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Mycological Research News[1]

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Preserved specimens suggest non-native origins of three species of *Phytophthora* in California

Phytophthora ramorum, which causes the foliar and canker disease Sudden oak death (SOD), was first detected in North America in 1995 following an extensive dieback of tanoak (*Lithocarpus densiflorus*) in Marin County, California (Garbelotto *et al.* 2001). Genetic studies identified a single mating type with mostly clonal population structure (Ivors *et al.* 2006), suggesting that *P. ramorum* was likely introduced to the USA, possibly through the nursery trade (Brasier 2003). At least two other recently described species of *Phytophthora* (*P. nemorosa* and *P. pseudosyringae*) cause limited tree mortality but identical foliar symptoms in tanoak and California bay laurel (*Umbellularia californica*) (Martin & Tooley 2003). Unlike *P. ramorum*, *P. nemorosa* and *P. pseudosyringae* are hypothesized to be of native origin (Hansen *et al.* 2003). We used PCR on specimens in the University and Jepson Herbaria of the University of California (UC), Berkeley to examine whether any of the three species of *Phytophthora* were present historically in California.

We focused our analysis on *L. densiflorus* and *U. californica*, two major host tree species that are common throughout the contemporary California range of *P. ramorum* (Rizzo & Garbelotto 2003). We visually inspected 225 *L. densiflorus* and 193 *U. californica* specimens, and collected foliar samples from 50 different trees (6 *L. densiflorus*, 44 *U. californica*[2]). For each tree species, half of the samples originated from symptomatic and half from asymptomatic ones. In an effort to control for false positives, we paired each symptomatic specimen with an asymptomatic negative control specimen of the same species. Symptomatic and asymptomatic specimens were collected between 1861 and 1984 in 21 of the 58 counties in California, including 11 of the 14 counties where SOD is now present (Rizzo *et al.* 2002).

Using sterile techniques, we destructively sampled specimens for genetic analysis by clipping two 1 cm^2 sections of dried leaf (asymptomatic) or lesion-leaf interface (symptomatic), generally from two different leaves on the specimen. We estimate from prior field and laboratory studies that our sample sizes were sufficient to detect *Phytophthora* spp. in approximately 15 (60 %) of the symptomatic specimens (Garbelotto, unpubl.). Each sample was split prior to being pulverized and extracted using two different methods (Kong *et al.* 2007; Guglielmo *et al.* 2007). Herbarium extracts were screened for *P. ramorum* using a nested TaqMan assay capable of detecting the pathogen from as little 15 fg of target DNA (Hayden *et al.* 2006), and further screened for congenerics using ribosomal primers ITS1, ITS4, and DC6 (Cooke *et al.* 2000; Hansen *et al.* 2003). We accounted for false negatives by ensuring reliable amplification of both the plant and pathogen DNA after spiking the extracts with trace amounts of *P. ramorum* DNA.

All 25 symptomatic and 25 asymptomatic preserved herbarium specimens were negative for *P. ramorum*, *P. nemorosa*, and *P. pseudosyringae*; our amplification controls suggest that these were true negatives rather than false negatives resulting from high concentrations of either PCR inhibitors or fragmented DNA. These results corroborate findings by Linzer *et al.* (unpubl.[3]), which suggest non-native origins of *P. nemorosa* and *P. pseudosyringae* based on low modern AFLP diversity. Combined, these studies bolster support for the management of all three species of *Phytophthora* as recently

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[2] *Lithocarpus densiflorus*: UC 37737, 37746, 46233, 46308, 1076091, and 1135288. *Umbellularia californica*: UC 9862, 9876, 40104, 40111, 40117, 40119, 76953, 136028, 150087, 185550, 220707, 455309, 483307, 571574, 571579, 581348, 589385, 615102, 635499, 635504, 691943, 750643, 765968, 765969, 854883, 917183, 1094043, 1126314, 1126326, 1126331, 1126333, 1126339, 1177379, 1189990, 1193223, 1196278, 1196280, 1219834, 1222167, 1244743, 1350970, 1506117, 1576850, and 1608894.

[3] Paper submitted and currently under review.

introduced forest pathogens in California. The results also raise new and challenging questions concerning how best to detect and manage non-native *Phytophthora*'s that vary so dramatically in distribution and virulence.

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New scientific names in this issue

Alphamyces gen. nov.
A. chaetiferum comb. nov. (syn. *Rhizophydium chaetiferum*)
Alphamycetaceae fam. nov.
Angulomyces gen. nov.
A. argentinensis sp. nov.
Angulomycetaceae fam. nov.
Aquamyces gen. nov.
A. chlorogonii comb. nov. (syn. *Phylctidium chlorogonii*)
Aquamycetaceae fam. nov.
Coniolariella hispanica sp. nov.
C. limoniispora comb. nov. (syn. *Rosellinia limoniispora*)
C. limoniispora var. *australis* comb. nov. (syn. *Rosellinia australis*)
C. limoniispora var. *gamsii* (syn. *Coniochaeta gamsii*)
Globomyces gen. nov.
G. pollinis-pini comb. nov. (syn. *Chytridium pollinis-pini*)
Globomycetaceae fam. nov.
Gorgonomyces gen. nov.
G. haynaldii comb. nov. (syn. *Phlyctidium haynaldii*)
Gorgonomycetaceae fam. nov.
Lecanicillium flavidum comb. nov. (syn. *Verticillium fungicola* var. *flavidum*)
L. fungicola comb. nov. (syn. *Acrostalagmus fungicola*)
L. fungicola var. *aleophilum* comb. nov. (syn. *Verticillium fungicola* var. *aleophilum*)
L. wallacei comb. nov. (syn. *Simplicillium wallacei*)
Pateramyces gen. nov.
P. corrientinensis sp. nov.
Pateramycetaceae fam. nov.
Phytophthora parsiana sp. nov.
Protrudomyces gen. nov.
Protrudomyces laterale comb. nov. (syn. *Chytridium laterale*)
Protrudomycetaceae fam. nov.
Urceomyces gen. nov.
Urceomyces sphaerocarpum comb. nov. (syn. *Rhizidium sphaerocarpum*)

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