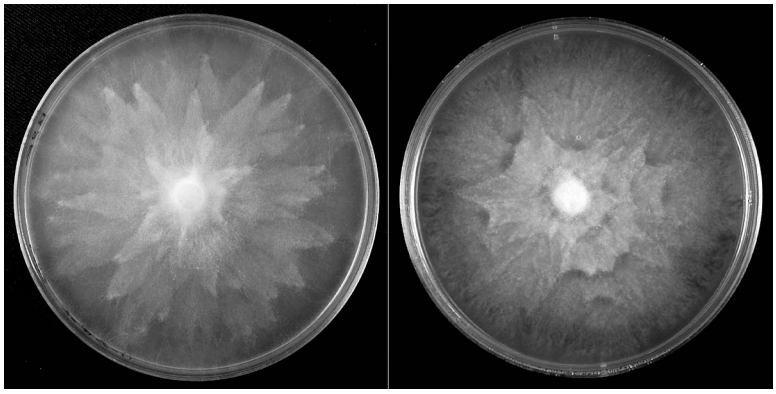

FIG. 6. Colony development by *P. riparia* isolate VI 3-100B9F on V8S (left) and CA (right) at 15 d.

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