

## Water sampling for Oomycetes

Oomycetes are fungus-like organisms found in marine, freshwater, and terrestrial environments. Some, such as *Phytophthora*, *Pythium*, and *Saprolegnia*, are parasites of plants and animals. DNA sequence data has revealed that these organisms are not fungi, but are more closely related to brown algae and diatoms. We will be “fishing” for these organisms by using baits from various plant species.

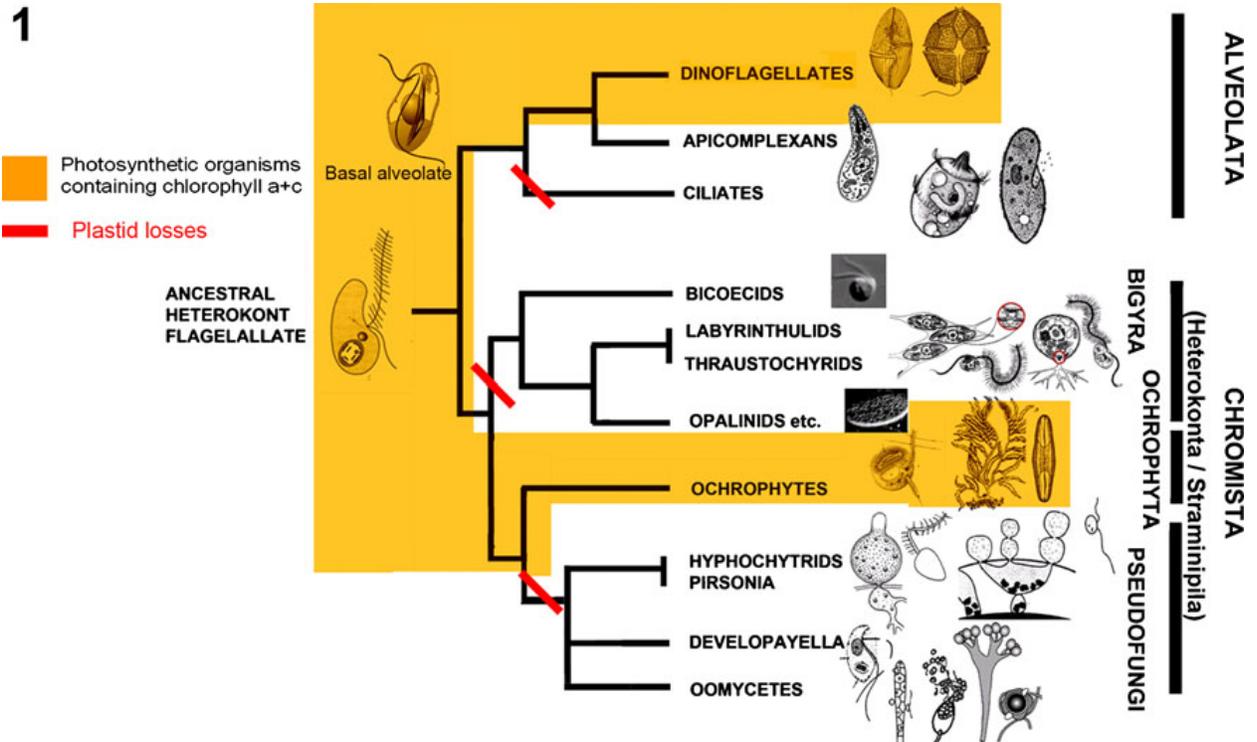


Fig. 1 Schematic phylogenetic tree summarizing the likely phylogenetic relationships between the diverse members of the “chromalveolate” superkingdom. The photosynthetic lines are shaded in orange and postulated plastid loss events indicated by the red bars. The terminology is mostly taken from Cavalier-Smith and Chao 2006 (2006) and the tree based on a phylogenetic analysis of conserved protein genes by Tsui et al. (2006)

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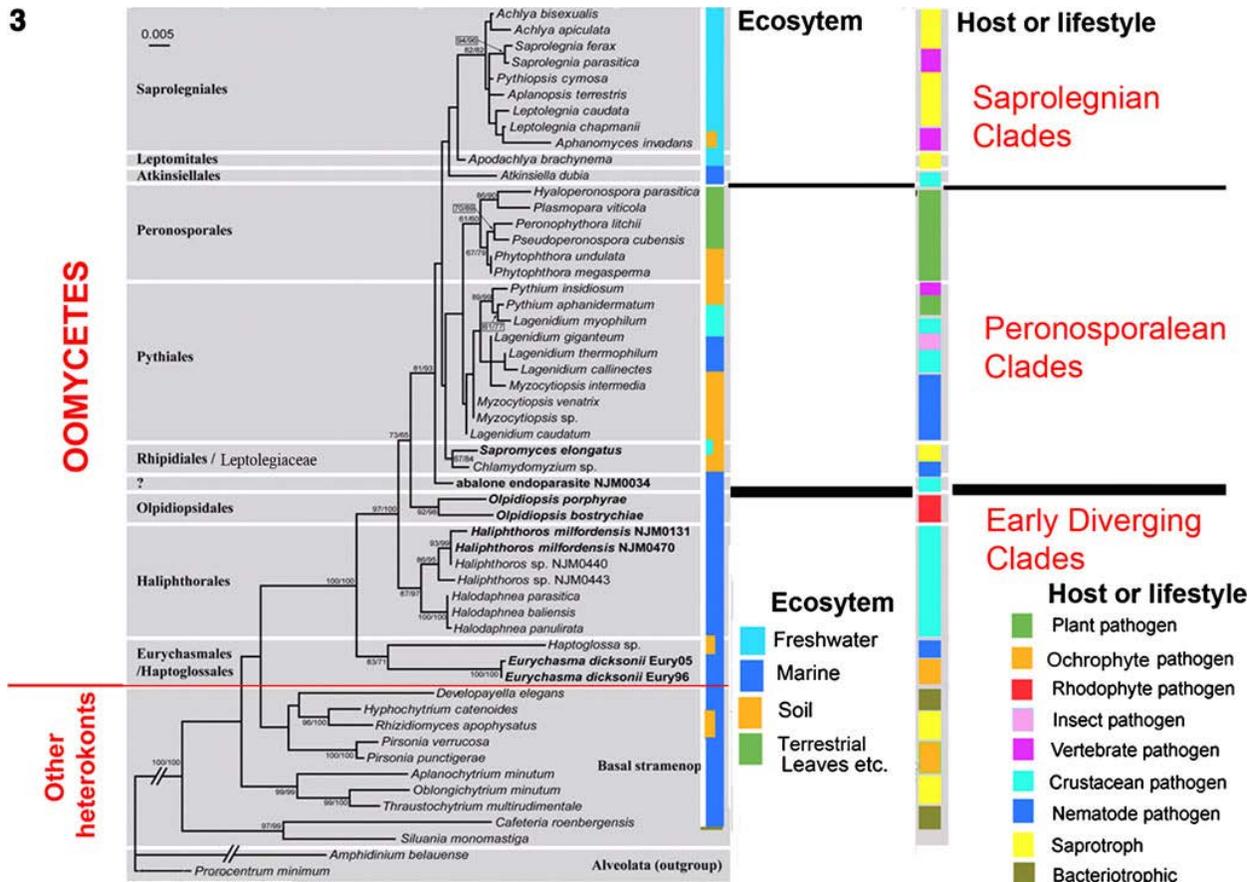


Fig. 3 Maximum likelihood tree (1,103 sites) based on 54 SSU rRNA tree sequences of oomycetes, other stramenopiles with two members of the alveolata as an outgroup. The main oomycete order clades are labelled on right. The two left hand bars map onto this tree the ecosystems from which genera come from and their host or life style respectively. Tree adapted from Sekimoto (2008)

Figures from Beakes, G.W., Glockling, S.L., and Sekimoto, S. 2011. The evolutionary phylogeny of the Oomycete “fungi”. *Protoplasma* DOI 10.1007/s00709-011-0269-2.

### Objectives

Is there a relationship between host bait material and Oomycete species isolated?

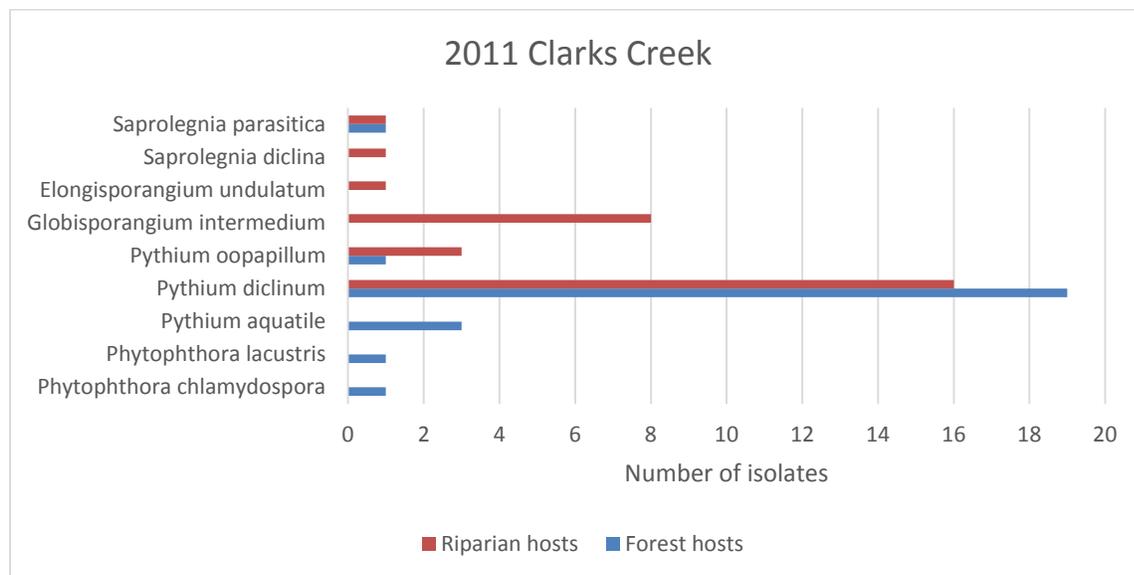
Riparian vs forest plants – are riparian plants resistant to infection?

What species are found in stormwater ponds that get runoff from urban landscaping? Are there plant pathogens?

## Some 2011 results

In 2011 the class sampled Clarks Creek near the headwaters and used 8 different plant hosts:

False lily of the valley	<i>Maianthemum dilatatum</i>	Forest
Grand fir	<i>Abies grandis</i>	Forest
Licorice fern	<i>Polypodium glycorrhiza</i>	Forest
Rhododendron	<i>Rhododendron 'Nova Zembla'</i>	Forest
Red alder	<i>Alnus rubra</i>	Riparian
Reed canary grass	<i>Phalaris arundinacea</i>	Riparian
Salmonberry	<i>Rubus spectabilis</i>	Riparian
Skunk cabbage	<i>Lysichiton americanum</i>	Riparian



Two *Phytophthora* species were isolated from bait of host plants found in forest environments, *P. chlamydospora* and *P. lacustris*, which did not occur on the riparian hosts. These two species are very common in streams and *P. chlamydospora* has also been found causing leaf blight on ornamental nursery plants such as *Rhododendron* and *Viburnum*.

## Methods

### **Materials:**

1L ml plastic bottle  
Small plastic cup  
Hole punch  
Thermometer

Kitchen strainer  
PARPH-V8 plates  
Parafilm strips  
Sampling pole

### **Plant material used:**

Leaves: *Rhododendron* 'Nova Zembla', reed canary grass (*Phalaris arundinacea*), salal (*Gaultheria shallon*), Douglas-fir (*Pseudotsuga menziesii*), Walter's sedge (*Carex striata*)

Fruits or seeds: Hemp (*Cannabis sativa*)\*

### **At the pond**

Rinse out 1L ml bottle and small plastic cup with pond water. Collect samples from various locations in the pond in small cup to fill large bottle to 1L. Avoid stirring up sediment and other debris at bottom of pond. Take temperature of pond water.

Each group will choose a plant species to use for bait. We will provide you with the plant material as we want all the ponds we sample to have the same conditions. We use fully expanded leaves and fruits or seeds that are relatively free of spots or other signs of infection. For leaves, punch out 20 leaf discs or cut blades of grass or sedge into 20 1 cm long pieces, and put in bottle. Include one intact leaf. For seeds or fruits, use 10 pieces. Put baits in bottle of pond water you collected and cap tightly. Rest bottle on its side for incubation.

### **In the lab**

Incubate bottle at room temperature in the dark for 3 days. Put in fridge until isolation day. Remove intact leaves from bottle and incubate in a moist chamber (Tupperware bin lined with wet paper towels) for a week.

Remove baits from bottle. This can be done by pouring through a kitchen strainer. Blot baits dry on paper towels to reduce bacterial contamination.

For each bottle, use 5 PARPH-V8 isolation plates. Insert 5 small leaf pieces in each of 4 plates. Cut 5 symptomatic areas out of intact leaf and insert into 5<sup>th</sup> plate. Label plates with the following

<b>Alder</b>	<b>1</b>	<b>5/17/15</b>	<b>JB/SK/AZ</b>
Plant species used	plate # (1-5)	date	isolator's initials

Parafilm edges of plates to reduce contamination and drying. Plates will be taken to WSU-P lab. A small group of students who are interested in isolating Oomycete spp. that grow are welcome to come after 3 days to do this. Two isolates per PARPH-V8 plate will be taken and plated on small PARPH-V8 plates. Try

to sample two colonies of different morphology on each plate. Avoid areas where several species may be mixed.

After 7 days of growth on the small plates, samples will be PCR'd and sequenced. The DNA sequences will be available for BLAST search and identification to species, if possible. Students can do BLAST searches on their computers.

\*Note: these seeds are autoclaved and not viable. Besides, it's not the kind that will get you high. But they make an excellent food source for oomycetes.

## Timeline

- 4/10/15 Morning and afternoon lab groups will collect and bait water samples from Bradley Lake.
- 4/17/15 Set up isolation plates in lab.
- 4/20 or 4/21 A small group from each lab can come to WSU-Puyallup to isolate oomycetes into pure culture.
- Week of 4/27 Small groups can come to WSU-Puyallup and look at cultures in microscope, take pictures, and possibly set up for PCR.
- Week of 5/11 Get sequence results, do BLAST search for ID.