



Phytophthora species infesting soil
in a California forest affected by
sudden oak death

Tyler Bourret

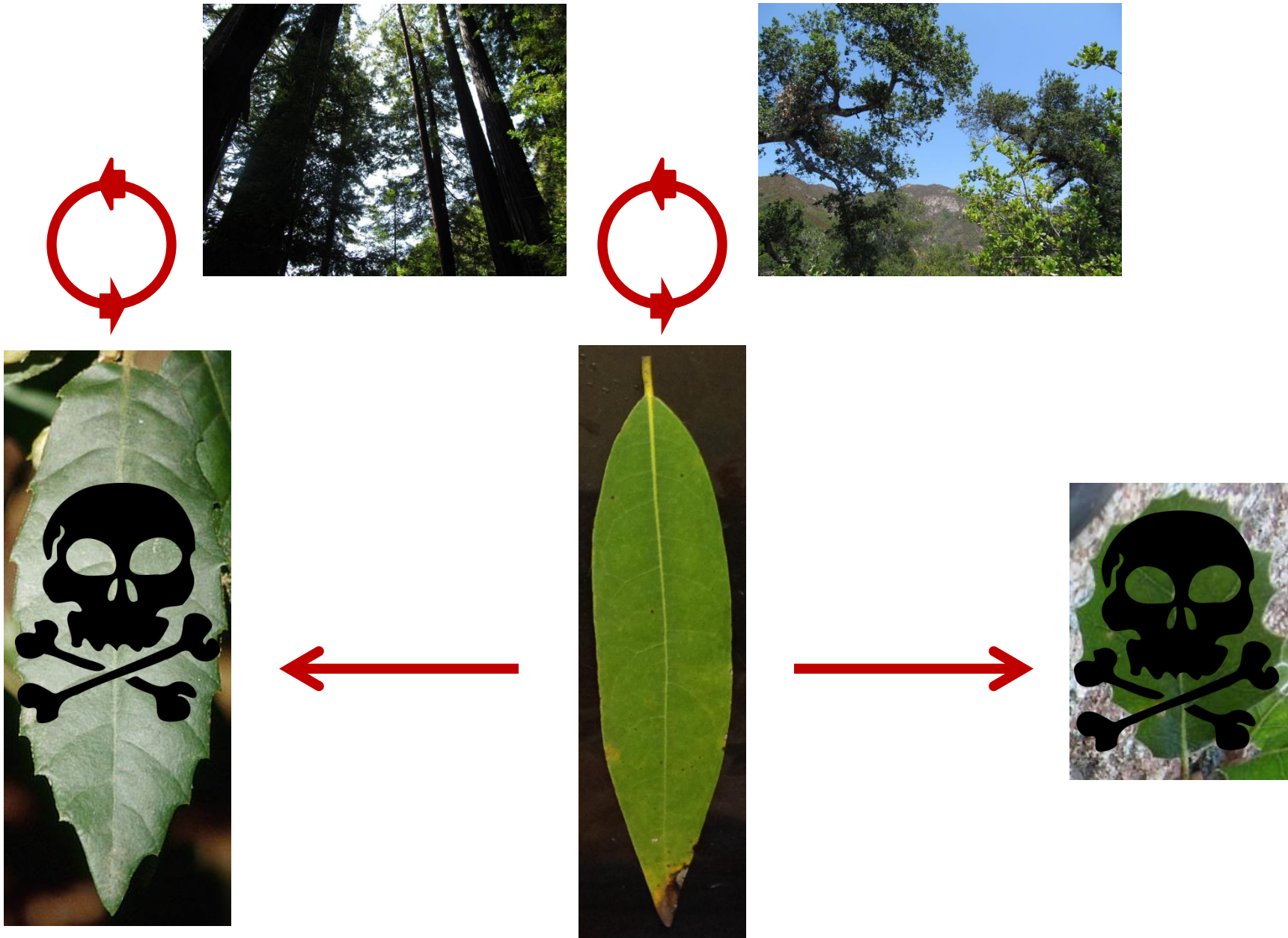
Rizzo Lab

UC Davis Plant Pathology

Sudden oak death (SOD)

- Caused by exotic oomycete *Phytophthora ramorum*
 - introduced to California via ornamental nursery trade
- Massive mortality of tanoak (*Notholithocarpus densiflorus*) and oaks (esp. *Quercus agrifolia*)
- California bay laurel (*Umbellularia californica*) supports high inoculum levels but is not negatively affected





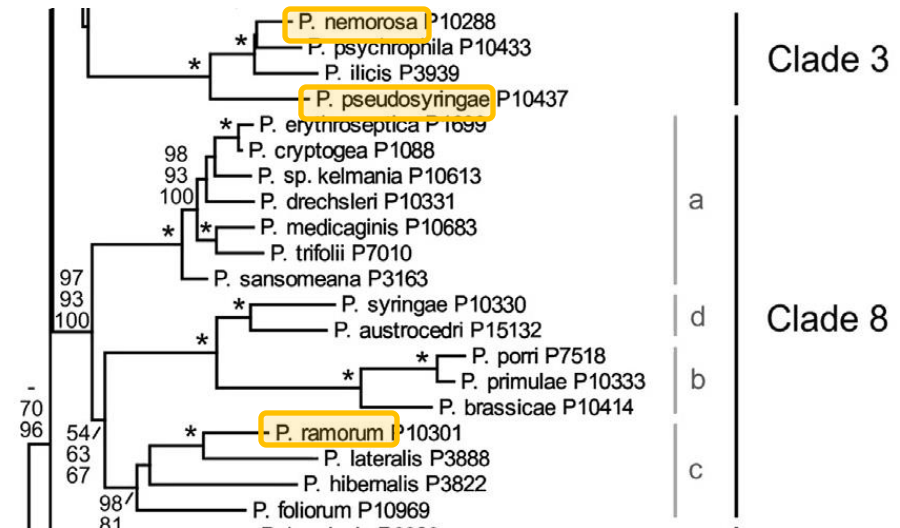
Tanoak

Bay

Live oak

Other species of *Phytophthora*

- Two other species co-occur with *P. ramorum*
- *P. pseudosyringae* and *P. nemorosa* cause identical symptoms on SOD hosts, but rarely tree mortality
- Unclear whether they are native to California



Big Sur SOD Plot network



- Plot network was established about a decade ago to study long-term effects of SOD
- 40 plots in redwood forest, 50 plots in mixed evergreen forests
- In 2008, two forest fires burned many of the plots

Effects of fire on *Phytophthora*

- Post-fire sampling revealed that *P. ramorum* persisted where infected bay trees survived
- *P. pseudosyringae* and *P. nemorosa* dominated post-fire resprouts, even in plots where they had never previously been detected
- Why did the fire have different effects on different species of *Phytophthora*?



Oospores in the soil?



- Oospores are the most resistant structures formed by *Phytophthora*
- *P. ramorum* is heterothallic, with only one mating present in CA forests
 - This means it can't complete its sexual cycle and form oospores
- *P. pseudosyringae* & *P. nemorosa* are homothallic, forming oospores readily without mating

Hypothesis & objectives

- If *P. pseudosyringae* & *P. nemorosa* survived the fire as oospores in the soil, they should be detectable in the soil of unburned plots
- Objective: Use Rhododendron leaves to “bait” *Phytophthora* out of soil
- Plots were to be visited during summer field seasons of 2013 & 2014
 - This meant sampling during an ongoing drought
- Plant tissue was also sampled from within plots



- The 40 plots containing coast redwood were sampled in 2013
- The 50 “mixed evergreen” plots were sampled in 2014

2013 results

<i>Phytophthora</i> species	Successful isolations	
	2013 (Redwood)	
	Plant Tissue	Soil Baiting n=360
<i>P. ramorum</i>	492	7
<i>P. pseudosyringae</i>	2	6
<i>P. nemorosa</i>	13	1
<i>P. "sequoiasoil"</i>		10
<i>P. syringae</i>		1
<i>P. chlamydospora</i>		1

- Due to the ongoing SOD epidemic, isolation of *P. ramorum* from foliage was extremely common
- *P. pseudosyringae* was more common in soil than in plant tissue
- Three plots yielded multiple species (up to 4)
- A previously undescribed species related to *P. cactorum* was most commonly baited
 - This species was given the placeholder taxon "sequoiasoil"

2014 results

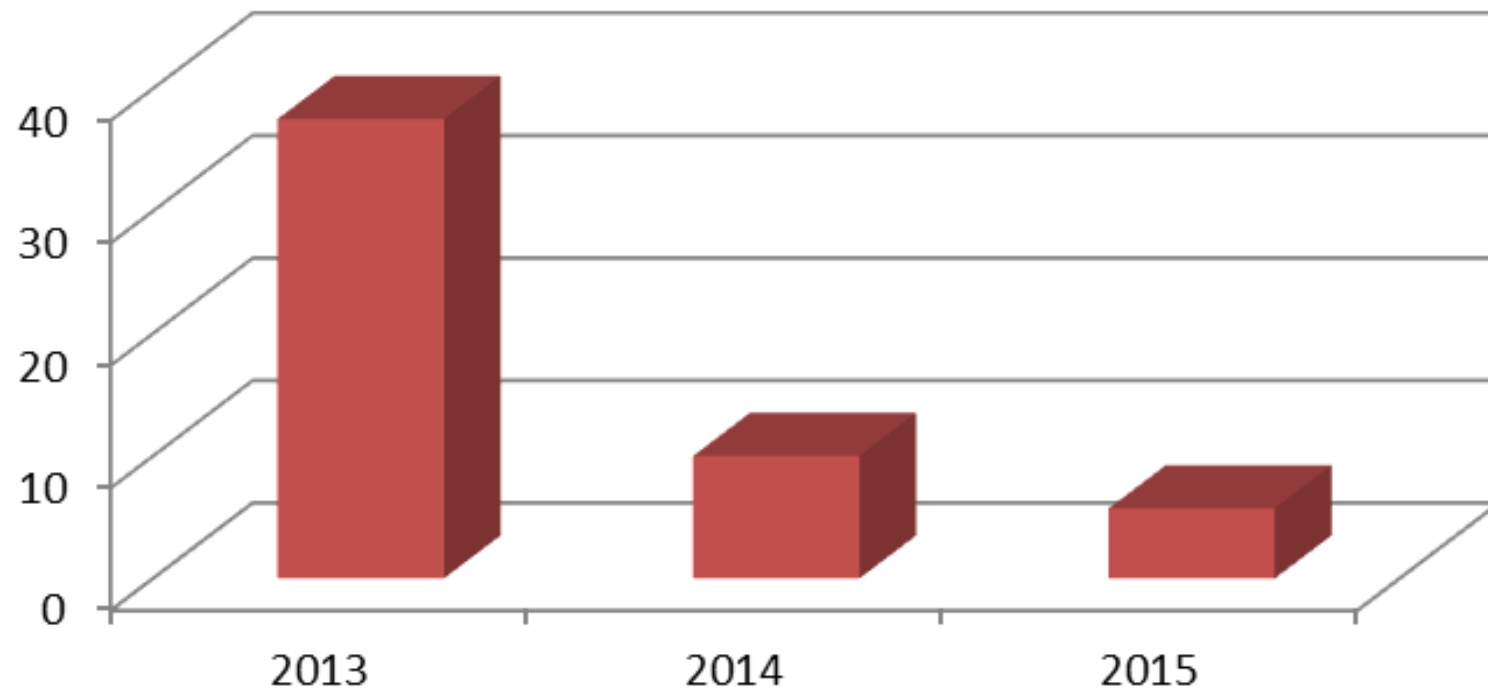
<i>Phytophthora</i> species	Successful isolations			
	2013 (Redwood)		2014 (Mixed evergreen)	
	Plant Tissue	Soil Baiting n=360	Plant Tissue	Soil Baiting n=300
<i>P. ramorum</i>	492	7	198	
<i>P. pseudosyringae</i>	2	6	3	4
<i>P. nemorosa</i>	13	1	1	
<i>P. "sequoiasoil"</i>		10		
<i>P. syringae</i>		1	1	2
<i>P. chlamydospora</i>		1		

- The drought increased to maximum severity in 2014
- *P. ramorum* was not recovered from soil
- Viable propagules of *P. pseudosyringae* were present in Big Sur forest soil during an extreme drought
 - These are likely the same propagules that allowed it to survive the 2008 fire
- Difficult to separate the effect of drought from the effect of forest type

<i>Phytophthora</i> species	Successful isolations					Total
	2013 (Redwood)		2014 (Mixed evergreen)		2015	
	Plant Tissue	Soil Baiting n=360	Plant Tissue	Soil Baiting n=300	Soil Baiting n=210	
<i>P. ramorum</i>	492	7	198		2	699
<i>P. pseudosyringae</i>	2	6	3	4		15
<i>P. nemorosa</i>	13	1	1			15
<i>P. "sequoiasoil"</i>		10				10
<i>P. syringae</i>		1	1	2		4
<i>P. chlamydospora</i>		1				1

- The drought continued into 2015
- Soil was baited from all 20 previously positive plots plus 15 previously negative plots

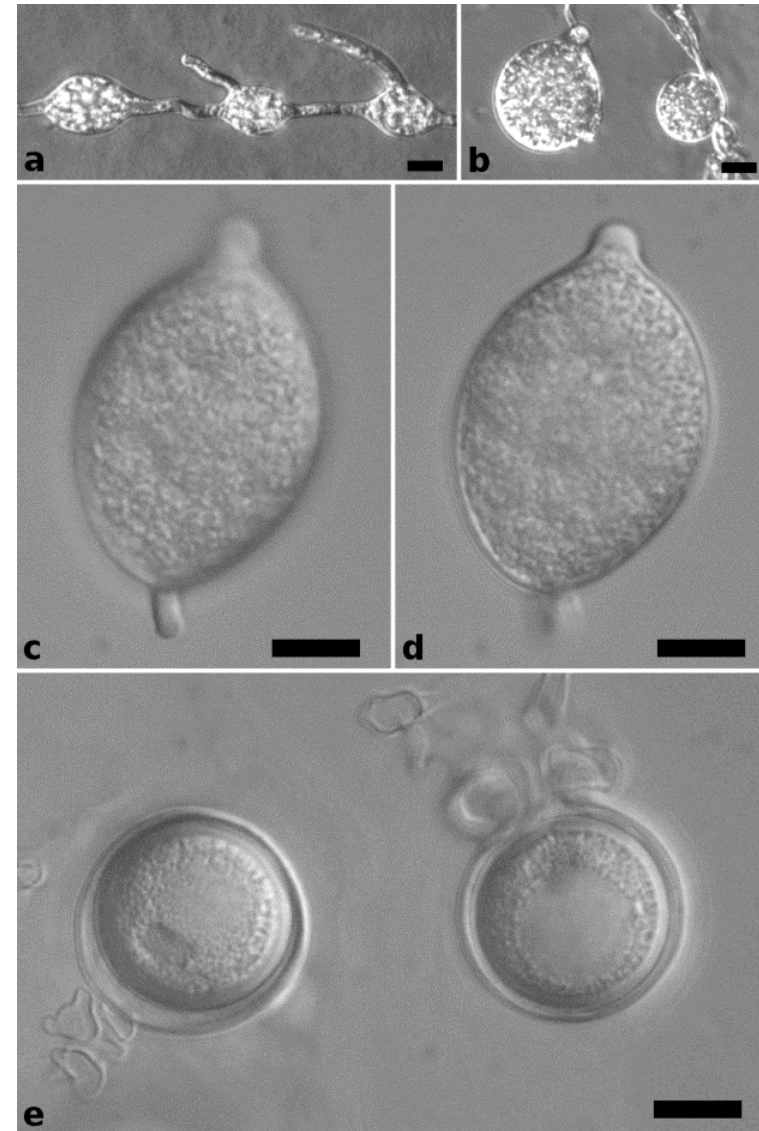
Percentage of plots from which *Phytophthora* was baited

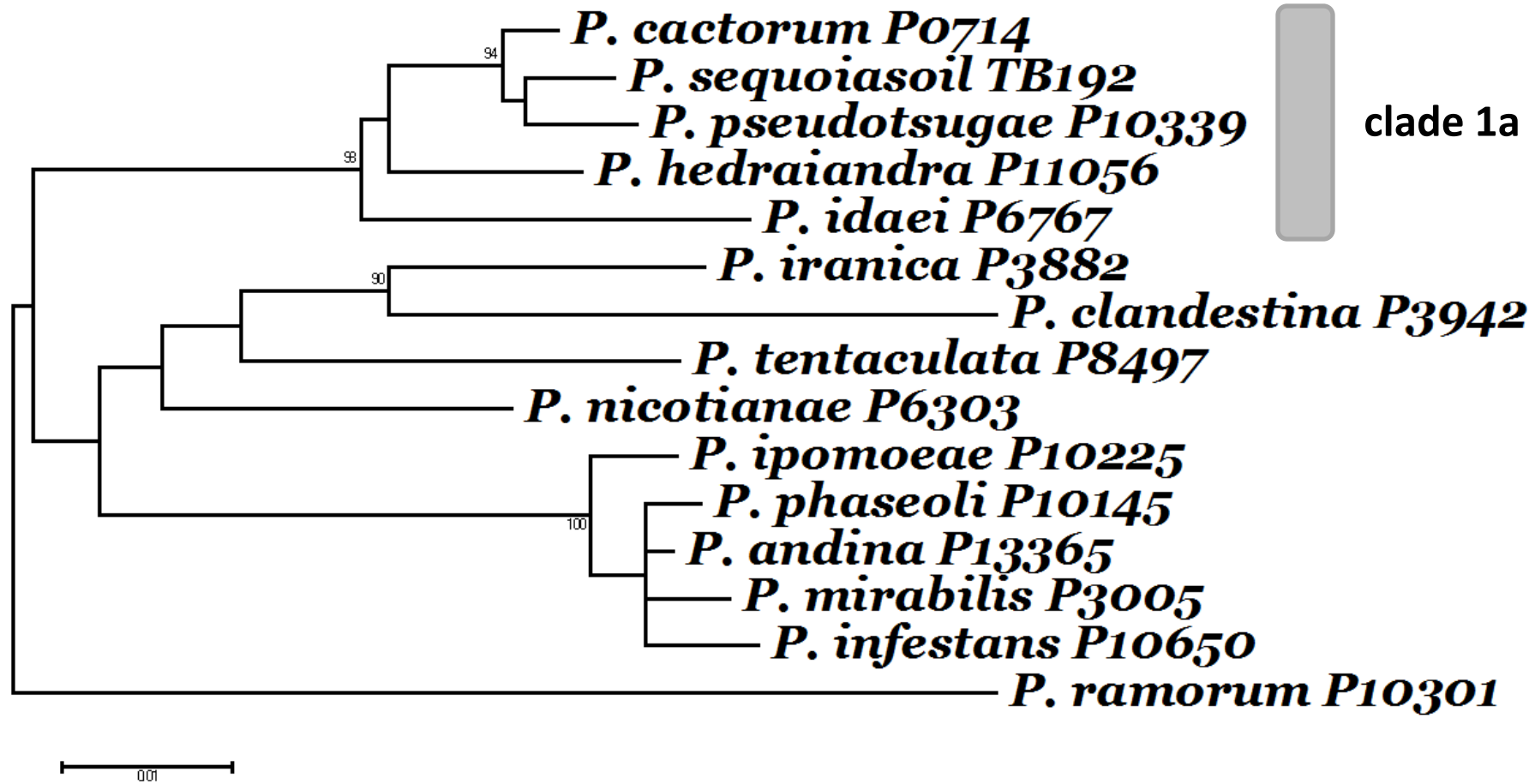


Phytophthora baited from $15/40 = 38\%$ of plots in 2013, $5/50 = 10\%$ of plots in 2014, $2/35 = 6\%$ of plots in 2015

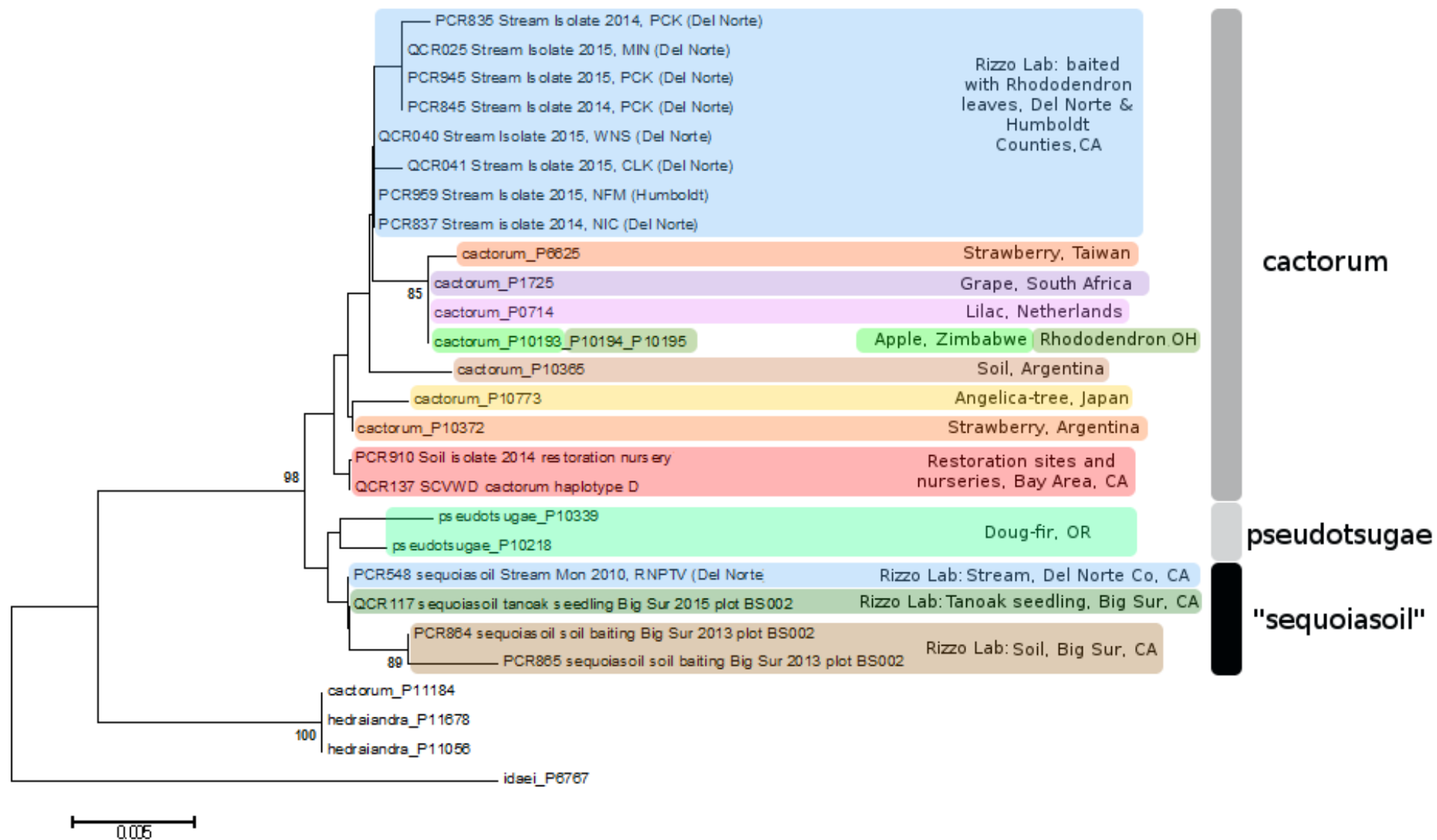
Ongoing work

- Correlate baiting success with edaphic factors, vegetation data & plot history
- Determine if the psychrophilic *P. nemorosa* can be reliably baited at room temperature
- Soil metagenomics
- In vitro investigations of spore hardiness: subject oospores to heat and desiccation stress followed by viability tests
- **Phylogenetic studies of *Phytophthora* clade 1a**





Maximum likelihood tree of *Phytophthora* clade 1 inferred from the mtCOXII locus. Support value percentages >70 from 1000 bootstrap iterations are shown. Tree created with MEGA6 using default settings with GTR+G nucleotide evolutionary model



Neighbor joining tree from mtCOXII + COXII-COXI spacer

Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases

T. Jung^{1,2,38}, L. Orlikowski³, B. Henricot⁴, P. Abad-Campos⁵, A. G. Aday⁶, O. Aguín Casal⁷, J. Bakonyi⁸, S. O. Cacciola⁹, T. Cech¹⁰, D. Chavarriaga¹¹, T. Corcobado¹², A. Cravador¹, T. Decourcelle¹³, G. Denton⁵, S. Diamandis¹⁴, H. T. Doğmuş-Lehtijärvi⁷, A. Franceschini¹⁵, B. Ginetti¹⁶, M. Glavendekić¹⁷, J. Hantula¹⁸, G. Hartmann¹⁹, M. Herrero²⁰, D. Ivic²¹, M. Horta Jung¹, A. Lilja¹⁸, N. Keca¹⁷, V. Kramarets²², A. Lyubenova²³, H. Machado²⁴, G. Magnano di San Lio⁹, P. J. Mansilla Vázquez⁷, B. Marçais²⁵, I. Matsiakh²², I. Milenkovic¹⁷, S. Moricca¹⁶, Z. Á. Nagy⁸, J. Nechwatal²⁶, C. Olsson²⁷, T. Oszako²⁸, A. Pane⁹, E. J. Paplomatas²⁹, C. Pintos Varela⁷, S. Prospero³⁰, C. Rial Martínez⁷, D. Rigling³⁰, C. Robin¹³, A. Rytönen¹⁸, M. E. Sánchez³¹, B. Scanu¹⁵, A. Schlenzig³², J. Schumacher³³, S. Slavov²³, A. Solla¹², E. Sousa²⁴, J. Stenlid²⁷, V. Talgø²⁰, Z. Tomic²¹, P. Tsopelas³⁴, A. Vannini³⁵, A. M. Vettraino³⁵, M. Wenneker³⁶, S. Woodward¹¹ and A. Pérez-Sierra³⁷

Discussion

- Variability within *P. “sequoiasoil”* suggest it is a native species
- *P. cactorum* represents many lineages;
 - isolates from streams in Northern CA form a unique lineage
 - so do Bay area nursery/restoration strains
- Are these lineages exotic or native?
- Is there gene flow?



Acknowledgments



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