Mycologia, 99(5), 2007, pp. 639–643.

Issued 8 January 2008

Phytophthora siskiyouensis, a new species from soil, water, myrtlewood (*Umbellularia californica*) and tanoak (*Lithocarpus densiflorus*) in southwestern Oregon

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Abstract: An unknown *Phytophthora* species was recovered in southwestern Oregon from rhododendron and tanoak leaf baits used for monitoring streams and soils for the presence of *Phytophthora ramorum*, from a blighted shoot of myrtlewood and from tanoak bark cankers. Isolates of this species yielded ITS-DNA sequences that differed substantially from other *Phytophthora* sequences in GenBank. Morphological features also differed from available descriptions of known *Phytophthora* species. Based on the combination of unique morphology and unique ITS sequences a new species is proposed. The new species, *Phytophthora siskiyouensis*, is homothallic with globose to subglobose oogonia, which may be terminal, sessile or laterally intercalary. Antheridia are capitate and mostly paragynous but sometimes amphigynous. Oospores are mostly aplerotic. Sporangia are variable but commonly ovoid to reniform, with apical, subapical or lateral semipapillae (occasionally more than one). Sporangia are terminal, subterminal or occasionally intercalary on unbranched sporangiophores, with basal, subbasal or lateral attachment. Sporangia are weakly deciduous, with variable length pedicels. This combination of characters clearly separates *Phytophthora siskiyouensis* from other known *Phytophthora* species.

Key words: oomycetes, phylogenetic clade 2

INTRODUCTION

Phytophthora species are well known as pathogens of agricultural crops and of some forest species. Ongoing surveys of *Phytophthora* species present in plants, streams and soils in the sudden oak death epidemic regions of Oregon and California are designed to develop some understanding of the diversity of *Phytophthora* species in natural environments. Undescribed or unexpected *Phytophthora* species have been reported from these surveys, including *Phytophthora nemorosa* from stem cankers in tanoak (*Lithocarpus densiflorus*) (Hansen et al 2003) and *P. pseudosyringae* from leaf lesions on myrtlewood (*Umbellularia californica*) (Martin and Tooley 2003).

We accumulated a large and diverse collection of unidentified *Phytophthora* isolates during these monitoring surveys of soil and water in SW Oregon. The collection of isolates initially was sorted by a modified single strand conformational polymorphism (SSCP) procedure (Kong et al 2003) of nuclear ribosomal internal transcribed spacer (ITS) and mitochondrial cytochrome oxidase (COX) spacer DNA fragments using capillary array electrophoresis to separate and visualize DNA fragment migration patterns (Kuhn and Schnell 2005). A number of isolates clustered in a unique group. ITS sequences from these isolates were identical and had no match in GenBank. When the isolates were cultured and examined they exhibited shared morphological features consistent with a single new species.

In this paper we describe *Phytophthora siskiyouensis*, which is found in soil and water in forested areas of coastal southwest Oregon. The new species also is found rarely in association with diseased myrtlewood and tanoak.

MATERIALS AND METHODS

Streams were sampled by immersing whole leaf baits (tanoak, myrtlewood, *Viburnum davidii* or *Rhododendron macrophyllum*) for 2 wk. Soils were sampled by flooding with deionized water and floating leaf bait pieces (*Rhododendron* sp. and *Chamaecyparis lawsoniana*) for 2–4 d or semi-immersing whole green pears for up to 1 wk. Isolates of *Phytophthora* were recovered from leaf bait or pear bait pieces plated in cornmeal agar (CMA) amended with 10 ppm natamycin (Delvocid®), 200 ppm Na-ampicillin, 10 ppm rifampicin, 25 ppm Benlate and 25 ppm Hymexazol (CARPBH). Additional isolates were recovered from diseased shoots of myrtlewood and from the margins of tanoak bark lesions plated in CMA amended with 10 ppm natamycin (Delvocid®), 200 ppm Na-ampicillin, and 10 ppm rifampicin (CARP). Isolates were grown on CMA amended with 20 ppm β-sitosterol (CMAβ) to obtain mycelium for DNA extraction and for storage as agar plugs in sterile de-ionized water.

Cultures for morphological study were grown on clarified V8 agar amended with 20 ppm β-sitosterol (V8S). Oogonia and oospores were observed on 20–30 d old cultures fixed

Accepted for publication 29 July 2007.
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and preserved in 3.7% formaldehyde. Sporangia were produced by transferring disks from margins of mycelium grown on V8S into natural stream water. Sporangia were fixed and preserved in 3.7% formaldehyde, 10% acetic acid, 0.03% gelatin in 0.05M Na-K buffer (FA-PBG). Specimens were mounted in the fixative and viewed with brightfield illumination on a Zeiss standard microscope with a 63× objective and measured with an eyepiece micrometer.

DNA extracts from eight *P. siskiyouensis* isolates were amplified with primers DC6 and ITS4 (Cooke et al 2000). Amplified products were sequenced with DC6, ITS2, ITS3 and ITS4 as sequencing primers (White et al 1990). All *P. siskiyouensis* isolates yielded identical sequences. Published sequences of other *Phytophthora* species were downloaded from GenBank and aligned with Clustal X v1.81. The phylogenetic tree (FIG. 8) was generated with TreeView (Win32).

TAXONOMY

Phytophthora siskiyouensis Reeser et E. M. Hansen, sp. nov.

Homothallis, oosporis in culturis procreans, antheridiis pro parte maxima paragyneis. Oogonia globosa ad ovoidea, in medio 28(25–30) μm diam. Sporangia in culturis aquosis procreans, in forma variabilia, ovoidea, reniform, et irregularia, semi-papillata, in medio 55 × 36 μm; ratio longitudo ad altitudinem in medio 1.5. Coloniis in agaro crescentibus 7.5 mm/d 25 C.

Type: WA5-030403, collected in Mar 2003 from a seasonal tributary to the Chetco River, Curry County, Oregon. ATCC MYA-4187, GenBank EF523386.

Etymology: ''siskiyouensis'' refers to the Siskiyou Mountains in SW Oregon where the new species was first identified.

Sporangia (FIGS. 1–3) were formed sparsely in V8S and abundantly on agar culture pieces placed in stream water. Sporangia varied widely in shape but were typically ovoid, reniform or some misshapen variant of these. Sporangia were scarcely semipapillate with prominent thickening. Semipapillae were applied apically, subapically or laterally, occasionally with two and more rarely with three semipapillae. Sporangiophores were simple, unbranched, short or long, attached basally, subbasally or laterally to the sporangium. A small hyphal swelling often was associated with the sporangiophore. Sporangia were typically terminal but were often subterminal and occasionally intercalary, and weakly deciduous with variable pedicel length (average 29 μm, individual pedicels 0–133 μm.) Sporangia for individual isolates averaged 46–70 μm long and 30–51 μm wide, (overall average 55 × 36 μm). Overall length to breadth ratio was 1.5, with a range of 1.1–2.0 for individual isolates.

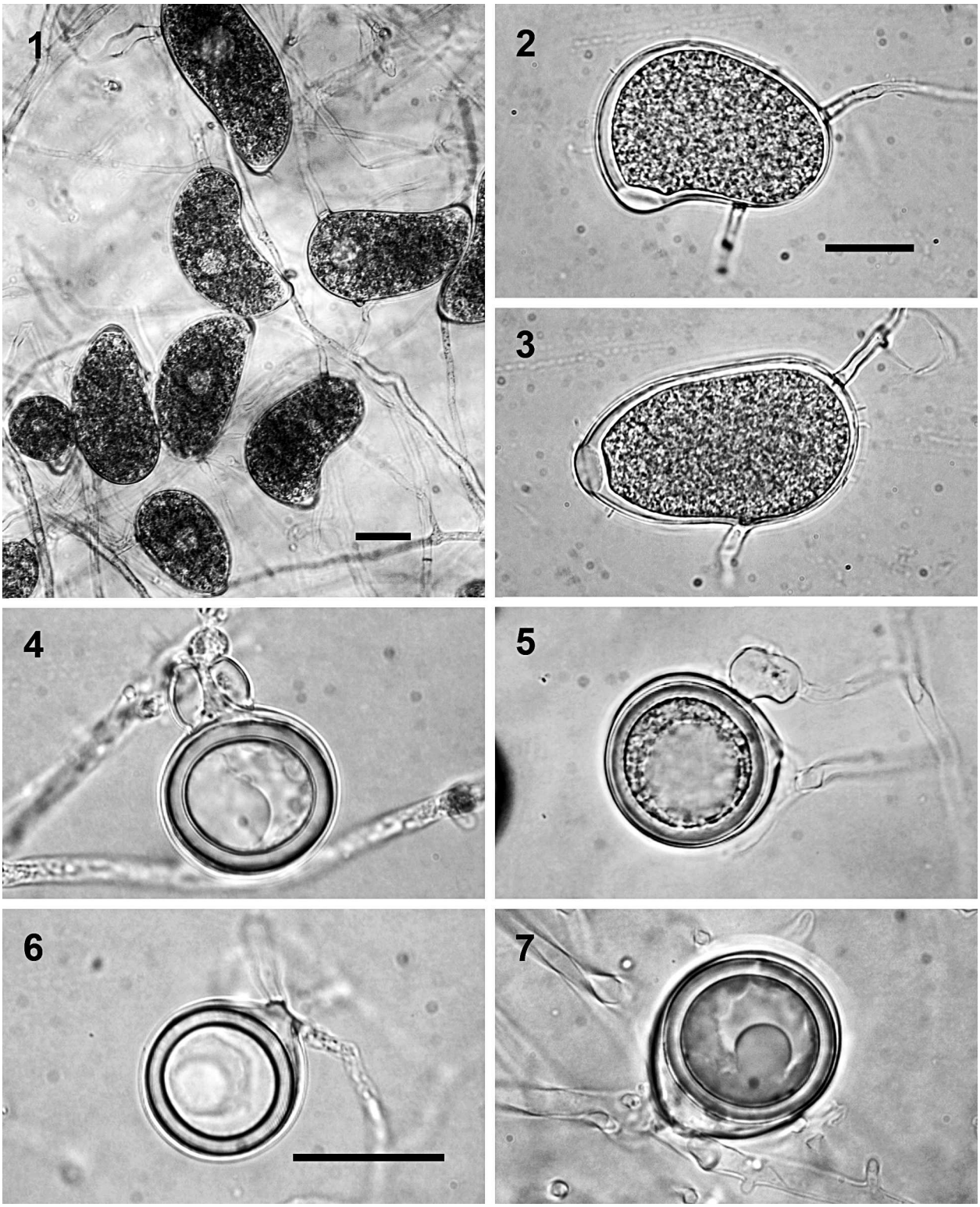
Oogonia (FIGS. 4–7) were formed abundantly on V8S in single culture and were usually globose to subglobose (or oblate), occasionally much elongated or with a funnel shape tapering toward the stalk. Oogonia were terminal on long or short stalks (often bent or kinked) and were frequently sessile and occasionally laterally intercalary. Average oogonial diameter for individual isolates was 24.8–30.3 μm (overall average 27.8 μm). Antheridia were predominately paragynous and capitate, with around 10% amphigynous. Antheridia were typically terminal, occasionally intercalary and usually diclinous, attached anywhere on the oogonium. Antheridia averaged 8.6–11.6 μm wide by 9.5–13.3 μm long. Oospores were globose to subglobose and usually aplerotic, sometimes markedly so in subglobose oogonia. Average oospore diameter for individual isolates was 22.5–25.8 μm (overall average 24.6 μm). Hyphal swellings or chlamydospores were not observed.

Colonies on V8S were submerged with a faint radiate pattern and little or no aerial mycelium. Optimal temperature for growth of the nine isolates tested was 25 C. Radial growth rate averaged 7.5 mm/d at 25 C (individual isolates were 6.2–8.5 mm/d at 25 C). Growth was slow at 5 C (average 0.84 mm/d) and almost nonexistent at 30 C (average 0.13 mm/d). Cultures that did not grow at 5 C or 30 C recovered when removed to 20 C. No growth was observed at 35 C, and cultures did not recover when removed to 20 C.

DISCUSSION

With its semipapillate sporangia and predominately paragynous antheridia, *Phytophthora siskiyouensis* would be placed in Waterhouse's (1963) Group III, and its irregular sporangia are reminiscent of *P. citricola*. It is distinguished readily from this species however by having deciduous sporangia with variable-length pedicels, variable orientation of semipapilla and sporangiophore attachment, intercalary and sessile attachment of some oogonia and mostly aplerotic oospores. *P. siskiyouensis* sporangia more resemble those depicted for *P. quercina*, (Jung et al 1999) except that they are slightly larger, semipapillate, weakly deciduous, and are formed singly on simple, unbranched sporangiophores. Sexual structures also resemble *P. quercina*, except that oogonia may be sessile or intercalary, and antheridia may be paragynous or amphigynous. The oogonial stalk and arrangement of paragynous antheridia are similar to those described for *P. hedraiandra* (de Cock and Levesque 2004).

ITS DNA sequence analysis places *P. siskiyouensis* in phylogenetic clade 2 of Cooke et al (2000). All eight isolates sequenced were identical. BLAST of GenBank



FIGS. 1–3. Sporangia of *Phytophthora siskiyouensis*. 1. Variety of sporangial shapes. 2. Reniform sporangium showing lateral semipapilla, subterminal insertion, subbasal attachment to sporangiophore. 3. Ovoid sporangium with apical semipapilla. FIGS. 4–7. Oogonia and oospores of *Phytophthora siskiyouensis*. 4. Terminal oogonium on short oogonial stalk and amphigynous antheridium. 5. Terminal oogonium on long oogonial stalk with paragynous, capitate, diclinous antheridium. 6. Subterminal, laterally intercalary insertion of oogonium. 7. Sessile oogonium. Bars = 20 μm.

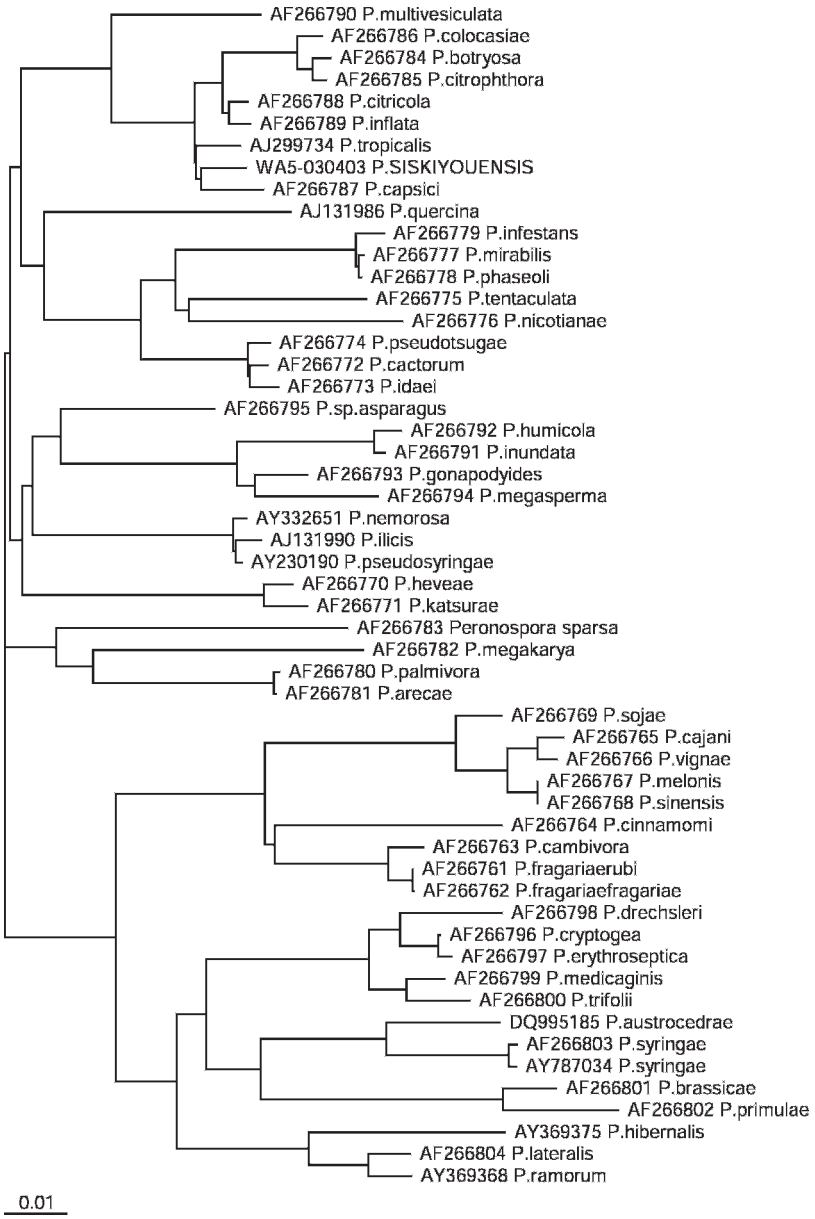


FIG. 8. Placement of *P. siskiyouensis* within the genus based on phylogenetic analysis of ITS rDNA sequences. Published sequences of other *Phytophthora* species were downloaded from GenBank and aligned with Clustal X v1.81. The phylogenetic tree was generated with TreeView (Win32).

ITS sequences showed greatest similarity to *P. tropicalis* and *P. capsici* but consistent differences were found among *P. siskiyouensis* and these related species. *P. siskiyouensis* is distinguished morphologically from these relatives (Aragaki and Uchida 2001) by its homothallic condition, predominately paragynous antheridia and semipapillate sporangia on unbranched sporangiophores.

Most species of *Phytophthora* historically were discovered by observation on or isolation from diseased plants. Little is known of endemic *Phytophthora* species in natural settings, especially in environments where disease is not evident. *Phytophthora siskiyouensis* was discovered first from streams and soil in areas dominated by native forest, and most isolates continue to come from these sources. Only later, and still infrequently, were isolates of the new species recovered from bole cankers on tanoak and only once from necrotic sprouts on myrtlewood. It appears to be part of the native forest mycobiota of SW Oregon, associated with occasional symptoms on a variety of plants. Studies are under way to determine the pathogenicity of *P. siskiyouensis* on tanoak and will be published later as part of a more general study of *Phytophthora* species associated with tanoak stem cankers.

TABLE I. List of isolates used in the study. All isolates were found in Curry County, Oregon

Isolate	Source	Year	GenBank	ATCC
33-2.7-soil-0603	soil	2003	EF523387	—
33-2-3.2-1102	soil	2002	—	—
WA1.1-021903	water	2003	—	—
WA1-030403	water	2003	—	—
WA1-051704	water	2004	—	—
WA4-111302	water	2002	—	—
WA4.6-031803	water	2003	—	—
WA5-030403 (type)	water	2003	EF523386	MYA-4187
WA32-040504	water	2004	—	—
4399.1	*Umbellularia californica*	2003	EF490683	—
9569	*Lithocarpus densiflorus*	2006	EF490684	—
9585.2	*Lithocarpus densiflorus*	2006	EF490682	—

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