

safety

BASIC LAB SAFETY

In this lab, there will be minimal exposure to hazardous chemicals. However, since we will be working with living and preserved specimens, it will be necessary to take a few basic safety precautions that are necessary in almost any biology labs.

MSDS

Material Safety Data Sheets (MSDS) for each of the chemicals used in the lab can be found in a labelled manila folder at the instructor's station at the front of the class. If you were somehow to accidentally be exposed to a chemical in a hazardous way, the exposure should be reported to the lab instructor immediately. The MSDS contains instructions on appropriate measures to be taken if a person is exposed to any of the chemicals being used in the lab.

Precautions

The best course of action to prevent any dangerous situation from arising is to take appropriate precautions. The use of **personal protective equipment (PPEs)** is appropriate at all times in the laboratory. Nitrile gloves are available for use during the lab period. These can be disposed of in the trash receptacle in the front of the classroom. You may wear goggles if you wish, but they are not provided by the lab. You may also wish to wear a lab coat, but lab coats are neither provided or required for the lab.

The specimens that we are using are preserved using alcohol, formalin, formaldehyde, or some proprietary preservative. Be aware that formaldehyde (and formalin) can pose health hazards. Students should avoid direct exposure to these chemicals by wearing gloves and/or protective eyewear when working with preserved specimens. Students should also avoid inhaling formalin or formaldehyde vapors.

Since we will be working with live and preserved specimens, it is not appropriate to eat or drink in lab. For reasons of sanitation, you should finish food or drink before entering the lab, and wash hands thoroughly before leaving the lab.

We will use scalpels and sharp probes, and you should exercise caution when carrying out a dissection. If you cut yourself, you should report it to the instructor as soon as possible. A first aid kit is mounted on the front wall of the classroom, and will include supplies necessary to clean and bandage the cut. If the cut is severe enough, the student should seek medical attention.

use of the microscope

Light / compound microscope

A light or **compound microscope** is used for viewing small organisms that will fit on a glass slide. They may be live or preserved specimens, but they must be small enough or thin enough for light to penetrate them. The basic principle used in a light microscope is that a light positioned on one side of a slide shines through the slide and the specimen into a system of lenses that magnifies the image, and passes the light into your eyes.

The lens closest to the specimen is called the **objective** lens, and the lens that you look through is called the **ocular** lens. This type of microscope is also called a "compound microscope," because of the compound effect of viewing the image through multiple lenses. For example, the **high power objective** has a magnification of 40X, but since the ocular lens has a magnification of 10X, the total magnification of the specimen is $40 \times 10 = 400X$. The highest total magnification of a compound microscope is usually 1000X, using an oil-immersion lens. The 100X objective requires that the specimen be viewed through a drop of mineral oil that actually makes contact with the lens itself. The oil-immersion objective is useful when viewing very small subjects like bacteria. We will not be using the 100X objective.

When viewing a subject with a compound microscope, one would normally begin with the lowest power objective and move to increasingly higher power objectives in succession. We will use the 4X, 10X and 40X objectives. Later in this section, we will discuss the steps to take when viewing a slide with the compound microscope.

Parts of the Compound Microscope

When retrieving a microscope from the storage area, one should be sure to place one hand under the **base** of the microscope, and hold the **arm** of the microscope with the other hand. Please keep in mind that these microscopes are very expensive instruments. Microscopes should be returned to storage after usage.

There are two adjustments for the light source on the microscope, an intensity adjustment (located on the base) and the **diaphragm**. The diaphragm adjusts the aperture through which the light is transmitted.

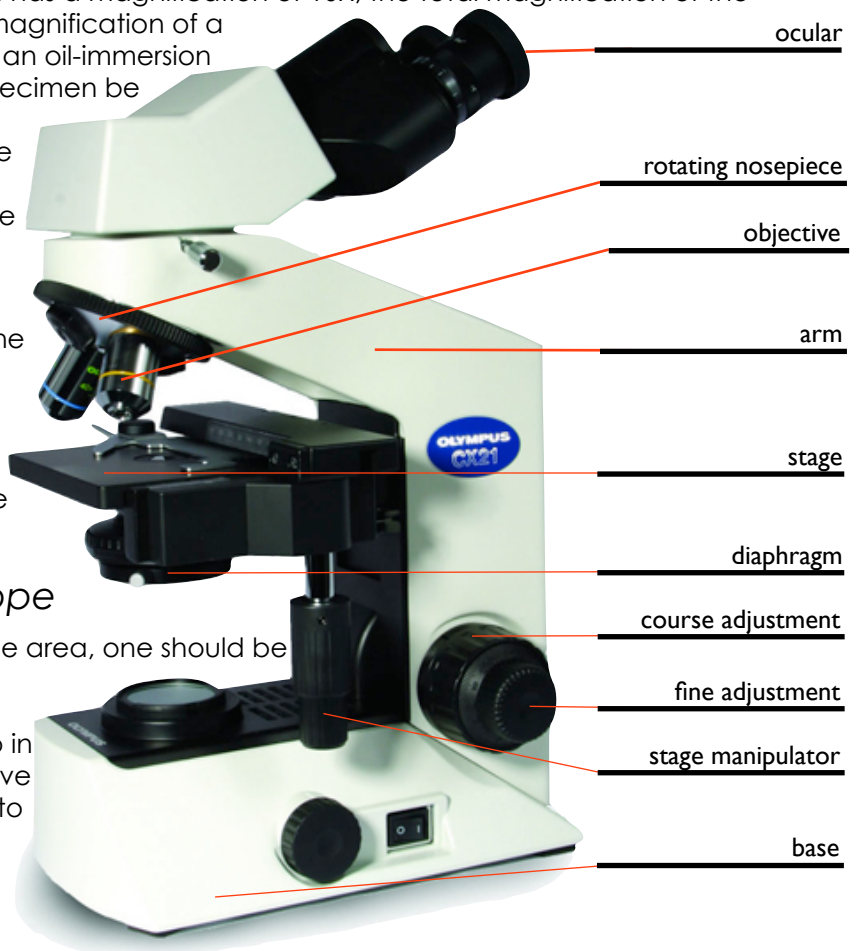
Objectives can be selected by rotating the **nosepiece** until it clicks into place. If the objective is not fully in place, the view through the ocular will be obstructed. When changing objectives, the nosepiece should always be visualized from the side to make sure that the objective is not going to make contact with the slide. If it does, it can damage the slide, the specimen, or the objective.

The focus can be changed by using the **fine** or **coarse adjustment** knobs located near the base of the scope.

Use of the Compound Microscope

* Obtain a sample slide from the box on your lab table. There is a caliper on the stage that holds the slide in place for viewing. Begin by placing the slide in the caliper so that it snaps into position. The slide can now be moved by using the stage manipulator knobs. Use the method described below to examine your specimen.

Often, if you hold the slide up to the light you will be able to see the specimen. You will want to position the slide so that the specimen is directly under the middle of the objective. Initially, you should begin with the scanning (4X objective) or the low power objective (10X). Never begin with the high power objective (40X). This is not because we do not trust you. This is not because we do not think you know what you are doing. As magnification increases, the field of view decreases and it becomes more difficult to focus. As a result, it will be too difficult to find what you are looking for under high power.



If you turn the coarse adjustment knob, you will notice that it actually moves the stage up and down. The fine adjustment does the same thing, but slower. The most efficient way to begin is by turning the coarse adjustment until the stage is as close to the objective as it will go. Notice that the low power objective will not touch the slide. This is not the case with the high power objective. **Never ever use the coarse adjustment knob with the high power objective.** If you turn the coarse adjustment under high power, you will most likely break the slide, and possibly the objective. Using the low power or scanning objective, look through the ocular and see if you can visualize the specimen. Now, rotate the coarse adjustment until the image starts to come into focus. When it is almost in focus, use the fine adjustment to sharpen the image. Keep in mind that the specimen you are viewing is three-dimensional. You can use the fine adjustment to focus up and down through the specimen.

You may also notice that when you move the slide left, the image through the oculars moves right, and vice-versa. Also, when you move the slide up, the image moves down. This can make it challenging to follow a moving specimen on a wet-mount slide.

Once you have found the specimen under low power, you may wish to switch to high power to view it under increased magnification. When an object is in focus under low power, it should remain in focus when switching to a higher power objective. This is because the objectives of compound microscopes are **parfocal**. When switching to the high power objective, be sure to visualize the objective as it nears the slide, to assure that it does not make contact with the slide. Once the objective is in position, you may look through the ocular. You may need to adjust the focus using the fine adjustment. Again, do not touch the coarse adjustment while under high power. If the specimen is not visible, you may wish to move the stage by using the stage manipulation knobs. In doing so, be very careful. Since the field of view is now smaller, even the smallest movement of these knobs will have an exaggerated effect. If for some reason you cannot find the specimen under high power, you still should not use the coarse adjustment. That will only make matters worse. Instead, go back to low power, find the specimen, center it in your field of view, and then go back to high power.

Some of the specimens that we will view under the microscope will be stained and others will not. In order to get the best possible view of a specimen, you may want to adjust the aperture with the diaphragm lever. If you turn the light source up all the way, and make the aperture as small as possible you will achieve the best contrast. This will allow viewing of even unstained specimens with as much detail as possible.

Wet-Mount Specimens

* In order to prepare a wet-mount slide, you will need an empty glass slide and a coverslip. Using either the eyedropper from your dissection kit or one of the plastic pipettes supplied by the lab, obtain a drop of pond water. You will get better results if you try to make sure some of the visible items from the water into your pipette. Place two or three drops of the pond water together onto the middle of your slide. To apply the coverslip, first apply just one edge of the coverslip so that it makes contact with the drops of pond water; then drop the coverslip so that it covers the pond water sample. It is important to make sure that there is not too much water. If there is too much water, it will run off of the slide, making it hard to move the slide on the stage of the microscope. Once you have your slide prepared, place it on the stage of the compound microscope and use the steps outlined above to see what you can find. Organisms commonly seen include cyanobacteria, algae, protozoans (e.g. *Paramecium*, *Euglena*, *Vorticella*, *Amoeba*), rotifers, and freshwater annelids.

Dissecting Scopes

The other type of microscope that we will be using in this class is the **dissection scope**. These microscopes are designed to magnify small specimens that are too large to fit on a slide, or too thick and/or opaque to be viewed with a light microscope. The magnification of dissection scopes varies greatly from model to model, but may be as high as 500X. With dissecting scopes it is generally not necessary to change objectives. Instead, magnification is usually controlled by a knob that changes the zoom of the image. These scopes also generally only have one adjustment knob for focus. Often, a dissecting scope will have dual light sources. One light source is positioned above the specimen for viewing opaque items while the other is below the specimen for transparent or translucent subjects. Not all of our scopes have a light source. For most of the items we will be viewing, there will be enough ambient light in the room to illuminate the specimen. (note: the amount of light necessary to view a object increases as magnification increases. This is why the visible field of a light microscope will seem dimmer when you switch to the high power objective.)

- * Obtain a specimen in plastimount or a microscope slide with a larger organism, and observe the specimen under the dissecting scope. Try the using each of the light sources to see which one works best with your specimen. You may find that your discover different features using the alternate light sources.

systematics

Systematics is the branch of biology that attempts to use information about phylogenies to construct biologically meaningful groups for use in taxonomy. **Taxonomy** is the system of naming that is used by biologists. Traditionally, organisms were grouped on the basis of shared similarities. Today, biologists attempt to group organisms based on shared ancestry. While some attempt to use phylogenies to adjust existing nomenclature to reflect evolutionary relationships, others have suggested that the traditional taxonomy should be abandoned in favor of an entirely new system based on nested clades (e.g. PhyloCode) that do not necessarily use the traditional taxonomic ranks.

- Domain: Eukarya
- Kingdom: Animalia
- Phylum: Arthropoda
- Class: Arachnida
- Order: Araneae
- Family: Lycosidae
- Genus: *Hogna*
- specific epithet: *carolinensis*

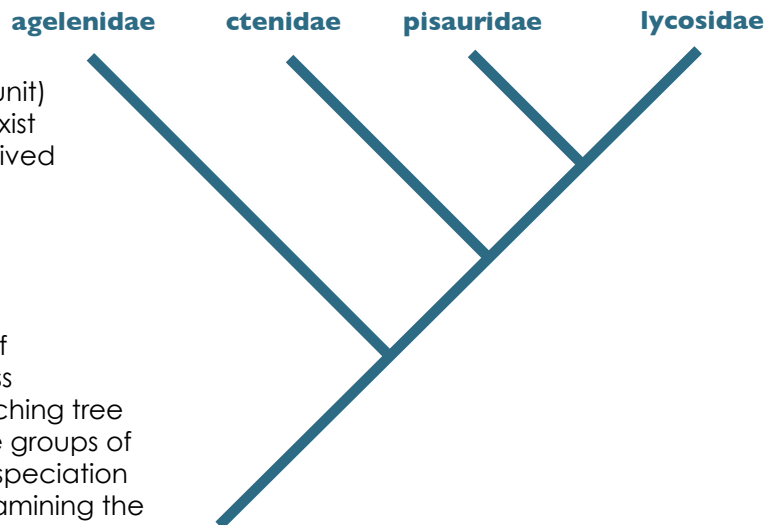
species name: *Hogna carolinensis*
common name: carolina wolf spider

In taxonomy, we use a nested hierarchy of names to represent groups of organisms. Traditional taxonomic ranks include: Domain, Kingdom, Phylum, Class, Order, Family, Genus, and specific epithet. It should be noted, however, that these are simply ordinal ranks. In other words, the ranks simply describe order of the hierarchy, not the amount of evolutionary difference among ranks. Therefore, one phylum is not the same size as another, and the amount of difference between a phylum and one of its orders is not the same as the distance between some other phylum and any one of its orders.

Taxonomists use a **binomial system of nomenclature** to represent species. The species name comprises the genus (pl. genera) and the specific epithet. The species name (scientific name) of an organism is always written in italics or underlined. These are generally latin, for the sake of standardization. Species are also often given common names, but these may differ in different regions of the world. Different languages may also use different common names. For these reasons, common names are not generally used for scientific purposes.

cladistic analysis

One of the ways that scientists have sought to resolve the discrepancies between evolutionarily meaningful groups and traditional taxonomies is through the use of cladistic analysis. Cladistic analysis is a method of examining the sets of characteristics that a group of organisms (or any hierarchical subunit) possesses, to determine what meaningful groups exist within a hierarchy. This is done by using shared derived characters (**synapomorphies**) to determine the constituents of groups.



Understanding trees

If we consider the evolutionary history of a group of organisms, we can think of the evolutionary process using the metaphor of a branching tree. This branching tree would show how speciation events have led to the groups of organisms that we are currently evaluating. Each speciation event is represented by a node on the tree. By examining the nodes of the tree, we can better understand how the organisms are related to one another. For example, the node that connects the families “ctenidae,” “pisauridae,” and “lycosidae” in this example tree demonstrates that these families share a common ancestor that is not shared

by the most basal group, the "agelenidae." Since this tree has three nodes, it implies three speciation events that must have existed between these four families of spiders. It is important to keep in mind that there were many other speciation events that occurred in the evolutionary history of these families, but there are only three that are relevant to this tree. The more taxa that we add to the tree, the more nodes it will have. It is also worth noting that although each node represents a species that existed at one time, the exact species that it represents is for all practical purposes unknowable. Furthermore, the cladistic analysis used to construct the tree does not in any way evaluate what species is represented by a node.

Once a tree is constructed, it is important to interpret the tree accurately. Unfortunately, inexperienced students will often have misconceptions about what information is actually conveyed by a tree. These implications can be avoided by remembering one very important fact about trees: **the only information conveyed by a simple phylogenetic tree is in the nodes.** Anything that seems to be implied by the horizontal or vertical arrangement of taxa names is erroneous. For example, some would look at the tree above and assume that "ctenids" are more closely related to "agelenids" than to "lycosids." Not only is this not true, but the opposite is actually explicitly stated by the tree: "ctenids" are more closely related to "lycosids" than to "agelenids." The node that joins "ctenids," "pisaurids," and "lycosids" defines this relationship.

This mistaken assumption outlined above can be avoided if one realizes that the tree can pivot on any node, without changing its topology. In other words, if "pisauridae" and "lycosidae" were switched, this would still be the exact same tree. Since the nodes did not change, the tree did not change.

It is also possible to format a hierarchy represented by a tree using only parentheses and text. The tree above could also be written:

(agelenidae(ctenidae(pisauridae, lycosidae)))

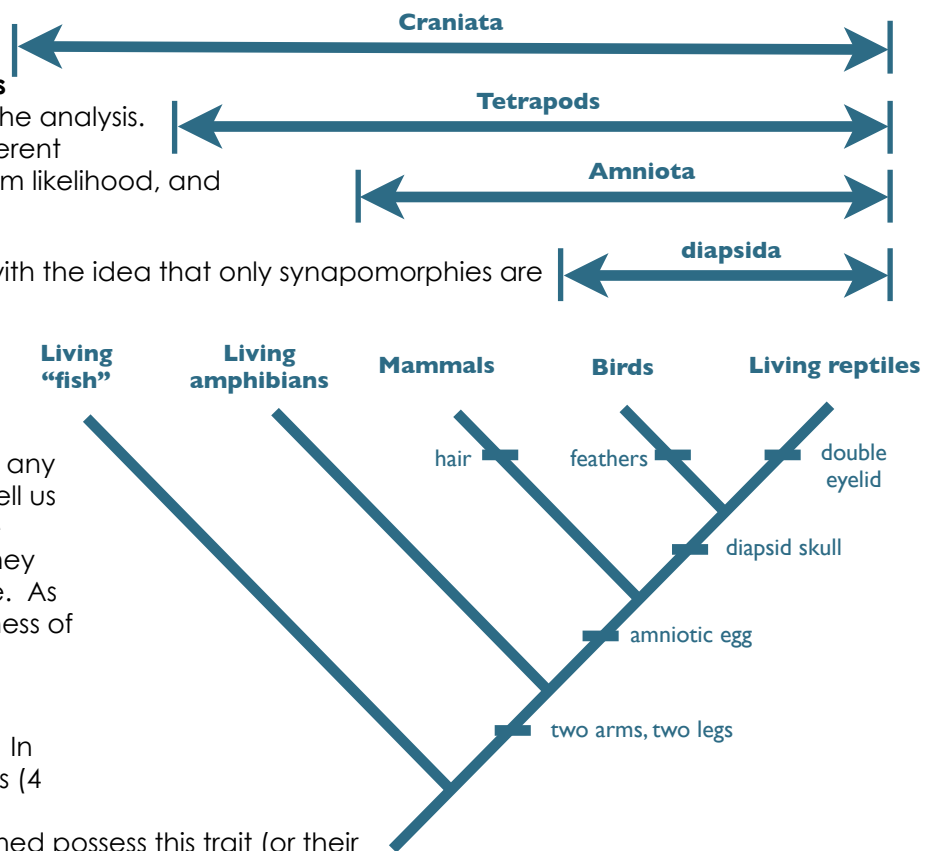
The same information is contained in the tree and the formula written above.

Cladistic analysis

In a cladistic analysis, relationships are determined on the basis of synapomorphies only. **Autapomorphies** and **plesiomorphies** are excluded from the analysis. Trees are constructed using several different methods, including parsimony, maximum likelihood, and neighbor-joining.

At first, some people have a problem with the idea that only synapomorphies are informative in determining relationships. However, if you consider other types of characters, you will understand why they are not informative. Autapomorphies are characteristics that are not shared with any other group. As a result, these do not tell us about relationships. Plesiomorphies are primitive characters. This means that they are characters that all the groups share. As such, they do not tell us about relatedness of groups, since all the groups share these characters.

Consider the example tree to the right. In this tree, the presence of tetrapod limbs (4 legs) is not an informative character because all of the groups being examined possess this trait (or their ancestors possessed it). This trait would be considered to be a



synplesiomorphy. This trait would not be included in a cladistic analysis, since it is not a synapomorphy. The characteristic feathers of birds would also be an uninformative trait for this tree. Since birds are the only group included in the tree that possess feathers, this trait is an autapomorphy. (i.e. it does not tell us which groups are more closely related to birds.)

Traits such as the diapsid skull and amniotic egg are probably synapomorphies. They are informative characters that give us insight into the relationships among groups. Among the groups represented in this tree, only the mammals, birds and reptiles have an amniotic egg. The diapsid skull is only found in the birds and the rest of the reptiles. This implies two relationships. First, mammals, birds and reptiles are more closely related to each other than they are to amphibians and fish. Secondly, birds and reptiles are more closely related to each other than either of them is to mammals. These two relationships are represented by two nodes on the tree.

Implications for systematics

If the goal of phylogenetic systematics is to group organisms in such a way that it reflects the evolutionary history, then a phylogenetic tree (once it is well supported) can be used to define these groups. If groups are to reflect evolutionary relationships, they must be **monophyletic**. This means that the group must contain all of the descendants of a common ancestor. If the group is missing a descendant, it is considered to be **paraphyletic**. If the group contains members that are descendants of different common ancestors (i.e. multiple separate lineages), it is considered to be **polyphyletic**. Either case poses a problem for systematic taxonomy. If a group is paraphyletic or polyphyletic, it is no longer considered to be valid.

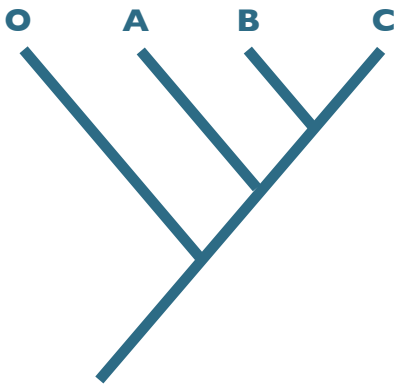
questions

microscope use

1. What does it mean when a microscope is parfocal?
2. What knob should you never touch when using the high power objective? why?
3. How can you increase the detail of an unstained specimen when viewing under a microscope?

cladistics

1. Why is it bad for a group to be polyphyletic?
2. What type of traits can be used in a cladistic analysis?
3. Draw two equivalent versions of the following tree:



4. Find the most parsimonious tree based on the following character matrix:

<i>taxon/ character</i>	1	2	3	4	5	6
outgroup	0	0	0	0	0	0
A	1	1	1	0	0	1
B	0	0	1	1	1	0
C	1	1	1	0	0	1
D	1	0	1	0	0	0
E	0	0	1	1	1	0

5. Find three monophyletic groups that could be made for the character matrix above.

Drawings

pond water sample (please draw to scale)

Magnification: _____

objective: _____

