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Systematics Lab

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Systematics is fundamental to all modern biology and all science students should and will see phylogenetic trees at some point. However, there are few opportunities for students to understand the science behind systematics. This lab serves as an introduction to "reading" phylogenetic trees and the processes that go into making the trees themselves from databases by utilizing the free, user-friendly program Geneious©. Helping our students learn the systematics language and understanding what goes into an analysis increases their appreciation for the importance of systematics and how it affects not only science but also their lives.

Keywords: systematics, phylogenetics, bioinformatics, BLAST, Geneious, Genbank, evolution

Introduction

Systematics is the classification and study of organisms with regard to their evolutionary history. It is fundamental to all modern biology and all science students should and will see phylogenetic trees at some point in their education. However, there are few opportunities for students to understand the science behind systematics and its importance. Systematics is essential to many branches of science and technology including: human health, pharmaceuticals, agriculture, as well as understanding and conserving biodiversity. Projects such as the Human Genome Project (HGP) are based on systematic analyses and the growing database of genomic data represents a multi-billion dollar investment of resources. This lab is designed to serve as an introduction to the concepts of how to unfold the information phylogenetic trees impart to us as well as the processes that go into making the trees themselves from databases such as the HGP and the National Center for Biotechnology Information's (NCBI) Genbank.

Systematics is now widespread throughout science and all science students should and will see phylogenetic trees at some point in their education. Helping our students on the road to start learning the systematics language and understanding what goes into an analysis increases their appreciation for the importance of systematics as well as why we have changes in our nomenclature over time due to new discoveries in systematic research.

Generally, several distinct programs are required to create phylogenetic trees that most accurately depict "true" evolutionary relationships. This is problematic due to the time and effort needed to learn and use the programs (some can take hours or days to do one step in the analysis!). Geneious© streamlines this process and makes it more tractable for teaching students by encompassing every aspect needed for a systematics analysis in one user-friendly program. There is a tutorial built into the program itself ("Help" in taskbar or under as "?" icon) as well as a pdf manual for your reference ("Help" in taskbar and select "Download Manual". If you are interested in exploring the Geneious© Pro version or if you want to update to the latest freeware program and manual, visit their website at **www.geneious.com**. There are also forums on the website with FAQs and Q&A sections. There are constantly newer versions of the program that you can update to, however, this lab exercise is written for version 3.5.4, although some of the screen shots in the lab handout are from previous versions. If you update to a more recent version of the program, then you will need to re-familiarize yourself with new options and settings in the newer version.

I have included my presentation notes of the background of systematics to state explicitly what I discuss at each slide so that you can better prepare yourself for introducing the terminology to your students. The purpose of having all of the terminology and diagrams is to give the students ideas that they might be able to relate and to use the systematics language to describe what could be occurring in the trees that they create in the hands-on-minds-on section of the lab and discuss in their analyses. The total estimated time for the entire lab is 120-180 minutes.

Student Outline

Introduction to Systematics Powerpoint Presentation

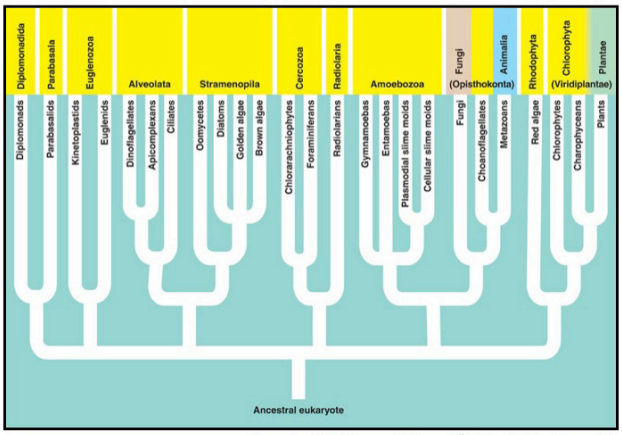
Systematics Laboratory

1

Some Background…

- What is systematics?
  - the classification and study of organisms with regard to their evolutionary history
- Why is it important?
  - Human health, Medicine & Pharmaceuticals
  - Agriculture & Genetic Resources
  - Forestry & Fisheries
  - Understanding & Conserving Biodiversity
- How do you study the systematics of a gene or organism?
- What are the concepts behind systematics?

2



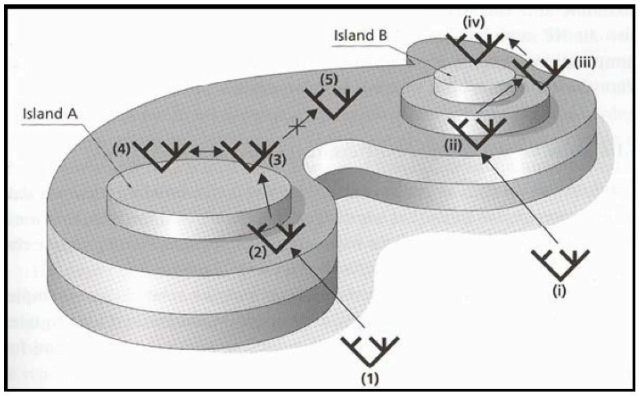
Campbell & Reece, Biology 6th Ed., 2001: Figure 28.4

A tree is just a hypothesis of the phylogenetic relationships of the organisms analyzed!

It is only as "good" as the data you choose to put in and the analysis you perform!

4

"Tree Space"



There is a lot of terminology related to phylogenetic trees!

We are going to go over some of the basics so we are all on the same page, but please refer to the explanations of terms at the back of the Systematics Lab assignment for future consultation.

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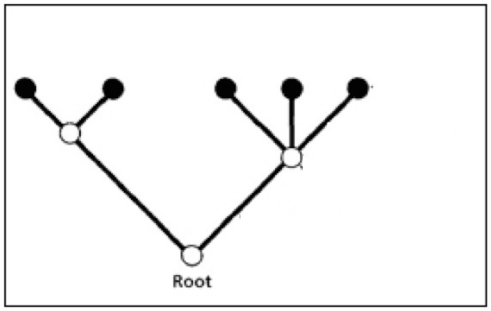
Tree-Related Terms



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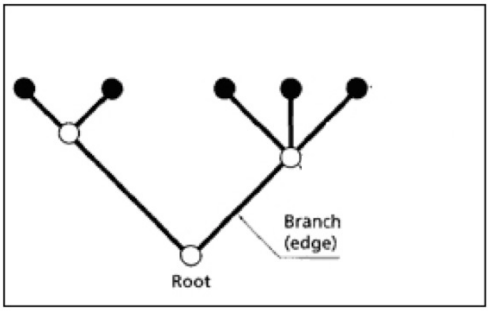
Page & Holmes, Molecular Evolution, 1998, Blackwell

Tree-Related Terms



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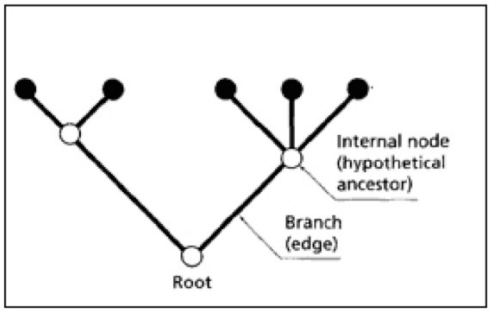

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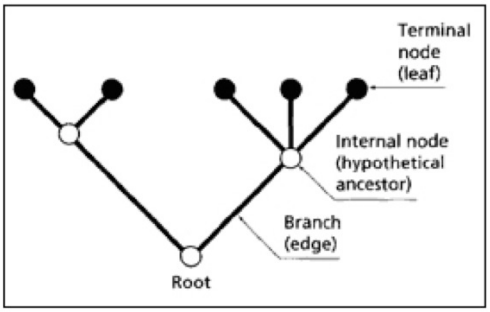

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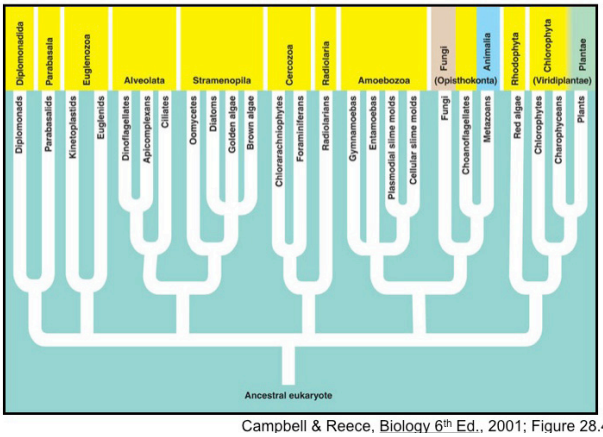
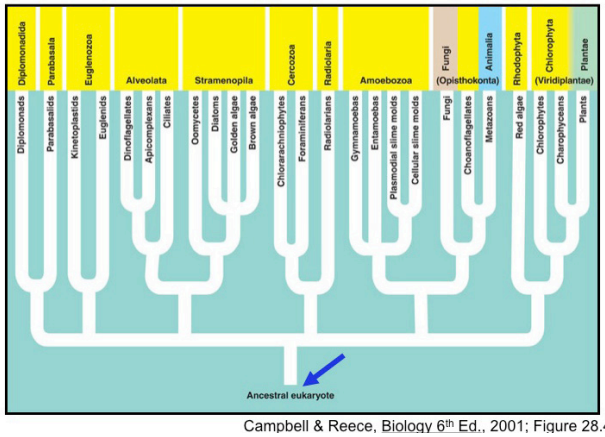
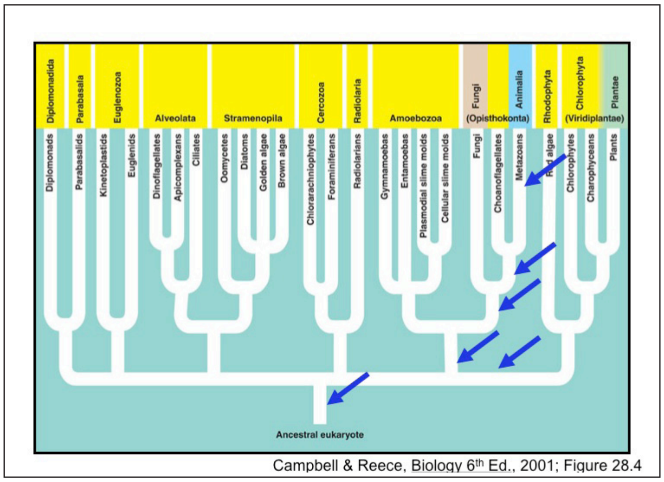


Campbell & Reece, Biology 6th Ed., 2001; Figure 28.4



Campbell & Reece, Biology 6th Ed., 2001; Figure 28.4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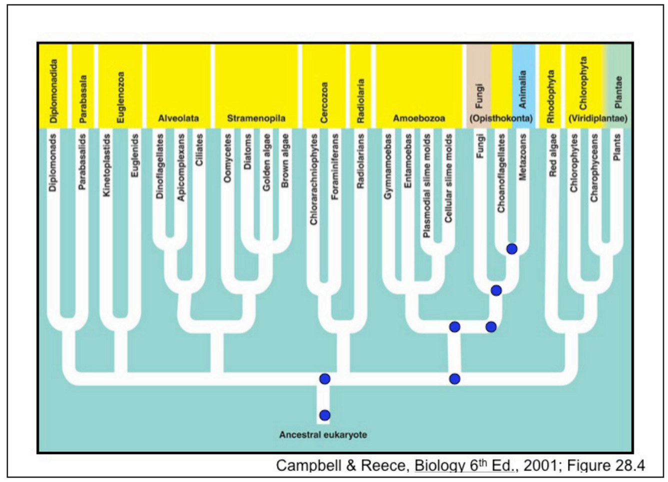
Parabasala
Euglenozoa
Alveolata
Stramenopila
Cercozoa
Radiolaria
Amoebozoa
Fungi (Opisthokonta)
Animalia
Rhodophyta
Chlorophyta (Viridiplantae)
Plantae
Diplomonads
Parabasalids
Kinetoplastids
Euglenids
Dinoflagellates
Apicomplexans
Ciliates
Oomycetes
Diatoms
Golden algae
Brown algae
Chlorarachniophytes
Foraminiferans
Radiolarians
Gymnamoebas
Entamoebas
Plasmodial slime molds
Cellular slime molds
Fungi
Choanoflagellates
Metazoans
Red algae
Chlorophytes
Charophyceans
Plants
Ancestral eukaryote
Campbell & Reece, Biology 6th Ed., 2001; Figure 28.4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Alveolata
Stramenopila
Cercozoa
Radiolaria
Amoebozoa
Fungi (Opisthokonta)
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Chlorophyta (Viridiplantae)
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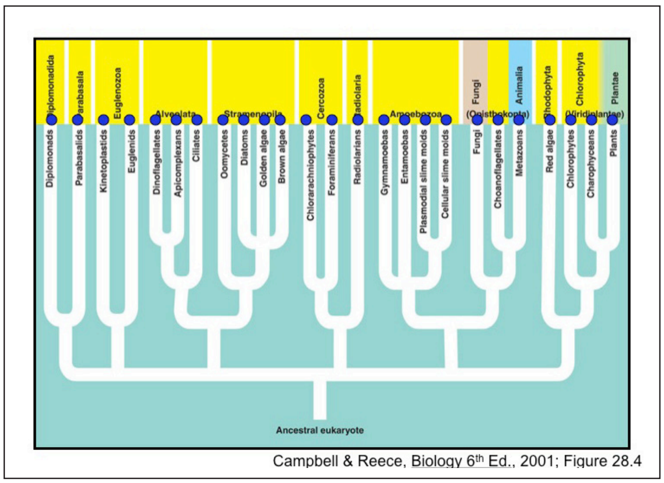
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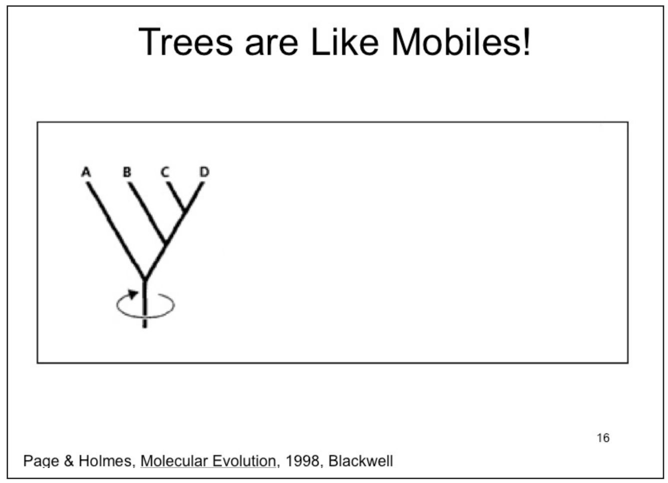
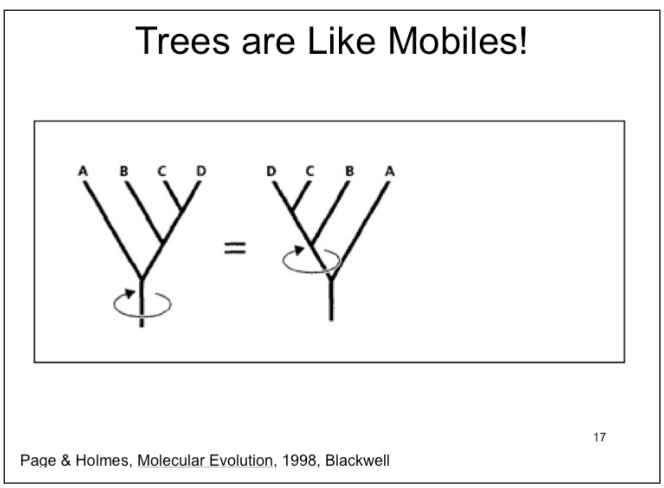

Trees are Like Mobiles!
A B C D
16
Page & Holmes, Molecular Evolution, 1998, Blackwell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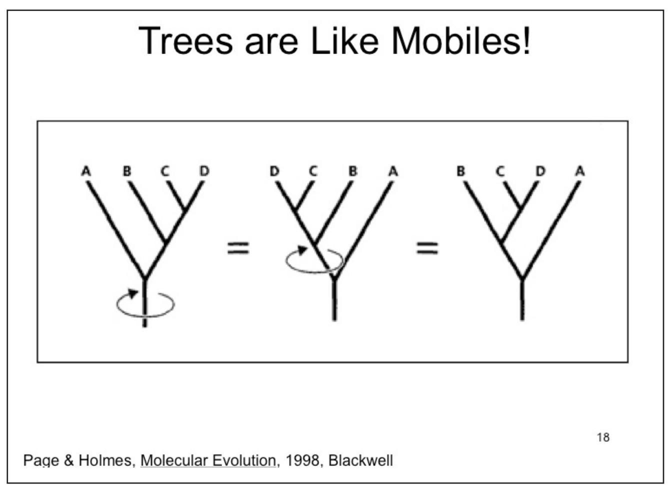
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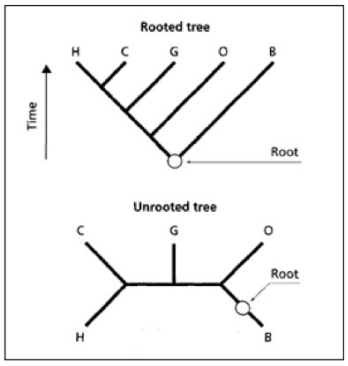
Tree-Related Terms



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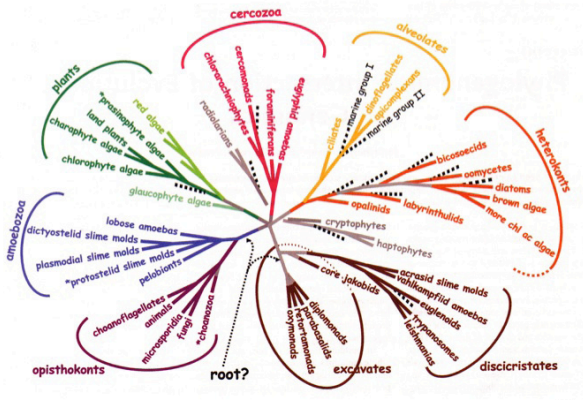

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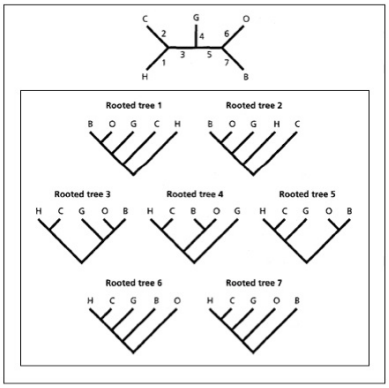
Baldauf, 2003, Science

Tree-Related Terms



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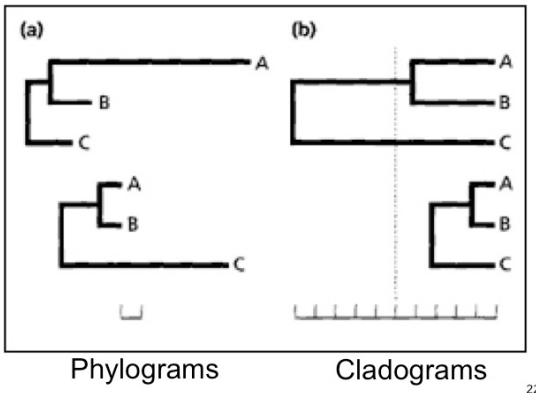

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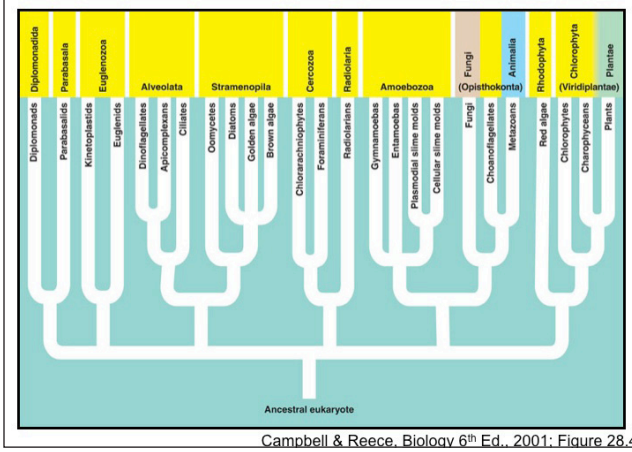
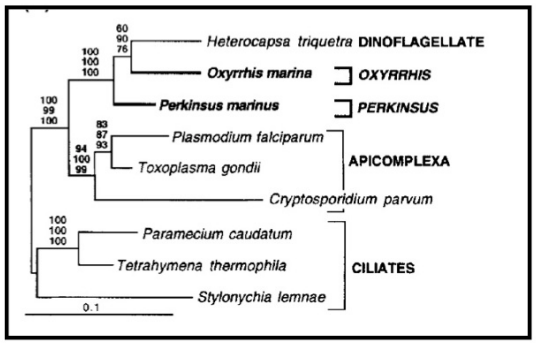


Campbell & Reece, Biology 6th Ed., 2001: Figure 28.4



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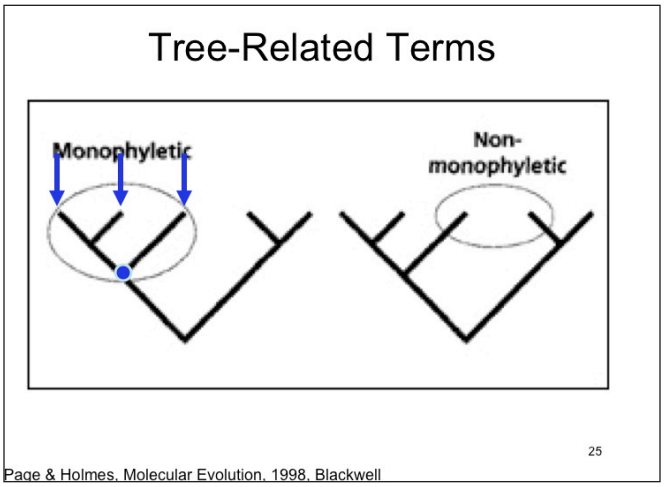
Saldarriaga, *et al.* 2003



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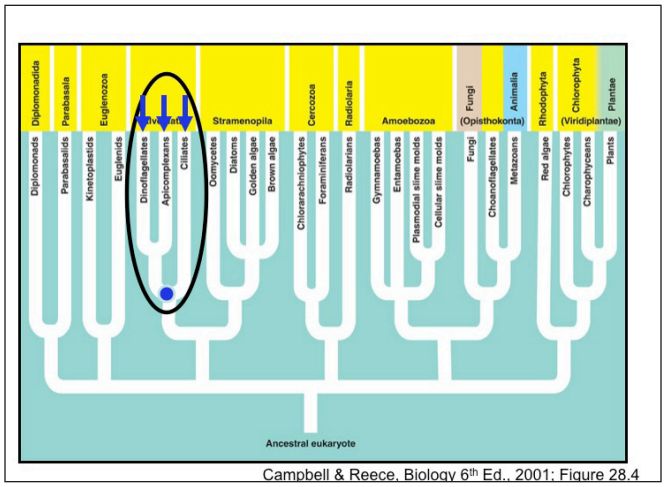
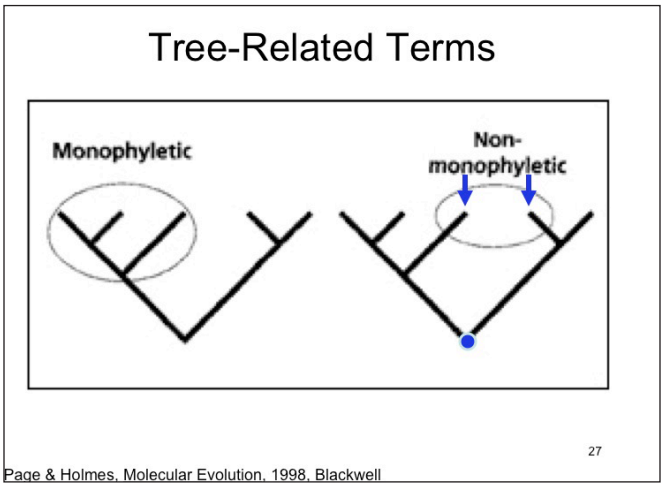


Campbell & Reece, Biology 6th Ed., 2001; Figure 28.4



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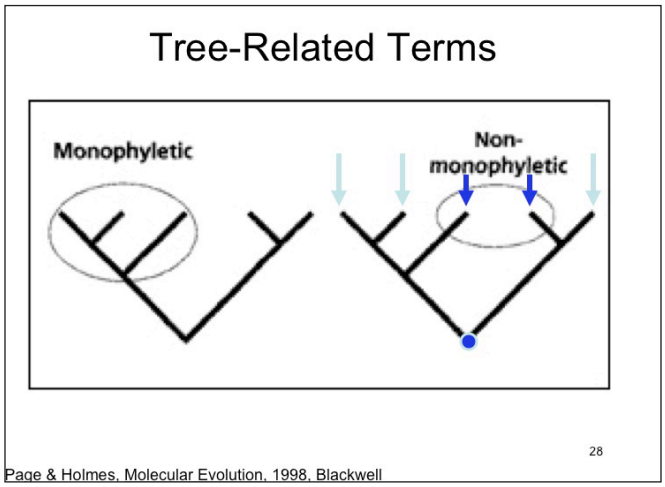

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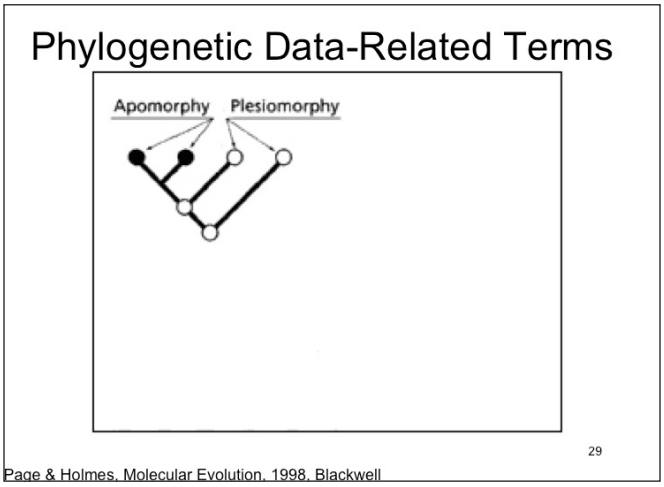

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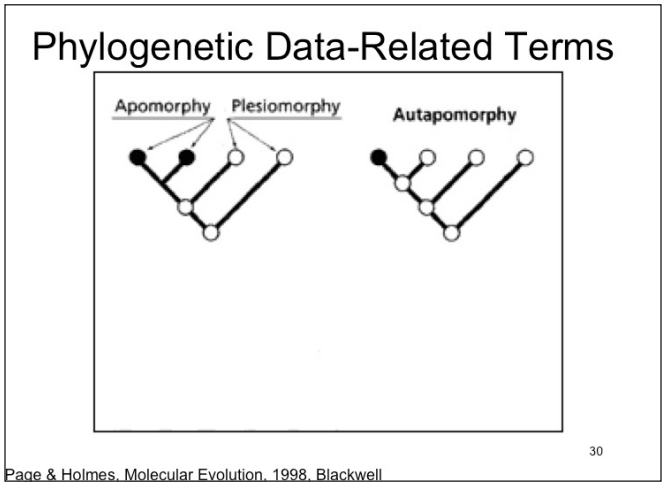
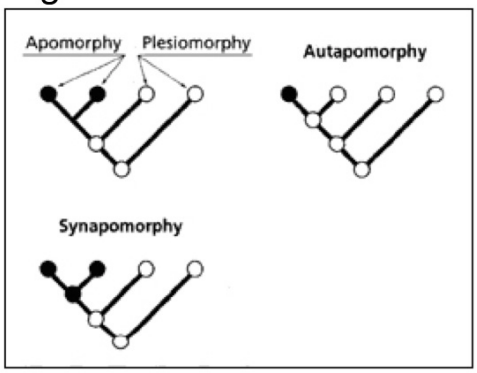


Page & Holmes, Molecular Evolution, 1998, Blackwell

Phylogenetic Data-Related Terms



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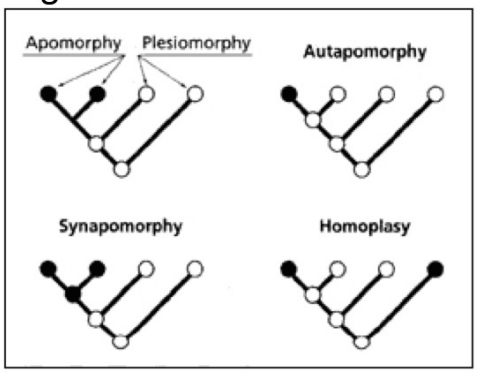
Page & Holmes, Molecular Evolution, 1998, Blackwell

Phylogenetic Data-Related Terms



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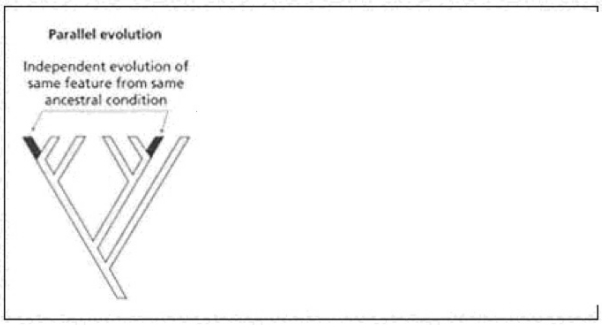

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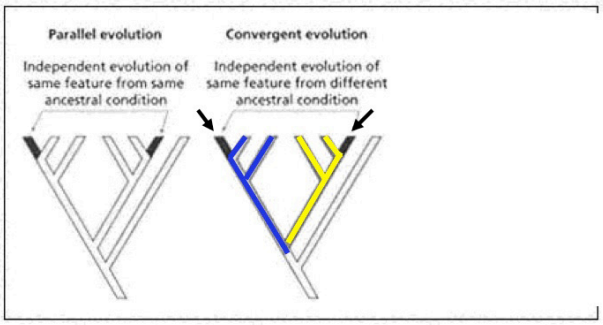

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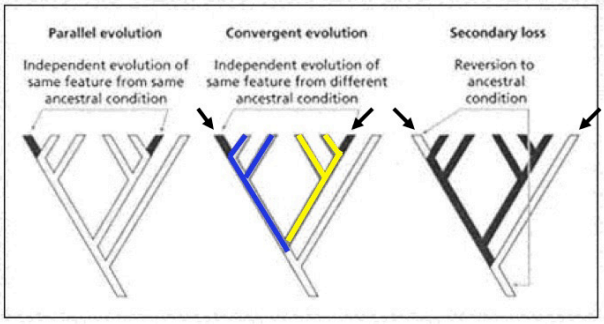

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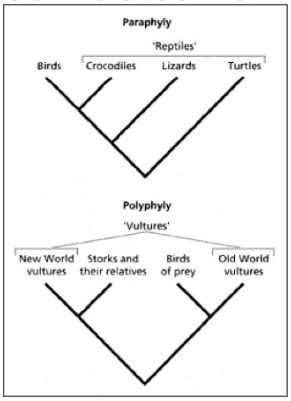

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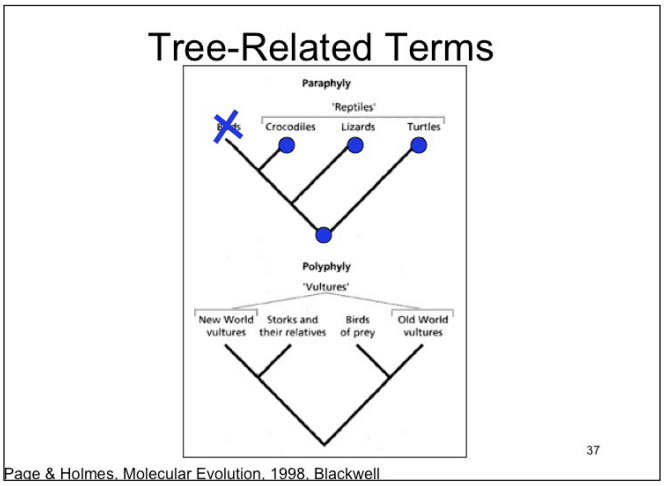

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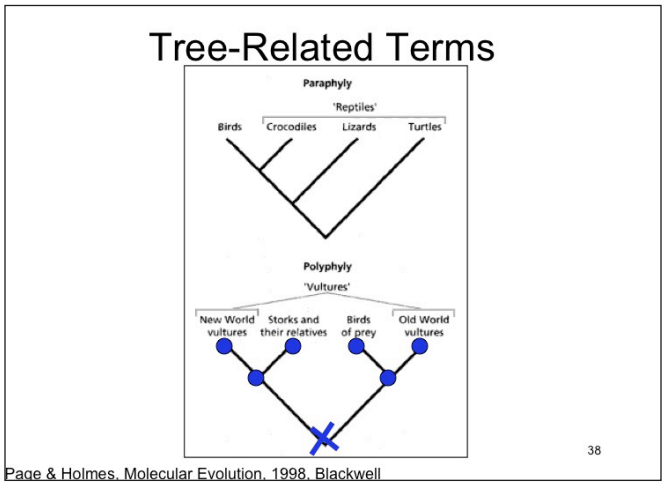

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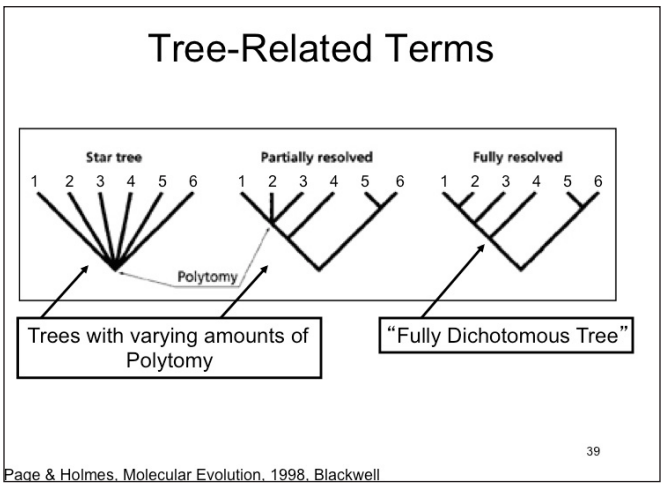

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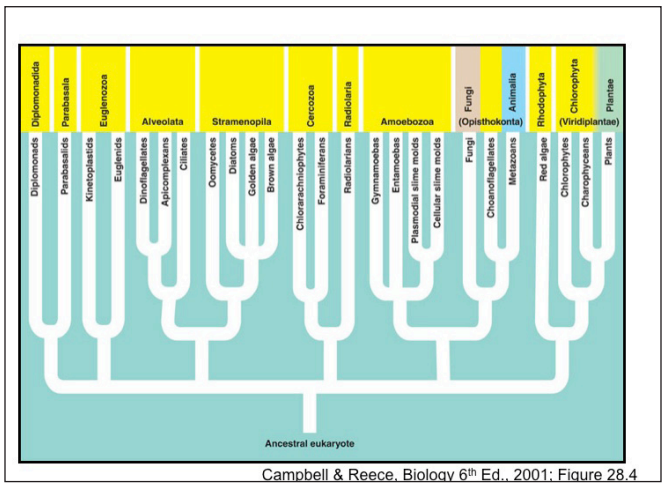
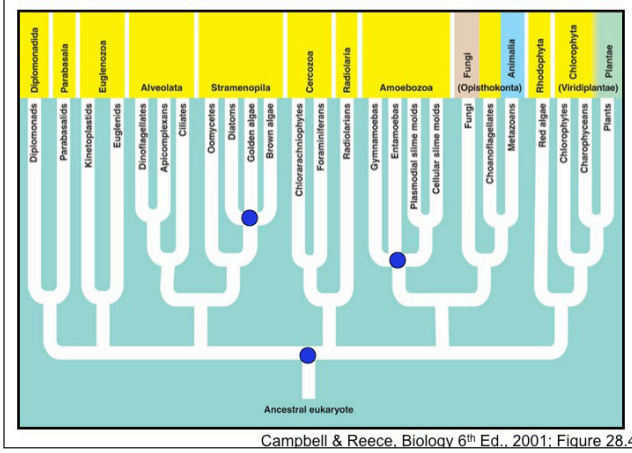
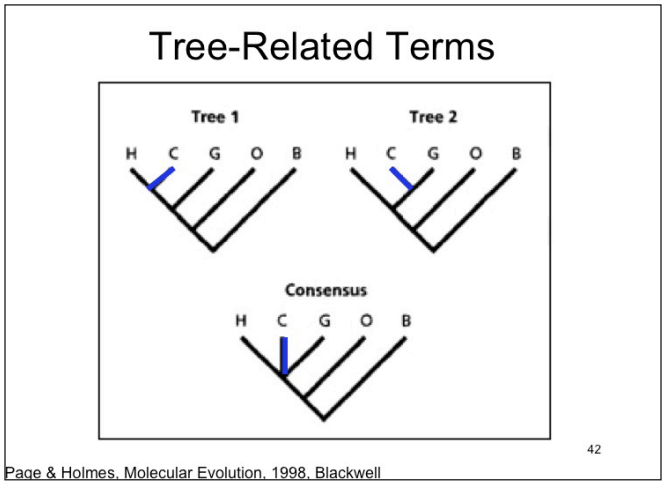


Campbell & Reece, Biology 6th Ed., 2001; Figure 28.4



Campbell & Reece, Biology 6th Ed., 2001; Figure 28.4



Page & Holmes, Molecular Evolution, 1998, Blackwell

Basic Outline of Systematic Analysis

- Acquire sequence data
- Perform multiple sequence alignment (MSA) of sequence data
- Create a tree using the MSA
- Find support values for relationships on the tree

43

Any Questions?

Let's start the lab and find out how this process works and what we can learn about systematics!

44

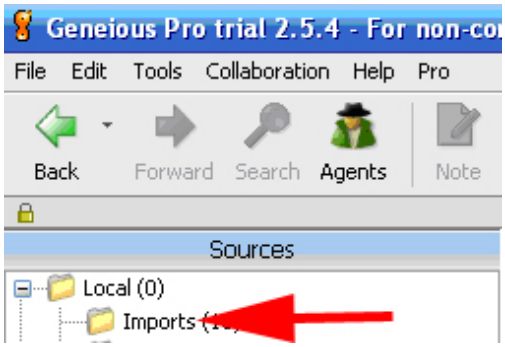
Systematics Hands-On-Minds-On with Geneious©

Lab Handout

1. There is a document entitled "Gene and Organism Assignment.doc" on your computer (ask instructor if location is unclear). Open the document. There are three possible genes and sets of organisms associated with them, but you will only be assigned one. From your assigned sequence sheet, which was handed to you, pick out 15 taxa and cross out the remaining taxa that you won't be using on the handout worksheet.

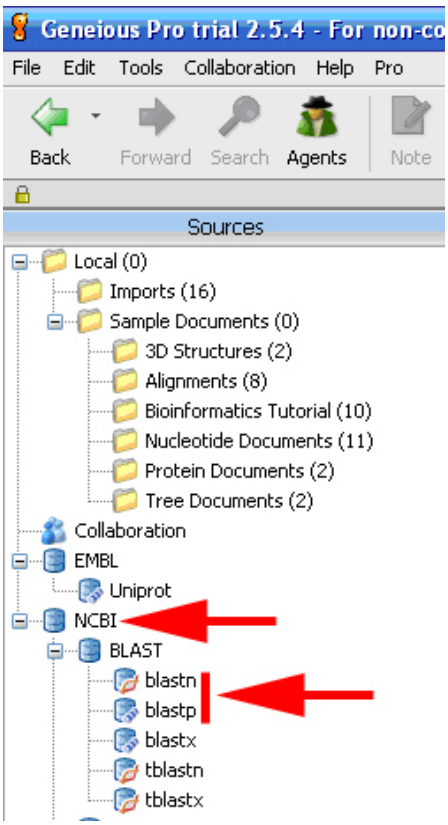
2. Open Geneious. (If you want to download this on your own computer you can download it at **www.geneious.com,** but be sure that you only get the academic version because the professional one costs money!). There is a tutorial in the program that you can go through at any time and there is also a user manual that you can download from the same website listed above if you need any help.

3. Create a new folder under the "Local" folder under the "Sources" window on the far left of the program window and name it "Imports". You can do this by either right clicking on the folder and selecting "New Folder" or by going up to File → new folder.



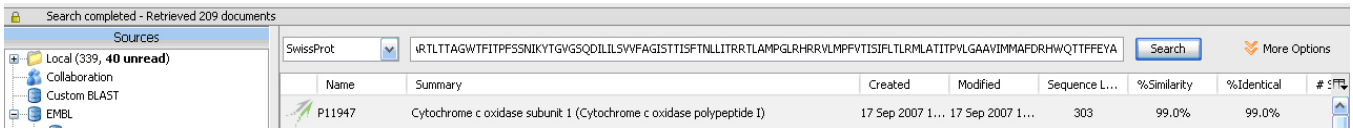
4. If you have a DNA sequence, then go under "NCBI → blastn", which is also under the "Sources" window on the far left of the program window. Otherwise, you have a protein, so go under "NCBI → blastp."

   How can you tell the difference between DNA and protein sequences? What do the letters represent? Circle either DNA or Protein on your gene and organism assignment sheet.

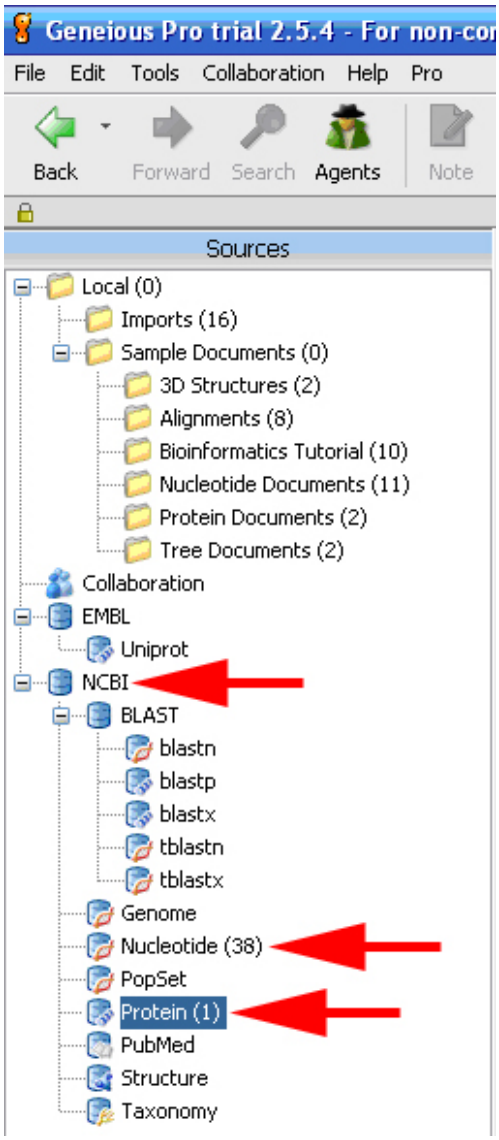


5. Select a database to search. The default nr (nonredundant) is fine. Type, or copy and paste, in your search criteria (i.e. your gene sequence), hit "Search", and wait until the message "Search Complete" comes up.

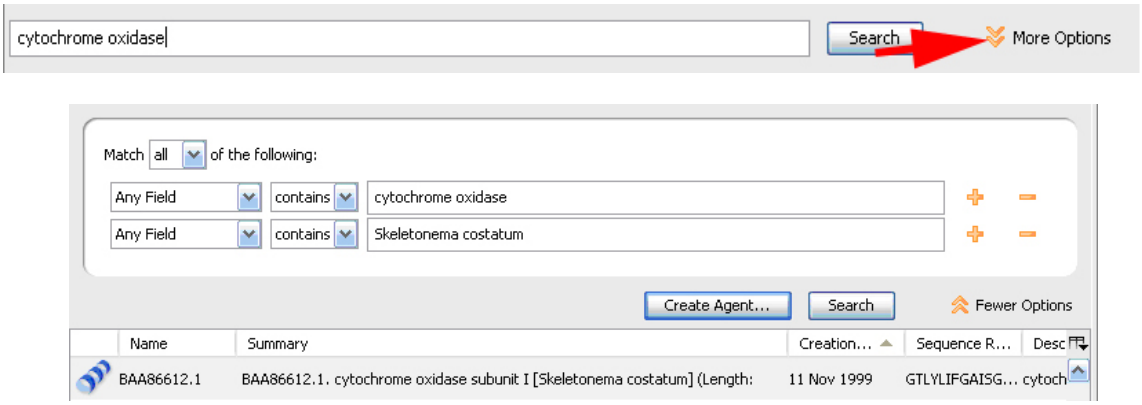
   What is the most likely "hit" to your sequence? What is the name of the gene that your sequence probably encodes? Write the name of the gene on your organism and gene assignment sheet.



6. Now that you know the name of the gene that your sequence encodes, you can go about searching for the sequences of that gene for the 15 organisms you chose. If you have a DNA sequence, then go under "NCBI → Nucleotide", which is also under the "Sources" window on the far left of the program window. Otherwise, you have a protein, so go under "NCBI → protein".



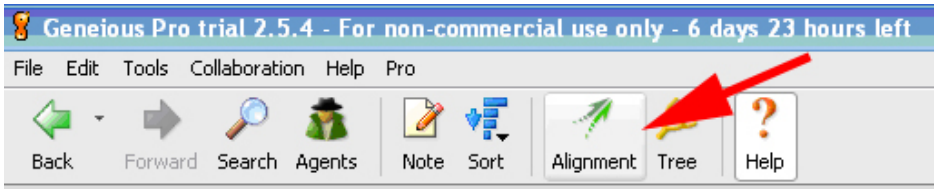
7. Type in search criteria (i.e. your gene name). If you want to put in more search criteria, then click the "More Options" button and you will get another line to put in search information (i.e. your organism name).



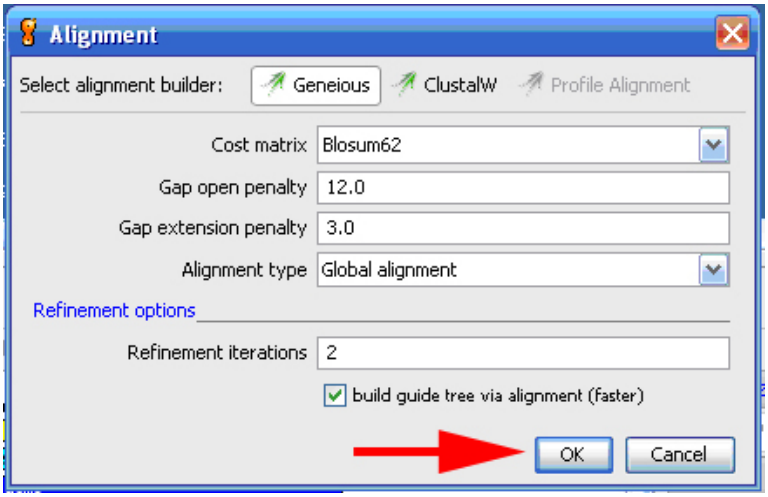
8. From the list of available sequences make sure you choose the longest, most recent entry with the correct gene and organism name, because there may be more than one entry for each gene for a specific organism. Do not choose partial sequences or ones that say "putative" or "hypothetical", only those that are known and are labeled "complete" or seem to be the same length as the other sequences you have downloaded for your other organisms. Click on the desired sequence and hit the control button while dragging the sequence into your newly created "Imports" folder from step 3.

9. Repeat steps 7 & 8 until you have one sequence for each of the 15 organisms you chose. At this time you should also rename each sequence name so that it reads as the organism's name (at least the genus) and not the NCBI identifying code. Write down the NCBI codes of the sequences you downloaded in your notes. That way you won't have to search for them again if you need them.

10. Go into the "Imports" folder and select all the sequences you have chosen (Ctrl + A, or click on the top sequence and scroll down and then while holding the Shift key click on the bottom sequence). Then, hit the "Alignment" button on the top toolbar.

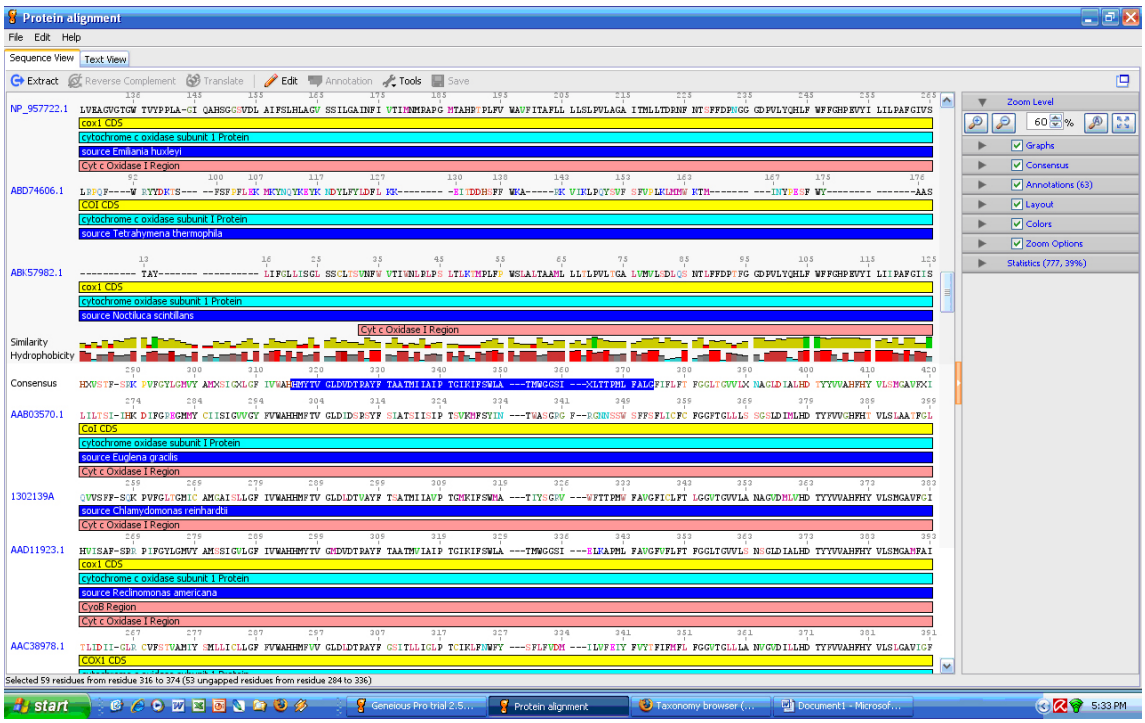


11. A new window will open up. Click "OK" because the default settings are fine for now.

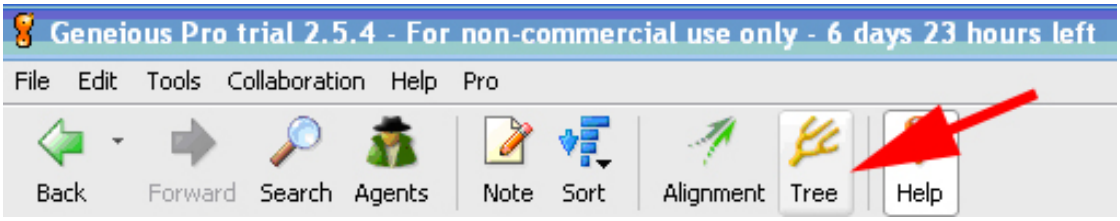


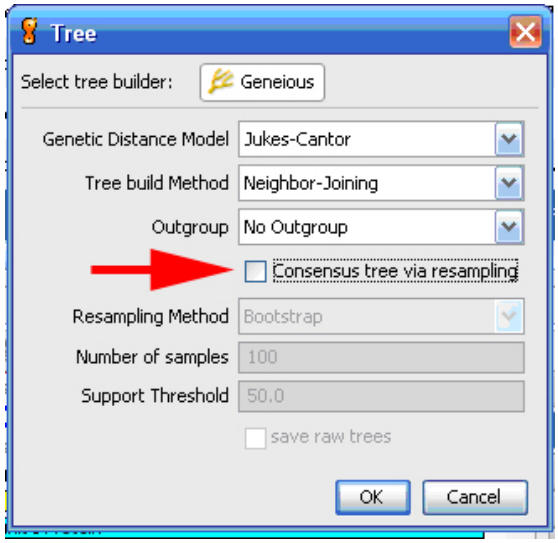
12. When the multiple sequence alignment (MSA) is finished (it should only take a couple of minutes at the most), a new line will appear among the rest of your sequences in the "Imports" folder. Click on this alignment to have it show up in the bottom panel of the program window (sequence view) or double click on it to have it come up in its own new window. You can play with this alignment and see the actual sequences.

    Do you notice anything about the different sequences? Are they all the same length, same code? Just by looking at the MSA try hypothesizing which organisms will be closely "related" in the tree you will make from the MSA. What did you notice that gave you a hunch or idea about the relationships?



13. Now, single click on the alignment line so that it is selected (blue) and hit the "Tree" button on the top toolbar. To start out with (for fast results) just do a simple Neighbor-Joining (NJ) tree *without* "consensus tree via resampling" box checked. The tree should be made in just a few seconds and this will appear as a new line in your "Imports" folder that you can click on to view.



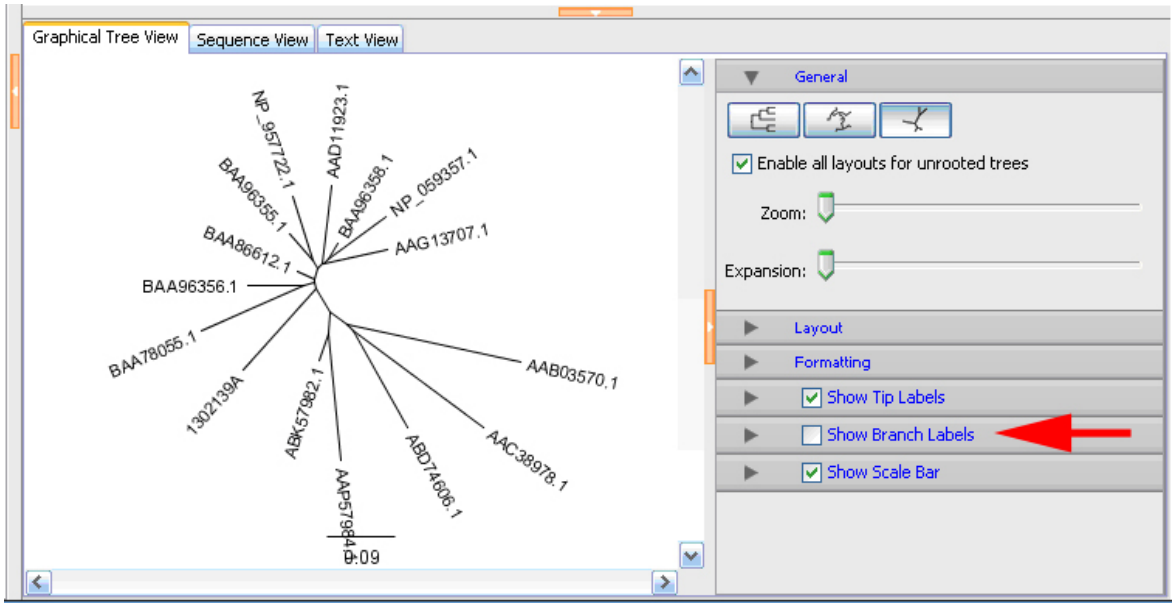


14. Play with the settings of the tree either in the "Graphical Tree View" in the bottom panel of the program window or double click on the tree line for a new window to open. Figure out how best to view the tree so that you can analyze the relationships. It will help if you make sure that you uncheck the "Show Branch Labels" button.

    Print out this tree and label it with your name and "NJ". Were your hunches about relationships from step 10 correct? Which ones?

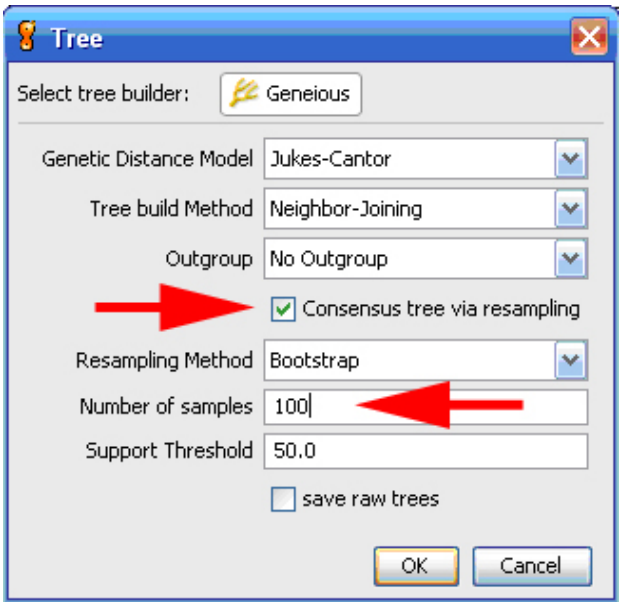
    To save the tree: Double click on tree line in main window and then be sure that "Graphical Tree View" tab is selected. In taskbar select File→Save as Image. Name the image appropriately and save it in jpeg format (.jpg) into a New Folder with your initials on the computer's desktop.

    To print the tree: Double click on tree line in main window and then be sure that "Graphical Tree View" tab is selected. In taskbar select File→Print. Check that the settings are OK and that the entire tree is visible and then print to the appropriate printer.



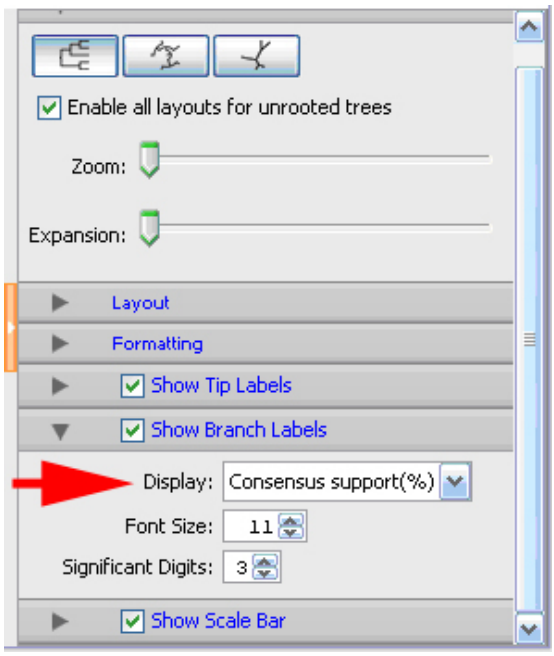
15. Now, click on the alignment line and hit the tree button again, but this time *check* the "Consensus tree via resampling" box. For the first time, just put 100 in the "Number of samples" box and hit "OK".

    If you have time, you can try putting in larger numbers (i.e. 1000; 10000) and see how those trees vary from each other. Print these trees out and label accordingly if you are going to use them to discuss your answers.



16. Play with this tree. It might be informative to check the "Show Branch Labels" button, but toggle to "Consensus support(%)" as the display. Otherwise, make this tree the same format as your "NJ" tree for easy comparison purposes.

    Print out this tree and label it with your name and "NJ consensus tree via resampling-100". If you compare your preliminary "NJ" and "NJ consensus tree via resampling-100" trees for your gene, do they give the same topology? If not why do you think the results may have differed?



17. *How does this tree compare to fig. 28.4 in Campbell and Reece (2001)? What "group" and "supergroup" does each organism you chose belong in? Write those group and supergroup names on the worksheet that was handed out to you next to the appropriate organism name and turn it in with the rest of your papers. Possible "group" names are the names on the white branches for each terminal node (eg.* Euglenids, Fungi, Plants). *The "supergroup" names are mostly in the yellow portion above the end terminals (e.g. Euglenozoa, Fungi/Opisthokonta, Plantae/Viridiplantae, respectively, for the example "groups" mentioned).*

18. Make sure you did everything we asked and you turned in all the required materials with your name at the top of each page. (See rubric for expectations and points.)
    - Labeled "NJ" tree from step 14 (be sure you have changed the accession number to the organism name for the branch labels)
    - Labeled "NJ consensus tree via resampling-100" from step 16 (be sure this is in the same format as the labeled "NJ" tree from step 14)
    - Original "Gene & Organism Assignment" handed to you with the sequence type circled, gene name, as well as the "group" and "supergroup" names filled in (for the organisms you chose) from steps 4,5 and 17
    - Typed up responses from questions in steps 4, 12, 14, and 16.

Glossary

Tree-Related Terms

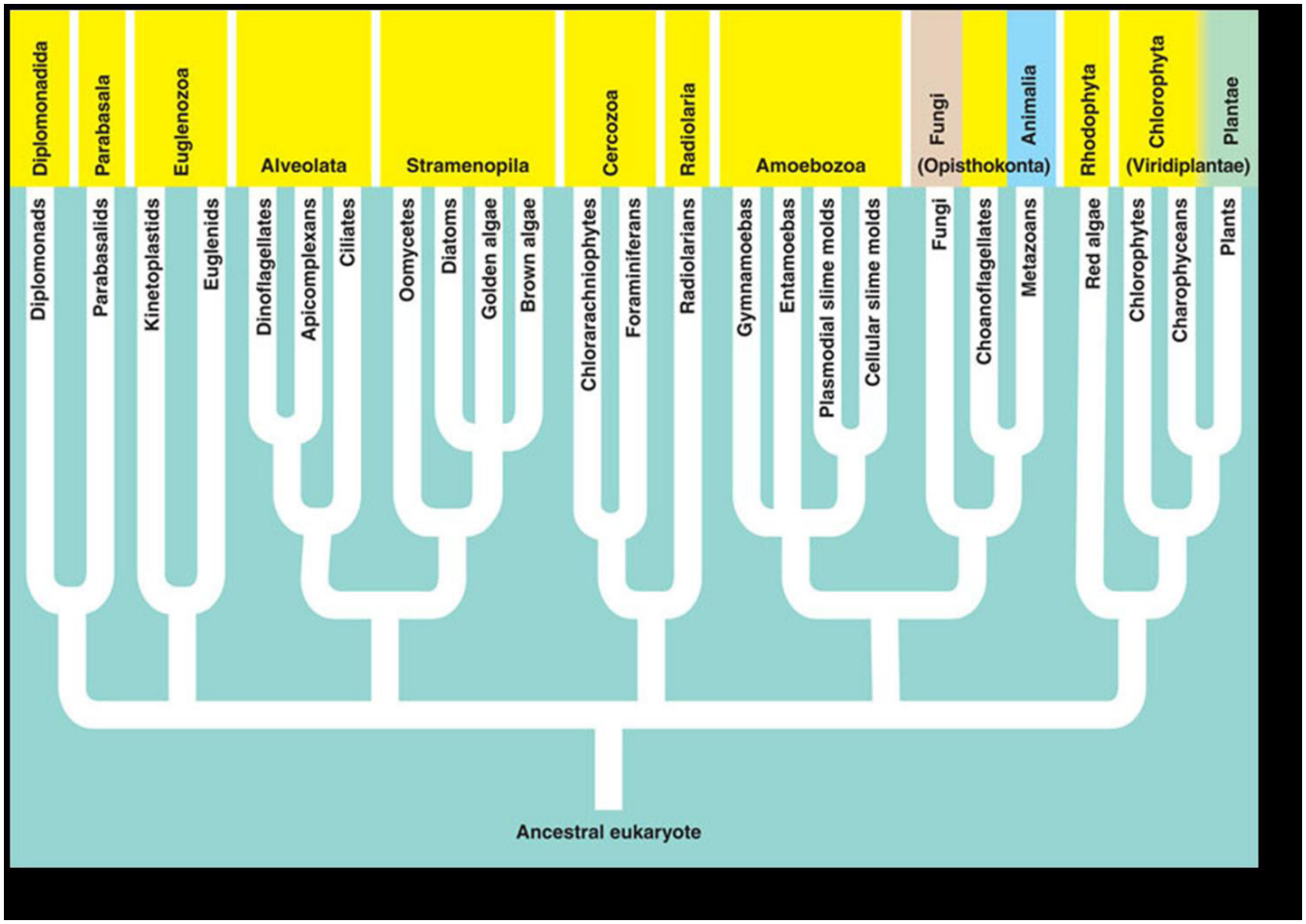
OUT	Operational taxonomic unit: an individual, population, species, genus, etc., in a phylogenetic analysis; graphically, these are represented by the tips (or leaves) of branches in a tree
Terminals/terminal nodes	The outermost tips of branches in a tree or network that represent OTUs in an organismal tree or molecules in a phylogeny of molecules (sometimes called a "gene tree")
Branches	The lines that connect present day OTUs, and ancestral entities to one another in a phylogenetic network or tree
Nodes/internal nodes	The points in a network or tree where two (or more) branches coalesce, moving from branch tips toward the center of the figure; these represent ancestral individuals, populations, species, etc. (Note: A node in a tree can be rotated freely on the branch that is below it or to its left in the horizontal orientation)
MRCA/most recent common ancestor (also LCA)	For any group of terminals in a tree, it is the node in the tree at which all those terminals coalesce; it represents the individual, species, etc., that is the last ancestor common to all the terminals in question
Root	The ancestral most point in a tree, commonly located on the far left side or bottom of a tree, depending on how the figure is drawn
Tree/rooted tree	A figure made up of OTUs, branches, and nodes, that specifies the nested (hierarchical) set of phylogenetic relationships among OTUs and internal nodes and which may or may not indicate the ancestral most point in the tree (i.e., the root)
Cladogram	A tree or network in which the branches are not proportional to the amount of evolutionary change that has taken place along them
Phylogram	A tree or network in which the branches are proportional to the amount of evolutionary change that has taken place along them (a scale bar is usually given)
Monophyletic Group/ clade	An ancestor and all its descendants
Paraphyletic Group	An ancestor and some (but not all) of its descendants
Polyphyletic Group	More than one ancestor and some or all of their descendants
Fully Dichotomous Tree	One in which all nodes are bifurcating; in other words two branches derive from every node in the tree (moving towards terminals)
Polytomy	Node in a tree that is not bifurcating (looks like a comb); in other words more than two branch are derived from this node (moving towards terminals)
Soft Polytomy	A polytomy in a consensus figure ("consensus tree") created by conflict among fully bifurcating source trees
Consensus Tree	Not really a tree; a figure that summarizes the extent of agreement among several phylogenetic trees (source trees) from one or more analyses

Phylogenetic Data-Related Terms

Homology	Aspects or features of organisms or molecules that are similar because they evolved from an aspect or feature of a common ancestor; vertebrate eyes are homologous
Homoplasy	Aspects or features of organisms or molecules that are similar, despite the fact that they evolved in parallel (independently; convergent evolution) in separate lineages and not because of common ancestry
Homologs	For molecular data, this term usually refers to genes in the same gene family
Ortholog	Usually used to refer to the corresponding gene in two or more species that is derived from descent through speciation events; orthologs duplicate during speciation events
Paralog	Usually refers to corresponding genes in one (or more?) species that are derived through at least one gene duplication event; paralogs duplicate during gene duplication events not associated with speciation
Character	Homologous aspect of organisms or sequences; e.g. eye color, or position #243 in a sequence alignment
Character State	Particular expression of a character; e.g., blue, or A
Plesiomorphy	Primitive character state; e.g., eyes, with respect to birds
Apomorphy	Derived character state; e.g., wings among birds
Synapomorphy	Shared, derived character state; e.g., flowers among angiosperms
Symplesiomorphy	Shared, primitive character state; e.g., xylem among angiosperms
Autapomorphy	Unique, derived character state; e.g., feathers in birds
Data Matrix	Matrix in which all character states have been recorded for numerous characters from numerous OTUs or sequences
Infile	Aligned data matrix that is supplied as the input file for a phylogenetic analysis (or any other computational analysis)
Outfile	File that results from a computational analysis – the output file
Morphological Data	Homologous characters and character states based on the morphology and anatomy of OTUs under study
Molecular Data	Aligned homologous DNA, RNA or protein characters (typically corresponding positions among aligned sequences), which may have been sampled from OTUs under study
Taxon Sample	OTUs chosen for inclusion in a phylogenetic analysis
Character Sample	Characters chosen for inclusion in a phylogenetic analysis
Long Branch Attraction (LBA)	An artifact of highly derived sequence to attract together to form an erroneous relationship based on stochastic change and not true evolutionary history
Indel	An instance in an alignment or comparison of two or more sequences of a gene where either an insertion of a base(s) or a deletion of a base(s) occurred in one at least one sequence to cause an apparent gap in an alignment

Website and Reference

- National Center for Biotechnology Information (NCBI): **http://www.ncbi.nlm.nih.gov/**
- Campbell & Reece, 2001, *Biology 6th Ed*., Fig. 28.4



Gene and Organism Assignment Handouts

GeneSequence#1:

VDAGKSTTTGHLIYKCGGLDKRKLAAIEKEAEQLGKSSFKYAFVMDSLKAERERGITIDISLWKFEGQKFSFTIIDAP-
GHRDFIKNMITGTSQADAAILVIDSTLGGFEAGIAEQGQTREHALLAFTLGIKQVIVAVNKMDDKTVNYNKARFDEI-
TAEMTRILTGIGYKPEMFRFVPISGWAGDNMTEKSPNMPWYNGPYLLEALDSLQPPKRPFDKPLRLPLQDVYKIN-
GIGTVPVGRVESGTMKPGMIVNFAPSTVTAEVKSIEMHHESLPEALPGDNIGFNVKNVSTADVKRGYVVGDTKRDP-
PVECASFTAQMIISNHPGKIHAGYQPVFDCHTAHIACKFDKLIQRIDRRHGKKATENPEYIQKDDAAIVEVVPSKPLV-
VESFQEYPPLGRFAIRDMKQTVAVGVIRSVNKKPNPIK

Type of Sequence: Circle one DNA (nucleotides) or Protein (amino acids)
Gene Name: _____

Table 1. Gene 1 organism information.
Pick 15 organisms and put an x by the ones you do not choose.

Common Name	Species	Group(s)	Supergroup
n/a	*Antonospora locustae*		
honeybee	*Apis mellifera*		
n/a	*Aspergillus fumigatus*		
silkworm	*Bombyx mori*		
agent of lymphatic filariasis	*Brugia malayi*		
nematode	*Caenorhabditis elegans*		
n/a	*Candida albicans*		
n/a	*Coccidioides immitis*		
n/a	*Cryptococcus neoformans*		
causative agent of cryptosporidiosis	*Cryptosporidium parvum*		
zebrafish	*Danio rerio*		
a cellular slime mold	*Dictyostelium discoideum*		
fruit fly	*Drosophila melanogaster*		
causative agent of amoebiasis	*Entamoeba histolytica*		
causative agent of giardiasis	*Giardia intestinalis (= G. lamblia)*		
soybean	*Glycine max*		
human	*Homo sapiens*		
yuca/tapioca	*Manihot esculenta*		
mouse	*Mus musculus*		
rice	*Oryza sativa*		
n/a	*Ostreococcus lucimarinus*		
n/a	*Pichia stipitis*		
causative agent of malaria	*Plasmodium falciparum*		
n/a	*Puccinia graminis f. sp. tritici*		
baker's yeast	*Saccharomyces cerevisiae*		
bread wheat	*Triticum aestivum*		
n/a	*Xenopus tropicalis*		
n/a	*Yarrowia lipolytica*		
corn/maize	*Zea mays*		

GeneSequence#2:

MAKIKIGINGFGRIGRLVARVILQRDDVELVAVNDPFITTDYMTYMFKYDSVHGQWKHHELKVKDEKTLLFGEK-
PVSVFGCRNPEEIPWSQTGADFVVESTGVFTDKDKAAAHLKGGAKKVVISAPSKDAPMFVVGVNEHEYKSDL-
HIVSNASCTTNCLAPLAKVINDKFGIVEGLMTTVHSITATQKTVDGPSAKDWRGGRAASFNIIPSSTGAAKAVGKVL-
PALNGKLTGMAFRVPTVDVSVVDLTVRLEKAATYEDIKAAIKAESEGKMKGILGYTEDDVVSTDFIGDNRSSIFDAK-
AGIALNDHFAKLVSWYDNEWGYSSRVVDLIVHMSKTE

Type of Sequence: Circle one: DNA (nucleotides) or Protein (amino acids)
Gene Name: _____

Table 2. Gene 2 organism information.
Pick 15 organisms and put an x by the ones you do not choose.

Common Name	Species	Group(s)	Supergroup
n/a	*Aspergillus fumigatus*		
silkworm	*Bombyx mori*		
cow	*Bos Taurus*		
Chinese cabbage	*Brassica rapa subsp. pekinensis*		
nematode	*Caenorhabditis elegans*		
n/a	*Candida albicans*		
domesticated dog	*Canis lupus familiaris*		
n/a	*Ciona intestinalis*		
causative agent of cryptosporidiosis	*Cryptosporidium parvum*		
a cellular slime mold	*Dictyostelium discoideum*		
fruit fly	*Drosophila melanogaster*		
n/a	*Encephalitozoon cuniculi*		
causative agent of amoebiasis	*Entamoeba histolytica*		
domesticated horse	*Equus caballus*		
soybean	*Glycine max*		
human	*Homo sapiens*		
n/a	*Kluyveromyces lactis*		
causative agent of cutaneous leishmaniasis	*Leishmania infantum*		
duck-billed platypus	*Ornithorhynchus anatinus*		
rabbit	*Oryctolagus cuniculus*		
rice	*Oryza sativa*		
n/a	*Ostreococcus lucimarinus*		
n/a	*Phanerochaete chrysosporium*		
n/a	*Pichia stipitis*		
causative agent of malaria	*Plasmodium falciparum*		
pig	*Sus scrofa*		
zebra finch	*Taeniopygia guttata*		
n/a	*Tetraodon nigroviridis*		
causative agent of East Coast Fever	*Theileria parva*		
wine grape	*Vitis vinifera*		
n/a	*Xenopus tropicalis*		
n/a	*Yarrowia lipolytica*		
corn/maize	*Zea mays*		

GeneSequence#3:

MREIVHVQGGQCGNQIGAKFWEVISDEHGVEPTGAYHGDSDLQLERINVYYNEATGGRYVPRAVLMDLEPGTMDS-
VRAGPFGQLFRPDNFVFGQTGAGNNWAKGHYTEGAELIDSVLDVVRKEAEGCDCLQGFQITHSLGGGTGSGMGTL-
LISKIREEYPDRIMETFSVFPSPKVSDTVVEPYNATLSVHQLVENADEVMVIDNEALYDICFRTLKLTTPTYG-
DLNHLVSACISGVTACLRFPGQLNSDLRKLAVNLIPFPRLHFFMIGFAPLTSRGSQQYRALTVPELTQQMFDAKNMM-
SASDPRHGRYLTASAMFRGRMSTKEVDEQMLNVQNKNSSYFVEWIPNNIKSSVCDIPPKGLKMSSTFVGNSTAI-
QEMFKRVAEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESNMNDLVSEYQQYQDATAEEEGEMDEEEGAME

Type of Sequence: Circle one: DNA (nucleotides) or Protein (amino acids)
Gene Name: _____

Table 3. Gene 3 organism information.
Pick 15 organisms and put an x by the ones you do not choose.

Common Name	Species	Group(s)	Supergroup
human	*Homo sapiens*		
rice	*Oryza sativa*		
n/a	*Ostreococcus lucimarinus*		
chimpanzee	*Pan troglodytes*		
n/a	*Pichia stipitis*		
causative agent of malaria	*Plasmodium falciparum*		
rat	*Rattus norvegicus*		
n/a	*Rhizopus oryzae*		
baker's yeast	*Saccharomyces cerevisiae*		
tomato	*Solanum lycopersicum*		
purple sea urchin	*Strongylocentrotus purpuratus*		
pig	*Sus scrofa*		
zebra finch	*Taeniopygia guttata*		
torafugu	*Takifugu rubripes*		
n/a	*Tetraodon nigroviridis*		
causative agent of East Coast Fever	*Theileria parva*		
causative agent of toxoplasmosis	*Toxoplasma gondii*		
red flour beetle	*Tribolium castaneum*		
bread wheat	*Triticum aestivum*		
causative agent of African sleeping sickness	*Trypanosoma brucei*		
causative agent of Chagas disease	*Trypanosoma cruzi*		
n/a	*Ustilago maydis*		
wine grape	*Vitis vinifera*		
n/a	*Xenopus tropicalis*		
n/a	*Yarrowia lipolytica*		
corn/maize	*Zea mays*		

Rubric

Neighbor-Joining Tree (4pts)	**Pts. Possible**
Overall tree	2
Terminals changed to genus name from NCBI code	1
Tree labeled properly (NJ and your name)	1

Neighbor-Joining Consensus Tree Via Resampling (4pts)	
Overall tree	2
Consensus support % values displayed on branches	1
Tree labeled properly (NJ Consensus and your name)	1

Gene and Organism Assignment Handout (9pts)	
(These columns filled out for your 15 organisms chosen for tree analysis.)	
15 Groups (0.3 points each)	4.5
15 Supergroups (0.3 points each)	4.5

Responses to Questions (8pts)

Level	Explanation	Pts. Earned
1	no questions answered	0
2	some, but not all questions answered understanding of material minimal thoughts unclear	2
3	all questions answered some understanding evident elaboration necessary to explain thoughts	4
4	all questions answered greater understanding evident coherent thoughts for most responses some provided or outside resources probably used	6
5	all questions answered very high levels of understanding evident deductive skills evident logical reasoning well explained clearly used provided or outside resources for responses	8

Total Points Earned =

Total Points Possible = 25

Note: The first three parts of the assignment are graded on a presence/absence basis, but the points earned in the *Responses to Questions* section will be based on how you explain your responses and the amount of thought and effort shown in them. If there are responses that fall between two levels, then the points will be half way between them (e.g., some evidence of answers in level 3 and others in level 4, then 5 points will be earned).

Materials

- Enough computers with high speed internet connection and at least 40 megabytes of memory available. Each student should work at his/her own computer, but teams of two or three students can work collaboratively at a single computer if resources are limited or if the instructor prefers students to work together. Also, because access to all of the needed software and web sites is free, students can conduct these activities on their home or dorm computers. (Note: whichever files the student downloads or creates will stay on that particular computer, so the files must be saved and transferred if work will be done on more than one computer.)
- A projector and screen with another computer connected to it for the instructor to use throughout the lab is preferred but not essential.
- Geneious© version 3.5.4 freeware downloaded from their website **(www.geneious.com).**

Notes for the Instructor

Lab Outline

- Give the students the "Introduction to Systematics" lecture so that they will have some background into understanding systematics. Be clear to state that there is a glossary of terms at the end of the lab. This can be handed out as a separate document in the beginning for them to take notes on, but notes shouldn't be necessary. Listening and interacting is usually the best use of this time. Generally, it is better to wait to give the lab and glossary out later to ensure the students pay attention and interact instead of starting on the assignment or just reading. This should take approximately 30-45 minutes.
- Walk students through the first couple of steps using a gene other than the genes assigned, but then allow them to work on their own and encourage them to consult their fellow lab mates and/or the internet (NCBI, Wikipedia, Google, etc.) if they are confused. Also, if a question keeps coming up, ask for everyone's attention and ask questions and guide them through the logic and how to figure it out as a group without directly giving them answers they should work for. If it is a technical issue, then this is best clarified using the instructor's computer and projector set up. This is a time where they are not only learning the methods and intricacies of the systematics process, but also about how this program operates. Let them explore it on their own for the most part to discover all that the program offers (visual and technical parameter settings) to allow them to get the most out of the experience independently. However, be wary so the students don't get so caught up in the details that they forget about the broader objectives of the lab.
- The students should be able to complete the entire hands-on-minds-on portion of lab within ~1-2 hours. The students should have access to the same computer and program at a later date after lab hours if necessary or during a second lab period. If multiple students in different lab sections need to use the same computer, then instead of having the general "Imports" folder created in Step 3 it should be specified by their initials (or lab day and time) instead so that other students do not overwrite or add extra files to a previous student's work. Sign up sheets can be set up by each computer so that the students don't try to use the same computers at conflicting times if after lab hours are required.
- Since most of the assignment (creating trees, answering questions, etc.) is finished during the lab session, only a few questions require time after the lab (although most students need to finish filling out their organism "group" and "supergroup" names after lab time). Be sure to specify that they can research using online resources (NCBI, etc.) outside of lab time and during lab time they should focus on working through the entire lab before trying to fill out the organism sheet. Completing all aspects of the assigned work does not require much time and so the assignment can be turned in after a week or two depending on how much time the instructor wants to allow and whether or not the instructor added extra facets to the assignment (see below). Be sure that a copy of the rubric is attached to the students' sheets so that the students understand the expectations and formatting that they will be graded on.
- The instructor should fill out the rubric accordingly and return it to the students with their graded/corrected sheets and trees. If there were several common misconceptions, the instructor can create a handout specifying the misconceptions and the clarifications. Also, it is up to the instructors discretion as to whether they will accept corrected trees and/or clarified answers from the students for full (or partial) credit after the first grades are distributed.

Possible Alternatives and Additions to the Lab

- Have students work with a partner and turn in the assignment as partners. Part of the rubric can be to have each student rate themselves and the other student as to how much they contributed to the assignment to help make it equal.
- Have each student do the entire lab on their own and come up with their own answers and turn them in. Then, have a follow up session to have students either with different genes and/or different organism choices discuss the similarities and differences of their resulting trees and what could have caused those differences.
- For higher level courses, have the student find a paper that has a phylogenetic tree in it and have them explain the results of the tree and describe what it says (support

values, polytomies, sister-relationships, etc.) to the rest of the class. The instructor can assign specific papers, or merely hand out a packet of several trees with no background information besides the figure legend and assign one figure per student so that more discussion is possible instead of just reporting results.

- The instructor could pick a certain system (eg. Fungi, Animals, Plants) that have been very well studied over time and select a previously published phylogenetic tree (or paper) and contrast it with a more recent phylogenetic tree (or paper) and have discussion with the students on what has changed in the relationships of the groups. Why did the changes occur? What different analyses, genes/characters were used, etc.? Are the changes just at the terminal nodes of the tree or are there deeper internal node changes as well? How does that information affect our idea of classification of that system?
- Add an extra column to the organism and gene assignment sheet to say "common name" next to the "Species" column and have the students fill this in so that they can recognize the commonly used names for these organisms. This makes the organisms more relevant to the students' knowledge base. It can also be used as a start of another extension assignment to this lab in which they can choose to present the importance of the organism and why it was chosen to have its genome sequenced. It could also just be a general and open discussion among the class as to why so many animal, fungi and plant species have been sequenced so far. The genome size issue can be brought up as well to indicate how it is much more difficult (generally) to sequence plants because their genomes are so large. Also, most protists whose genomes have been sequenced are either model organisms for their group and/or have serious impacts on human health.

Helpful References

Phylogenetics & the Systematics Process

Baldauf, Sandra L. 2003. Phylogeny for the Faint of Heart. *Trends in Genetics* 19(6):345-351.

Page, Roderic D. and Edward C. Holmes. 1998. *Molecular Evolution: A Phylogenetic Approach.* Blackwell Science.

Hall, Barry G. 2007. *Phylogenetic Trees Made Easy: A How-To Manual, Third Edition.* Sinauer Associates, Inc.

BLASTing and Technical Information

NCBI general: **http://www.ncbi.nlm.nih.gov/**
NCBI Education (Info. & tutorial links): **http://www.ncbi.nlm.nih.gov/education/**
NCBI BLAST Info. & Terms: **http://www.ncbi.nlm.nih.gov/BLAST/blastcgihelp.shtml#expect**

Powerpoint Presentation Notes for Introduction to Systematics Lab

(Note: Generally anything in parentheses is extra information, notes to instructors or answers to questions and timing and what is actually said can be moderated as the instructor sees fit. Also, be sure to cover anything said on the slide that is written out even if not stated explicitly in the notes below.)

- Slide 2 - Systematics is the classification and study of organisms with regard to their evolutionary history. Why is systematics important? (let students come up with ideas before you reveal the rest of the slide contents) Systematics allows scientists to determine relatedness between organisms (e.g., closest living relatives), study relationships of evolutionary change in parasite-host systems, discover which metabolic pathways would be good drug therapy targets to combat parasites or treat diseases, the origin of eukaryotes and life, etc. It is also the basis for our nomenclature of all organisms and how we communicate between members of scientific fields and between educators and students! (Systematics Agenda 2000: Charting the Biosphere Technical Report; pub. 1994 says systematics is important for "human health, species economics, medicines & pharmaceuticals, agriculture, agriculture & genetic resources, forestry, fisheries, understanding and conserving Earth's life support system, enhancement of the quality of everyday life, enhancement of scientific research"). How do you study the systematics of a gene or organism? What are the important concepts behind systematics? That is what we will explore in this lab.
- Slide 3 - This is a figure that describes the evolutionary relationships of all of the Eukaryotes (from a general introductory college-level biology textbook). We will use it throughout this introduction to help explain the process of systematics.
- Slide 4 - There are many possible trees that could show the phylogenetic relationships of the organisms studied…all of these possible trees together make up the tree space…
- Slide 5 - … which is depicted here three-dimensionally. The best tree here is denoted roman numeral "iv" because it is at the highest peak in tree space, which means that it is the most likely to explain the data, the most probable, etc. All other trees are possibilities, but in this analysis are lower in space and probability to support the relationships between the organisms using the data in the analysis. [Background: - Tree space is a theoretical concept used to discuss properties of the collection of all possible trees for a given data set. The size of tree space is determined by the number of sequences. The shape of tree space depends on several factors, including: the size of the data matrix, the number of categories for character states (e.g., 5, 21, etc.), the distribution of character states in a data ma-

trix, and the weighting scheme used to score character substitutions (e.g., equal weights, BLOSUM62, etc.).]

- Slide 6 – (read what is said on the slide)
- Slide 7 - The root is the hypothetical last common ancestor (LCA) of all the members of the group being studied, which is by this time extinct. However, sometimes we can use a known closely related organism to serve as a root to another group, these are referred to as the most recent common ancestors (MRCAs).
- Slide 8 - Branches within the tree show relationships between ancestral and more diverged organisms.
- Slide 9 - Internal nodes represent hypothetical ancestors that could have given rise to…
- Slide 10 - …terminal nodes which are extant (currently living) creatures.
- Slide 11 - Where is the root?
- Slide 12 - Does this tree show where the actual root of the eukaryotes is (which lineage it is associated with)? How is this depicted so as to be honest about our knowledge of the origin of the eukaryotes?...Where are the branches? (let them answer, then…)
- Slide 13 - …Here are some branches. Where are internal nodes? (let them answer, then…)
- Slide 14 - …Here are some internal nodes. Where are terminal nodes? (let them answer, then…)
- Slide 15 - …Here are all of the terminal nodes. What do the terminal nodes represent in this tree (clones, species, genera, families, kingdoms, super kingdoms, etc.)? Terminal nodes depend on what questions or classifications the tree is trying to study or represent. If we are not sure what they terminal nodes are, then we refer to them as operational taxonomic units, or OTUs.
- Slide 16 - One thing that is very important to understand about trees is that they are like mobiles and can be drawn stylistically to seem like certain groups are more closely related to one another, when in truth that may not be the case. For instance, stylistically the OTUs here were arranged such that they are in alphabetical order the way that we read (left to right). However, if we rotate the lower branch…
- Slide 17 - …then it is opposite alphabetical, and if we rotate an internal branch connecting the ancestor of the DCB clade,…
- Slide 18 - …then it appears even more out of kilter with how we perceive these made-up groups should be organized.
- Slide 19 - These two diagrams represent the relationships between OTUs “H” (human), “C” (chimpanzee), “G” (gorilla), “O” (orangutan) and “B” (baboon), however the one on the top roots the tree between B and O and the lower tree doesn’t although it depicts where that root would be located to convert it into a rooted tree. A rooted tree allows us to get a better perspective on what lineages broke off from each other when, whereas an unrooted tree does not. The tree we have been using is a rooted Eukaryotic branch of the tree of life, but…
- Slide 20 - …this is another depiction of the Eukaryotic branch of the tree of life, which is unrooted, because as was noted here (point at where it say’s “root” with the dotted lines going to two different lineages)...there was and still is speculation as to where the root truly belongs. Unrooted trees typically look like starbursts (as seen here) or as several starbursts with branches connecting them.
- Slide 21 - There are many possibilities for rooting a tree as shown here…for one unrooted tree, which is shown at the top there are seven possible rooted trees that can be derived from it by placing a root on one of the seven branches within the tree. As more OTUs are added, the number of rooted trees grow exponentially to make tree space very large! However, you can force a root, but it might be incorrect and lead to misleading trees.
- Slide 22 - This figure depicts the differences between a phylogram (A) and a cladogram (B). A phylogram is a tree with branch lengths that are proportional the amount of evolutionary change and a scale bar, similar to how we use them on maps, is usually given at the bottom. Whereas, a cladogram’s branch lengths do not convey any information as to the amount of evolutionary change that has happened along them and generally just draws all of the OTUs out to be even at an imaginary endpoint. They do not have a scale bar with any meaning associated.
- Slide 23 - Does this tree show us how much evolutionary change has occurred on each branch? (answer – no, there is no scale bar and all the terminal nodes end at the same place.) So is this tree a phylogram or a cladogram? (answer - it is a cladogram.).
- Slide 24 - Does this tree show us how much evolutionary change has occurred on each branch? (answer - yes, there is a scale bar at the bottom and the terminal nodes do not all end at the same point arbitrarily.) How can we determine the actual value of how much change has occurred on each branch? (answer - measure the difference of the branch and compare it to the scale bar…much like you do on a map to determine distance between two places.) So is this tree a phylogram or a cladogram? (answer - it is a phylogram.)
- Slide 25 - There are two ways to start out looking at set of OTUs and determining what sort of grouping it is. If it is monophyletic, then it is known as a clade, which is defined as being an ancestor (an internal node) and all of its descendents (OTUs and possibly some other internal nodes).
- Slide 26 - Alveolata is a monophyletic clade because it contains all extant members of the three groups that make it up, the Dinoflagellates, Apicomplexans and Ciliates as well as the common ancestor that they evolved from. Eukaryota is a monophyletic clade because it contains all eukaryotic organisms and the an-

cestor to all eukaryotes (even though we don't know what it was). It also does not contain any organism that is not a Eukaryote.

- Slide 27 - However, if a group of OTUs is not monophyletic then it does not include an ancestor and all of its descendents. In this case the non-monophyletic group is two OTUs that share a common ancestor if you go back in time enough,…
- Slide 28 - …but since not all of the other OTUs that are derived from that common ancestor are not included in the group it is not monophyletic. We will cover the different types of non-monophyly after we clarify how we can determine if a clade is monophyletic or non-monophyletic a little later on.
- Slide 29 - When describing one character, be it the shape or size of an arm bone, the presence or absence of eyes, or even an Adenine or a Guanine at a certain base position in a certain gene, a tree can be made for all of the organisms in the study to decide the evolutionary history of that single character. That helps us determine if what we see is a true relationship through descent or if it is a matter of convergent evolution due either to random chance or convergent evolution due to common evolutionary pressures (such as environment). If we assume that the ancestor of the four organisms studied had white eyes based on our analysis of eye color, then that would be considered the plesiomorphy for that character in the organisms that have it now because it was acquired directly without apparent evolutionary change from the ancestor. However, if along the way one node evolved black eyes, then it is considered an apomorphy because it is a derived trait from the ancestral organism character state, which has white eyes.
- Slide 30 - If only one external node has this trait, such as feathers on birds within the reptiles, then it is an autapomorphy because it is unique apomorphy to the group and not shared with other groups within the reptiles.
- Slide 31 - However, if two or more external nodes that are connected by branches to one or more internal nodes all share the black eye trait, then it is a shared derived character state, or synapomorphy.
- Slide 32 - One type of change in character state that can confound analysis is the case of homoplasy, where the same change (black eyes from white eyes) happened in two different lineages that are not monophyletic. Again, this can be due to convergent or parallel evolution… These next several figures should help make the difference between the various methods of homoplasic evolution of character states more clear.
- Slide 33 – Parallel evolution is what we saw in the last figure where there was independent evolution of the same feature from the same ancestral condition. For example all other members of the clade had white eyes, but these two unrelated organisms both evolved black eyes from an ancestor with white eyes.
- Slide 34 - Convergent evolution is the independent evolution of the same feature from different ancestral conditions. For example, the most recent common ancestor had white eyes, but then one lineage evolved blue eyes and another evolved yellow eyes. Then, an organism whose ancestor had blue eyes evolved black eyes and an organism whose ancestor had yellow eyes evolved black eyes.
- Slide 35 - Also, another form of homoplasy is secondary loss. In this case the white-eyed ancestor has one a derived lineage that directly received white eyes from it and another derived lineage that is more closely related to a common ancestor with black eyes, but reverted to the ancestral state of white eyes independent from the other white-eyed lineage. Homoplasy is very important to systematics because it can cause scientists to erroneously group organisms together because they study homoplasious characters instead of apomorphic characters. It is obvious when we view these organisms on these trees that the organisms with black eyes in the first two instances and white eyes in the last instance are not closely related to one another. However, if we looked at several characters that all had that same evolutionary history for those organisms the scientist would most likely group those organisms as being most closely related to one another to the exclusion of the other organisms. Therefore, it is important to know the evolutionary history of the character trait as well as the organisms being studied so that we can recreate the most accurate phylogeny for the group. There are other problems in systematics that can come about because we were not aware of the true evolution of organisms. Some of this is due to homoplasy and some is due to an enormous amount of autapomorphies.
- Slide 36 - We use the terms reptiles and vultures as if they were true groups, or monophyletic clades, when in fact they are not. Shown here are two specific cases of non-monophyly. Above is a cladogram of the four closely related groups, turtles, lizards, crocodiles and birds. However, birds have so many autapomorphies (feathers, wings, etc.) that they are not grouped with what we call "reptiles".
- Slide 37 - The "reptiles" therefore are known as paraphyletic because they contain a common ancestor and some but not all of the organisms that descended from it. If we wanted to keep the group name reptiles and still have it represent a monophyletic group, what would we have to do and teach to our students? Below is a cladogram of specific lineages of birds. It shows that what we call vultures actually evolved twice from two distinct lineages. The New World vultures as seen on the left are more closely related to storks and their relatives, whereas the Old World vultures on the right are more closely related to birds of prey, such as hawks and eagles.

- Slide 38 - Therefore, the term vulture is not monophyletic, but polyphyletic meaning that the group includes more than one ancestor and some or all of its descendents. Vultures seem to have several times by convergent evolution to fill the niche of carrion-eaters, so the term reflects ecology, not evolution.
- Slide 39 - Generally, in a relationship we would like to know which organisms or groups are more closely related to one another with certainty. However, sometimes we cannot resolve particular relationships because we don't have enough information to analyze those questions yet. These unresolved relationships are shown as polytomies. For these six OTUs there we see varying levels of resolution, from the left to the right. On the far left we see a star-shaped tree because there is absolutely no resolution and all of the OTUs are joined in one huge polytomy. In the middle we cannot resolve OTUs 1, 2 & 3, but we can resolve the relationships between 4, 5 and 6. On the far right we see a fully dichotomous tree which means all of the relationships between the 6 OTUs have been resolved.
- Slide 40 - Are there any polytomies in this tree? (answer – yes) Where are they? (let the students offer some ideas and then move on to the next slide for all of the labeled polytomies in this tree)
- Slide 41 – (be sure the students understand that these are the three and only instances of polytomy on this tree, however, the important basal relationships are completely unresolved!) However, just because there aren't many polytomies in this tree doesn't mean that the relationships that are resolved represent the actual evolution of the eukaryotes…only what our best supported hypothesis is given the data we currently have. Since this book was published there have already been several proposed changes between the major groups (example – Cercozoans and Radiolarians seem to be more closely related to the Alveolates than the Alveolates are to the Stramenopiles).
- Slide 42 - Sometimes when we perform analyses we get only one resulting tree, whether it is fully dichotomous or not. However, some analyses can find several equivalent "best" trees. In this case you can see the two possible trees from one such analysis. In Tree 1 "C" is more closely related to "H" than it is to "G", but in Tree 2 "C" is more closely related to "G" than it is to "H". One way to summarize the results of an analysis when there are two different trees is to create a consensus tree (point out the "consensus" tree on the slide). This consensus tree conveys the uncertainty of the relationships of "H", "C" and "G" by collapsing those relationships to a polytomy. A consensus tree is not a true tree and is always a cladogram rather than a phylogram because information of branch length cannot accurately be combined from two or more trees. However, they can also show us that a seemingly fully dichotomous tree is not actually well-supported and a consensus tree will show the nodes where there is low support for relationships. You will make consensus trees in the assignment and they will help you identify instances of low support.
- Slide 43 – (read off what the basics of a systematic analysis are)…(note for instructor: To acquire sequence data we usually BLAST (basic local alignment search tool) which uses a sequence you input that you can search for other similar sequences in a database that hundreds of scientists deposit their sequences into NCBI.) We will be using one program, called Geneious© that allows us to all of these steps.
- Slide 44 – (say what is on the slide and answer any questions that you deem appropriate)
- Extra Information For Instructors – You can provide screen shots of trees (with different genes, for instance cytochrome oxidase I) with support values on them. Explain that the bootstrap (or other support values) shown at nodes on phylograms are put on there by the authors from the consensus tree cladograms and they might have to do the same thing to show support on their NJ trees (has no values, but has varying branch lengths) from their NJ-consensus tree (shows values). Also, if there is more than one type of tree-building analysis or support calculation used to study a group of organisms, then there is usually one support value for each analysis run or support calculation type for that given node if it was recovered. You can also provide screen shots of alignments using a different gene. In the questions for alignments the easiest things for students to find are: differences in sequence lengths (if at the beginning or end of the sequence, this might just denote an incomplete Genbank sequence and not necessarily -the complete gene…therefore, missing information instead of just real indels, however this program does not differentiate by default), insertion/deletion (indels), and conserved regions (taller letters in sequence logo bit score at top of each line & the height of the "similarity" bars for each base position). All of these things can affect an alignment and therefore the tree that results from the alignment because it is the first step in determining which organisms are more closely related to one another by their sequence composition. As an example, if *Skeletonema* (a centric diatom) & *Cryptomonas* (a cryptomonad) are both the only organisms in a part of an alignment that are missing a large length of sequence bases ~410-500 in sequence alignment, then they might be more closely related to each other than either is to the other organisms that are not missing that segment of sequence. (Note: to find the reference base numbers in the alignment refer to the consensus line numbers above each section of the alignment…can deselect "wrap" on side if this is too confusing). These organisms are also both Stramenopiles so it makes sense that they should be grouped together.

Keys to Gene and Organism Assignments

(Note: I was unable to find a nucleotide gene, a gene that does not code for a protein, of appropriate size that was available for many eukaryotes and easy to search and BLAST for. However, the questions distinguishing between DNA and protein sequences remained in the lab so the students can learn the differences between them and be sure they are BLASTing using the correct search.)

GeneSequence#1: VDAGKSTTTGHLIYKCGGLDKRKLAAIEKEAEQLGKSSFKYAFVMDSLKAERERGITIDISLWK FEGQKFSFTIIDAPGHRDFIKNMITGTSQADAAILVIDSTLGGFEAGIAEQGQTREHALLAFTLGIKQVIVAVNKMD-DKTVNYNKARFDEITAEMTRILTGIGYKPEMFRFVPISGWAGDNMTEKSPNMPWYNGPYLLEALDSLQPPKRPFD-KPLRLPLQDVYKINGIGTVPVGRVESGTMKPGMIVNFAPSTVTAEVKSIEMHHESLPEALPGDNIGFNVKNVSTAD-VKRGYVVGDTKRDPPVECASFTAQMIISNHPGKIHAGYQPVFDCHTAHIACKFDKLIQRIDRRHGKKATENPEY-IQKDDAAIVEVVPSKPLVVESFQEYPPLGRFAIRDMKQTVAVGVIRSVNKKPNPIK

Type of Sequence: Protein
Gene Name: Elongation factor 1-alpha (EF1α) – nuclear-encoded gene

Table 4. Gene 1 (Elongation factor 1-alpha) answer key.
Pick 15 organisms and put an x by the ones you do not choose.

Common Name	Species	Group(s)	Supergroup
n/a	*Antonospora locustae*	Microsporidia/Fungi	Opisthokonta
honeybee	*Apis mellifera*	Insect/Metazoans/Animals	Opisthokonta
n/a	*Aspergillus fumigatus*	Ascomycota/Fungi	Opisthokonta
silkworm	*Bombyx mori*	Insect/Metazoans/Animals	Opisthokonta
agent of lymphatic filariasis	*Brugia malayi*	Nematoda/Metazoans/Animals	Opisthokonta
nematode	*Caenorhabditis elegans*	Nematoda/Metazoans/Animals	Opisthokonta
n/a	*Candida albicans*	Ascomycota/Fungi	Opisthokonta
n/a	*Coccidioides immitis*	Ascomycota/Fungi	Opisthokonta
n/a	*Cryptococcus neoformans*	Basidiomycota/Fungi	Opisthokonta
causative agent of cryptosporidiosis	*Cryptosporidium parvum*	Apicomplexans	Alveolata
zebrafish	*Danio rerio*	Teleostei/Metazoans/Animals	Opisthokonta
a cellular slime mold	*Dictyostelium discoideum*	Cellular Slime Molds	Amoebozoa
fruit fly	*Drosophila melanogaster*	Insect/Metazoans/Animals	Opisthokonta
causative agent of amoebiasis	*Entamoeba histolytica*	Entamoebas	Amoebozoa
causative agent of giardiasis	*Giardia intestinalis (= G. lamblia)*	Diplomonads	Diplomonadida
soybean	*Glycine max*	Eudicotyledons/Plants/Plantae	Viridiplantae
human	*Homo sapiens*	Mammalia/Metazoans/Animals	Opisthokonta
yuca/tapioca	*Manihot esculenta*	Eudicotyledons/Plants/Plantae	Viridiplantae
mouse	*Mus musculus*	Mammalia/Metazoans/Animals	Opisthokonta
rice	*Oryza sativa*	Poaceae/Plants/Plantae	Viridiplantae
n/a	*Ostreococcus lucimarinus*	Prasinophyceae/Chlorophytes	Viridiplantae
n/a	*Pichia stipitis*	Ascomycota/Fungi	Opisthokonta
causative agent of malaria	*Plasmodium falciparum*	Apicomplexans	Alveolata
n/a	*Puccinia graminis f. sp. tritici*	Basidiomycota/Fungi	Opisthokonta
baker's yeast	*Saccharomyces cerevisiae*	Ascomycota/Fungi	Opisthokonta
bread wheat	*Triticum aestivum*	Poaceae/Plants/Plantae	Viridiplantae
n/a	*Xenopus tropicalis*	Anura/Metazoans/Animals	Opisthokonta
n/a	*Yarrowia lipolytica*	Ascomycota/Fungi	Opisthokonta
corn/maize	*Zea mays*	Poaceae/Plants/Plantae	Viridiplantae

GeneSequence#2:MAKIKIGINGFGRIGRLVARVILQRDDVELVAVNDPFITTDYMTYMFKYDSVHGQWKHHE
LKVKDEKTLLFGEKPVSVFGCRNPEEIPWSQTGADFVVESTGVFTDKDKAAAHLKGGAKKVVISAPSK-
DAPMFVVGVNEHEYKSDLHIVSNASCTTNCLAPLAKVINDKFGIVEGLMTTVHSITATQKTVDGPSAK-
DWRGGRAASFNIIPSSTGAAKAVGKVLPALNGKLTGMAFRVPTVDVSVVDLTVRLEKAATYEDIKAAI-
KAESEGKMKGILGYTEDDVVSTDFIGDNRSSIFDAKAGIALNDHFAKLVSWYDNEWGYSSRVVDLIVHM-
SKTE

Type of Sequence: Protein
Gene Name: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) – glycolytic/cytosolic (not chloroplast!)

Table 5. Gene 2 (Glyceraldeyhyde 3-phosphate dehydrogenase) answer key.
Pick 15 organisms and put an x by the ones you do not choose.

Common Name	Species	Group(s)	Supergroup
n/a	*Aspergillus fumigatus*	Ascomycota/Fungi	Opisthokonta
silkworm	*Bombyx mori*	Insect/Metazoans/Animals	Opisthokonta
cow	*Bos Taurus*	Mammalia/Metazoans/Animals	Opisthokonta
Chinese cabbage	*Brassica rapa subsp. pekinensis*	Eudicotyledons/Plants/Plantae	Viridiplantae
nematode	*Caenorhabditis elegans*	Nematoda/Metazoans/Animals	Opisthokonta
n/a	*Candida albicans*	Ascomycota/Fungi	Opisthokonta
domesticated dog	*Canis lupus familiaris*	Mammalia/Metazoans/Animals	Opisthokonta
n/a	*Ciona intestinalis*	Chordata/Metazoans/Animals	Opisthokonta
causative agent of cryptosporidiosis	*Cryptosporidium parvum*	Apicomplexans	Alveolata
a cellular slime mold	*Dictyostelium discoideum*	Cellular Slime Molds	Amoebozoa
fruit fly	*Drosophila melanogaster*	Insect/Metazoans/Animals	Opisthokonta
n/a	*Encephalitozoon cuniculi*	Microsporidia/Fungi	Opisthokonta
causative agent of amoebiasis	*Entamoeba histolytica*	Entamoebas	Amoebozoa
domesticated horse	*Equus caballus*	Mammalia/Metazoans/Animals	Opisthokonta
soybean	*Glycine max*	Eudicotyledons/Plants/Plantae	Viridiplantae
human	*Homo sapiens*	Mammalia/Metazoans/Animals	Opisthokonta
n/a	*Kluyveromyces lactis*	Ascomycota/Fungi	Opisthokonta
causative agent of cutaneous leishmaniasis	*Leishmania infantum*	Kinetoplastids	Euglenozoa
duck-billed platypus	*Ornithorhynchus anatinus*	Mammalia/Metazoans/Animals	Opisthokonta
rabbit	*Oryctolagus cuniculus*	Mammalia/Metazoans/Animals	Opisthokonta
rice	*Oryza sativa*	Poaceae/Plants/Plantae	Viridiplantae
n/a	*Ostreococcus lucimarinus*	Prasinophyceae/Chlorophytes	Viridiplantae
n/a	*Phanerochaete chrysosporium*	Basidiomycota/Fungi	Opisthokonta
n/a	*Pichia stipitis*	Ascomycota/Fungi	Opisthokonta
causative agent of malaria	*Plasmodium falciparum*	Apicomplexans	Alveolata
pig	*Sus scrofa*	Mammalia/Metazoans/Animals	Opisthokonta
zebra finch	*Taeniopygia guttata*	Aves/Metazoans/Animals	Opisthokonta
n/a	*Tetraodon nigroviridis*	Teleostei/Metazoans/Animals	Opisthokonta
causative agent of East Coast Fever	*Theileria parva*	Apicomplexans	Alveolata
wine grape	*Vitis vinifera*	Eudicotyledons/Plants/Plantae	Viridiplantae
n/a	*Xenopus tropicalis*	Anura/Metazoans/Animals	Opisthokonta
n/a	*Yarrowia lipolytica*	Ascomycota/Fungi	Opisthokonta
corn/maize	*Zea mays*	Poaceae/Plants/Plantae	Viridiplantae

*GeneSequence#3:*MREIVHVQGGQCGNQIGAKFWEVISDEHGVEPTGAYHGDSDLQLERINVYYNEATGG
RYVPRAVLMDLEPGTMDSVRAGPFGQLFRPDNFVFGQTGAGNNWAKGHYTEGAELIDSVLDVVRKE-
AEGCDCLQGFQITHSLGGGTGSGMGTLLISKIREEYPDRIMETFSVFPSPKVSDTVVEPYNATLSVHQL-
VENADEVMVIDNEALYDICFRTLKLTTPTYGDLNHLVSACISGVTACLRFPGQLNSDLRKLAVNLIPFPRL-
HFFMIGFAPLTSRGSQQYRALTVPELTQQMFDAKNMMSASDPRHGRYLTASAMFRGRMSTKEVDEQML-
NVQNKNSSYFVEWIPNNIKSSVCDIPPKGLKMSSTFVGNSTAIQEMFKRVAEQFTAMFRRKAFLHWYT-
GEGMDEMEFTEAESNMNDLVSEYQQYQDATAEEEGEMDEEEGAME

Type of Sequence: Protein
Gene Name: Beta-tubulin (β-tubulin) – nuclear-encoded gene

Table 6. Gene 3 (Beta-tubulin) answer key.
Pick 15 organisms and put an x by the ones you do not choose.

Common Name	Species	Group(s)	Supergroup
human	*Homo sapiens*	Mammalia/Metazoans/Animals	Opisthokonta
rice	*Oryza sativa*	Poaceae/Plants/Plantae	Viridiplantae
n/a	*Ostreococcus lucimarinus*	Prasinophyceae/Chlorophytes	Viridiplantae
chimpanzee	*Pan troglodytes*	Mammalia/Metazoans/Animals	Opisthokonta
n/a	*Pichia stipitis*	Ascomycota/Fungi	Opisthokonta
causative agent of malaria	*Plasmodium falciparum*	Apicomplexans	Alveolata
rat	*Rattus norvegicus*	Mammalia/Metazoans/Animals	Opisthokonta
n/a	*Rhizopus oryzae*	*incertae sedis*/Fungi	Opisthokonta
baker's yeast	*Saccharomyces cerevisiae*	Ascomycota/Fungi	Opisthokonta
tomato	*Solanum lycopersicum*	Eudicotyledons/Plants/Plantae	Viridiplantae
purple sea urchin	*Strongylocentrotus purpuratus*	Echinodermata/Metozoan/Animals	Opisthokonta
pig	*Sus scrofa*	Mammalia/Metazoans/Animals	Opisthokonta
zebra finch	*Taeniopygia guttata*	Aves/Metazoans/Animals	Opisthokonta
torafugu	*Takifugu rubripes*	Teleostei/Metazoans/Animals	Opisthokonta
n/a	*Tetraodon nigroviridis*	Teleostei/Metazoans/Animals	Opisthokonta
causative agent of East Coast Fever	*Theileria parva*	Apicomplexans	Alveolata
causative agent of toxoplasmosis	*Toxoplasma gondii*	Apicomplexans	Alveolata
red flour beetle	*Tribolium castaneum*	Insect/Metazoans/Animals	Opisthokonta
bread wheat	*Triticum aestivum*	Poaceae/Plants/Plantae	Viridiplantae
causative agent of African sleeping sickness	*Trypanosoma brucei*	Kinetoplastids	Euglenozoa
causative agent of Chagas disease	*Trypanosoma cruzi*	Kinetoplastids	Euglenozoa
n/a	*Ustilago maydis*	Basidiomycota/Fungi	Opisthokonta
wine grape	*Vitis vinifera*	Eudicotyledons/Plants/Plantae	Viridiplantae
n/a	*Xenopus tropicalis*	Anura/Metazoans/Animals	Opisthokonta
n/a	*Yarrowia lipolytica*	Ascomycota/Fungi	Opisthokonta
corn/maize	*Zea mays*	Poaceae/Plants/Plantae	Viridiplantae

Table 7. Possible breadth of organisms with BLASTable genomes.

Common Name	Species	Group(s)	Supergroup
yellow fever mosquito	*Aedes aegypti*	Insect/Metazoans/Animals	Opisthokonta
n/a	*Allomyces macroogynus*	Blastocladiomycota/Fungi	Opisthokonta
African malaria mosquito	*Anopheles gambiae*	Insect/Metazoans/Animals	Opisthokonta
n/a	*Antonospora locustae*	Microsporidia/Fungi	Opisthokonta
honeybee	*Apis mellifera*	Insect/Metazoans/Animals	Opisthokonta
thale cress	*Arabidopsis thaliana*	Eudicotyledons/Plants/Plantae	Viridiplantae
n/a	*Aspergillus fumigatus*	Ascomycota/Fungi	Opisthokonta
n/a	*Batrachochytrium dentrobatidis*	Chytridiomycota/Fungi	Opisthokonta
silkworm	*Bombyx mori*	Insect/Metazoans/Animals	Opisthokonta
cow	*Bos Taurus*	Mammalia/Metazoans/Animals	Opisthokonta
Chinese cabbage	*Brassica rapa subsp. pekinensis*	Eudicotyledons/Plants/Plantae	Viridiplantae
agent of lymphatic filariasis	*Brugia malayi*	Nematoda/Metazoans/Animals	Opisthokonta
nematode	*Caenorhabditis elegans*	Nematoda/Metazoans/Animals	Opisthokonta
n/a	*Candida albicans*	Ascomycota/Fungi	Opisthokonta
domesticated dog	*Canis lupus familiaris*	Mammalia/Metazoans/Animals	Opisthokonta
n/a	*Ciona intestinalis*	Chordata/Metazoans/Animals	Opisthokonta
n/a	*Coccidioides immitis*	Ascomycota/Fungi	Opisthokonta
n/a	*Coprinopsis cinerea okayama*	Basidiomycota/Fungi	Opisthokonta
n/a	*Cryptococcus neoformans*	Basidiomycota/Fungi	Opisthokonta
causative agent of cryptosporidiosis	*Cryptosporidium parvum*	Apicomplexans	Alveolata
zebrafish	*Danio rerio*	Teleostei/Metazoans/Animals	Opisthokonta
a cellular slime mold	*Dictyostelium discoideum*	Cellular Slime Molds	Amoebozoa
fruit fly	*Drosophila melanogaster*	Insect/Metazoans/Animals	Opisthokonta
n/a	*Encephalitozoon cuniculi*	Microsporidia/Fungi	Opisthokonta
causative agent of amoebiasis	*Entamoeba histolytica*	Entamoebas	Amoebozoa
domesticated horse	*Equus caballus*	Mammalia/Metazoans/Animals	Opisthokonta
domesticated cat	*Felis catus*	Mammalia/Metazoans/Animals	Opisthokonta
chicken	*Gallus gallus*	Aves/Metazoans/Animals	Opisthokonta
causative agent of giardiasis	*Giardia intestinalis (= G. lamblia)*	Diplomonads	Diplomonadida
soybean	*Glycine max*	Eudicotyledons/Plants/Plantae	Viridiplantae
human	*Homo sapiens*	Mammalia/Metazoans/Animals	Opisthokonta
n/a	*Kluyveromyces lactis*	Ascomycota/Fungi	Opisthokonta
causative agent of cutaneous leishmaniasis	*Leishmania infantum*	Kinetoplastids	Euglenozoa
causative agent of visceral leishmaniasis	*Leishmania major*	Kinetoplastids	Euglenozoa
n/a	*Lotus japonicus*	Eudicotyledons/Plants/Plantae	Viridiplantae
rhesus monkey/macaque	*Macaca mulatta*	Mammalia/Metazoans/Animals	Opisthokonta

yuca/tapioca	*Manihot esculenta*	Eudicotyledons/Plants/Plantae	Viridiplantae
gray short-tailed opossum	*Monodelphis domestica*	Mammalia/Metazoans/Animals	Opisthokonta
mouse	*Mus musculus*	Mammalia/Metazoans/Animals	Opisthokonta
a wasp	*Nasonia giraulti*	Insect/Metazoans/Animals	Opisthokonta
duck-billed platypus	*Ornithorhynchus anatinus*	Mammalia/Metazoans/Animals	Opisthokonta
rabbit	*Oryctolagus cuniculus*	Mammalia/Metazoans/Animals	Opisthokonta
rice	*Oryza sativa*	Poaceae/Plants/Plantae	Viridiplantae
n/a	*Ostreococcus lucimarinus*	Prasinophyceae/Chlorophytes	Viridiplantae
chimpanzee	*Pan troglodytes*	Mammalia/Metazoans/Animals	Opisthokonta
human body louse	*Pediculus humanus corporis*	Insect/Metazoans/Animals	Opisthokonta
n/a	*Phanerochaete chrysosporium*	Basidiomycota/Fungi	Opisthokonta
n/a	*Pichia stipitis*	Ascomycota/Fungi	Opisthokonta
causative agent of malaria	*Plasmodium falciparum*	Apicomplexans	Alveolata
n/a	*Podospora anserine*	Ascomycota/Fungi	Opisthokonta
n/a	*Puccinia graminis f. sp. tritici*	Basidiomycota/Fungi	Opisthokonta
rat	*Rattus norvegicus*	Mammalia/Metazoans/Animals	Opisthokonta
n/a	*Rhizopus oryzae*	*incertae sedis*/Fungi	Opisthokonta
baker's yeast	*Saccharomyces cerevisiae*	Ascomycota/Fungi	Opisthokonta
n/a	*Selaginella moellendorffii*	Lycopodiophyta/Plants/Plantae	Viridiplantae
tomato	*Solanum lycopersicum*	Eudicotyledons/Plants/Plantae	Viridiplantae
purple sea urchin	*Strongylocentrotus purpuratus*	Echinodermata/Metozoan/Animals	Opisthokonta
pig	*Sus scrofa*	Mammalia/Metazoans/Animals	Opisthokonta
zebra finch	*Taeniopygia guttata*	Aves/Metazoans/Animals	Opisthokonta
torafugu	*Takifugu rubripes*	Teleostei/Metazoans/Animals	Opisthokonta
n/a	*Tetraodon nigroviridis*	Teleostei/Metazoans/Animals	Opisthokonta
n/a	*Thalassiosira pseudonana*	Diatoms	Stramenopila
causative agent of East Coast Fever	*Theileria parva*	Apicomplexans	Alveolata
causative agent of toxoplasmosis	*Toxoplasma gondii*	Apicomplexans	Alveolata
red flour beetle	*Tribolium castaneum*	Insect/Metazoans/Animals	Opisthokonta
bread wheat	*Triticum aestivum*	Poaceae/Plants/Plantae	Viridiplantae
causative agent of African sleeping sickness	*Trypanosoma brucei*	Kinetoplastids	Euglenozoa
causative agent of Chagas disease	*Trypanosoma cruzi*	Kinetoplastids	Euglenozoa
n/a	*Ustilago maydis*	Basidiomycota/Fungi	Opisthokonta
wine grape	*Vitis vinifera*	Eudicotyledons/Plants/Plantae	Viridiplantae
n/a	*Xenopus tropicalis*	Anura/Metazoans/Animals	Opisthokonta
n/a	*Yarrowia lipolytica*	Ascomycota/Fungi	Opisthokonta
corn/maize	*Zea mays*	Poaceae/Plants/Plantae	Viridiplantae

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