

**Title:** Plant Phylogenies Pathway through Florida

**Author(s):**

William J. Furiosi II

Oviedo High School

william\_furiosi@scps.k12.fl.us

**Abstract:**

Evolutionary biologist Theodosius Dobzhansky once said, “Nothing in biology makes sense except in the light of evolution.” Phylogenetic trees are means of hypothesizing the evolutionary relatedness of life. Consequently, a corollary was developed saying that, “Everything in biology makes more sense in light of a tree of relationships.” Using common Florida species native to a specific locale (in this case, Seminole County was used), students will explore evolutionary trends by developing phylogenetic trees and homology tables using sequence alignment tools. Each student groups’ species list will be a subset of a larger class set. Groups will have overlapping information and will see how their trees compare based on species differences. In summary of the activity, the class will attempt to compile their data into one larger tree; the result is that students will see that science doesn’t always involve clean data and emphasizes the need for more powerful computational methods to handle the vast amount of data available.

**Subject, Grade, Level:**

*Grades:* 9-12

Standard/Honors Biology

AP Biology

AICE Biology

IB Biology

**Learning objectives:**

Students will be able to...

- Utilize computational tools to determine sequence homology between species.
- Create cladograms from sequence identity data.
- Describe differences among plant species based on evolutionary patterns.

**Timeframe:**

**Advanced Preparation:** Approximately 5 to 10 minutes is necessary to divide species among student groups and an additional 15 minutes is suggested to explore use of National Center of Biotechnology Information (NCBI)’s Basic Local Alignment Search Tool (BLAST) and the molecular Evolutionary Genetics Analysis (MEGA7) software.

**Lesson:** The lesson should be completed within a 50-minute class period.

**List of materials:**

- Computers or tablets – 1 per every 2 students
- Post-it® Wall Pad sheets – 1 per every 2 students

## INSTRUCTOR: Procedure and General Instructions

Randomly assign students any **five** of the following species:

**A** Spanish needle (*Bidens alba*)

**A** Southern Live Oak (*Quercus virginiana*)

**A** St. Augustine Grass (*Stenotaphrum secundatum*)

**A** Southern Magnolia (*Magnolia grandiflora*)

**A** Crepe Myrtle (*Lagerstroemia indica*)

**A** Spanish Moss (*Tillandsia usneoides*)

**A** Cabbage Palm (*Sabal palmetto*)

**F** Barometer Earthstar (*Astraeus hydrometricus*)

**G** Longleaf Pine (*Pinus palustris*)

**G** Slash Pine (*Pinus elliottii*)

**A** Bahia Grass (*Paspalum rotatum*)

**A** Moses-in-the-Cradle (*Tradescantia spathacaea*)

**A** Stinging Nettle (*Urtica dioica*)

**A** Prickly Pear Cactus (*Opuntia stricta*)

**A** Sea Grape (*Coccoloba uvifera*)

Include at least one gymnosperm (**G**) in each grouping compared to angiosperms (**A**) and fungi (**F**).

Instruct students to BLAST the amino acid sequences of the rcbL (ribulose-1,5-bisphosphate carboxylase large subunit [RuBisCO]) or matK (maturase K). Students should identify sequence homology using a table, similar to the one below:

Scientific Name	<i>B. alba</i>	<i>Q. virginiana</i>	<i>S. secundatum</i>	<i>M. grandiflora</i>	<i>P. palustris</i>
<i>B. alba</i>	-				
<i>Q. virginiana</i>		-			
<i>S. secundatum</i>			-		
<i>M. grandiflora</i>				-	
<i>P. palustris</i>					-

Alternatively, students can perform a multiple sequence alignment with all species concurrently using MEGA software.

From the sequence data, students should then aim to create a cladogram. Emphasize the fact that cladograms and phylogenetic trees are *hypotheses*, and they are subject to change based on further evidence.

Once all students have completed their trees, they should recreate their trees either on a whiteboard with other students' work or on wall Post-it® notes. The purpose is to allow students to see the construction of multiple trees with plant species they may have thought were similar. Because the species were randomized, there should also be overlap that students may be interested in.

Students should also include their phylogenetic table in the LARGE whole-class data table (see appendix). The data students acquire will not be enough to satisfy the entire table. Utilize the incompleteness to identify how there still remain gaps in the Open Tree of Life produced in 2015. Also, reinforce the need for computational methods in order to process all the data into a single tree. Having 20 species involves  $2.22 \times 10^{20}$  possible tree products.

With the students stumped on how to compile all the data from the class, show the computational aspect of Open Tree of Life's production. Create a phylogenetic tree of the 15 species using RStudio through a script provided by M. Sun from Drs. Soltis' lab, and show how much simpler it makes it.

### **Recommended Software & Computational Tools**

**NCBI BLAST:** <https://blast.ncbi.nlm.nih.gov/Blast.cgi>

Nucleotide BLAST (blastn) is used for identifying sequence homology between nucleotide sequences. blastx translated nucleotide sequences automatically into protein sequences which it then aligns, while tblastn takes amino acid sequences and reverse translates it back into nucleotide sequences. Protein BLAST (the preferred method for this activity) takes amino acid sequences and determines alignment with other sequences.

**MEGA:** <https://www.megasoftware.net>

With sequences downloaded in FASTA format, multiple sequence alignments can be performed with various algorithms to determine homology.

**RStudio:** <https://www.rstudio.com/products/RStudio/>

RStudio is an integrated development environment (IDEA) for coding in R. R provides libraries and functional/algorithmic capabilities of extracting data from the [Open Tree of Life](#) to create a smaller subtree.

### **References**

Hinchliff, C. E., Smith, S. A., Allman, J. F., Burleigh, J. G., Chaudhary, R., Coghill, L. M., ... Cranston, K.A. (2015). Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proceeds of the National Academy of Sciences of the United States of America*. doi: 10.1073/pnas.1423041112



## Appendix A: Comprehensive Sequence Homology of 15 Florida Plant Species

Scientific Name	<i>B. alba</i>	<i>Q. virginiana</i>	<i>S. secundatum</i>	<i>M. grandiflora</i>	<i>L. indica</i>	<i>T. usneoides</i>	<i>S. palmetto</i>	<i>A. hydrometricus</i>	<i>P. palustris</i>	<i>P. elliotii</i>	<i>P. rotatum</i>	<i>T. spathacaea</i>	<i>U. dioica</i>	<i>O. stricta</i>	<i>C. uvifera</i>
<i>B. alba</i>	-														
<i>Q. virginiana</i>		-													
<i>S. secundatum</i>			-												
<i>M. grandiflora</i>				-											
<i>L. indica</i>					-										
<i>T. usneoides</i>						-									
<i>S. palmetto</i>							-								
<i>A. hydrometricus</i>								-							
<i>P. palustris</i>									-						
<i>P. elliotii</i>										-					
<i>P. rotatum</i>											-				
<i>T. spathacaea</i>												-			
<i>U. dioica</i>													-		
<i>O. stricta</i>														-	
<i>C. uvifera</i>															-