Mycorrhizae Workshop
University of Costa Rica
12-15 July, 2016

Course Objectives

- Gain appreciation of the ubiquity, diversity and ecological importance of mycorrhizae
- Learn how to identify different types of mycorrhizae visually
- Acquire basic knowledge of the ecology, evolution and physiology of mycorrhizae in the Orchidaceae
- Become aware of some of the key outstanding questions concerning orchid-fungus interactions

Course Format

- A combination of lectures, discussion of a few readings, and hands-on work with microscopic and molecular identification of mycorrhizal fungi in orchids
- If no students wish to simultaneously enroll in the statistics workshop, we will engage in a relaxed, full day format Tuesday through Friday (i.e. 9AM to 5PM with a lunch break). However, if students wish to participate in both workshops, we will carry out the mycorrhizae workshop in the afternoon in a more intensive format (1PM - 6PM)
- Students will have a choice of different orchid taxa with which to work and will generate novel data on mycorrhizae of orchids from Tapanti and Jardin Botanico Lankester
- A brief lab manual will be provided, along with access to powerpoints and assigned readings

Course Schedule

<u>Day 1</u>

Lecture:

- Introduction to the major categories of mycorrhizae: structure, taxonomy and ecology
    - Arbuscular mycorrhizae (AM)
    - Ectomycorrhizae (ECM)
    - Ericoid mycorrhizae
    - Orchid mycorrhizae
- Introduction to the use of molecular methods in fungal identification

Lab:

- Examine examples of AM, ECM and orchid mycorrhizae on dissecting and compound microscopes
- Learn to section orchid roots and identify mycorrhizal structures
- Attempt to isolate orchid fungi in pure culture
- Carry out “Extract-N-Amp” protocol on samples of orchid mycorrhizal tissue
- Carry out PCR amplification of fungal ITS region from orchid mycorrhizal DNAs

Day 2

Lecture:

- In depth review of the systematics and ecology of orchid-associated fungi
- Applying molecular methods to orchid mycorrhizae: pitfalls and prospects
    - Fungal taxonomy and PCR primer issues
    - Which fungi are really mycorrhizal?

Lab:

- Run gel to assess PCR success/failure
- Set up Restriction Length Fragment Polymorphism (RFLP) digestion of PCR products to run overnight
- Set up PCRs on additional samples and/or failed samples

Discussion:

- Assigned reading on the diversity of mycorrhizas

Day 3

Lecture:

- Introduction to cutting-edge analysis of fungal communities: next-generation-sequencing (NGS) & bioinformatics
- Developments in research on orchid mycorrhizae
- The problem of orchid co-existence - do fungi play a role? Description of our NSF-funded project with UCR, JBL and Tapanti.

Lab:

- Set up and run RFLP gel
- Run gel on PCRs from day 2
- Examine culture plates
- Visualize, compile and discuss RFLP results

Discussion:

- Assigned reading on orchid mycorrhizae

Day 4

Lecture:

- Potentials and pitfalls of profiling fungal communities using NGS
    - PCR biases
    - Sequencing error
    - OTU clustering and within species sequence variation
- Discussion of assigned reading

Lab:

- Computer lab:

- Examine ITS sequences obtained from orchid mycorrhizae (provided, not generated in workshop)
- Carry out BLAST searches of different databases
- Group sequences into species clusters
- Examine ITS sequence alignments
- Construct phylogenetic trees for different groups of orchid fungi

Discussion:

- Assigned reading on NGS sequencing pitfalls
- Ideas for interesting orchid mycorrhizae research projects