Ecol 483/583 – Herpetology
Lab 2: Phylogeny Exercise, Amphibia Diversity 1: Urodela & Gymnophiona
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Lab objectives
The objectives of today's lab are to:
1. Continue to familiarize yourselves with local herps and their external anatomy.
2. Use this knowledge to collect morphological character data from specimens.
3. Reconstruct a phylogeny using your collected character data.
4. Be able to discuss phylogenetic concepts introduced in lecture in the context of the lab.
5. Familiarize yourselves with extant diversity of the Urodela and Gymnophiona.

Collecting data from specimens and using it to reconstruct a phylogeny will reinforce your knowledge of local herps and accustom you to using the terminology from last week's lab. During today's lab you will also begin studying amphibian diversity. The lab will introduce the Urodela (salamanders) and Gymnophiona (caecilians).

Tips for learning the material
Take some time today to review some of the material that was covered last week. The same specimens are out to allow you to do this. Use the opportunity to quiz yourself or a partner by covering up species names and identifying specimens. Note which ones you find easy to ID and which ones give you trouble. Why do those give you troubles? Refine your criteria for differentiating them and take some more notes.

However, do not spend too much time reviewing last week's material – there are new things to cover as well. Today's phylogeny exercise will help you to reinforce some of this material, at least for the lizards, which will be the focus of that exercise. In addition, some new specimens are out – introduce yourself to the salamanders and caecilians. Remember the strategies you have been using to learn the species that are available in lab. The species names that are in bold represent Arizona natives, which you will have to know how to identify to species. However, the species that are not in bold are not local and you are only responsible for know which higher taxon ("family") they belong to.

Exercise 1: Collecting character data

A necessary step toward constructing a phylogeny is to collect character data. This means looking at specimens and figuring out which characteristics they posses. In practice, this is rarely straight forward. First, a researcher must look at many (possibly hundreds) of specimens and figure out which ones are variable enough but not too variable to be useful. Characters that don't vary among any of the species or **operational taxonomic units (OTUs)** are **plesiomorphic** and not informative of phylogeny. Likewise characters that characterize only one OTU are called **autapomorphic** and are also not useful to reconstructing phylogeny. Autapomorphies, however, are useful for identification or diagnosis of species. Throughout the labs, by learning how to identify species that you see, you will frequently rely on autapomorphies – what makes each species unique. At the opposite extreme are characters that vary too much. These are often **polymorphic** within species. Although they sometimes can be useful for phylogenetic reconstruction, they are typically too variable. For phylogenetic reconstruction, **synapomorphies** are the characters of interest. **Synapomorphies unite some but not all of the OTUs.**

Take a look at the anurans that are on display,

What is a synapomorphy of the genus *Hyla*?

What is a plesiomorphy of *Hyla*? (this is a tough one)

Now take a look at the rattlesnakes on display.

What is a plesiomorphy of *Crotalus*?

What is a synapomorphy of *Crotalus*?

What is an autapomorphy of *Crotalus cerastes*?

Repeat this exercise with other taxa on display so that you gain an understanding of what these terms mean and how they can help you learn features of the taxa you are learning.

In today's lab, you **DO NOT** need to spend time selecting characters. This is very time consuming and has been done for you. You can concentrate on examining a small number of specimens of each species and coding your findings into the table provided below. We will be working on a phylogeny of some local lizards to help you learn to differentiate them.

Characters generally come in two types: **binary** and **multi-state**. Binary characters only have two states (written in a matrix as 0 when **ancestral** and 1 when **derived**). All the characters we are using today are binary. Multi-state characters have more than two states. For example, if the character is number of scale rows, the states might be 48, 49, 50, or 51 (in this example there are four states). Multi-state characters also come in two types: **discrete** and **continuous**. Discrete characters include things like scale number, tooth number, and eye color. Continuous characters are measurements, such as snout-vent length, for which there is an infinite number of states. Because of this, dealing with continuous characters can be quite complicated.

For the turtles on display, come up with one example of each type of character listed:

Continuous:

Discrete binary:

Discrete multi-state:

What is meant by "ancestral" and "derived"?

Complete the table provided. Some of the lizard specimens in lab have capital letters beside them (A-H, I is a salamander). When you examine a specimen, fill in the species names in the appropriate column and then examine it to code all of the characters (1-10). Character descriptions are supplied below the table, as well as instructions on how to code each one. Pay close attention, as characters can be very subtle.

Character data worksheet

		Character									
ID	Species name	1	2	3	4	5	6	7	8	9	10
A											
B											
C											
D											
E											
F											
G											
H											
I											

Character descriptions

1. *If the ventral scales are rectangular and in regular rows and columns, code "1", if they are arranged in any other way, code "0".*
2. *There is an obvious spot on the side of the body, just posterior to the front limbs (1) or not (0).*
3. *The seams between the supralabial scales are vertical (oriented dorsal-ventral) (0) or diagonal (oriented from postero-ventral to antero-dorsal) (1).*
4. *External ear openings are present (0), or absent (1).*

5. *There are bony spines or crest on the posterior margin of the head (1), or not (0).*

6. *The median postmental scale is present (0), or absent (1). The mental scale is that which is at the tip of the lower jaw (like the rostral is on the upper jaw – see the diagram of the ventral view of the snake head at the back of your field guide, where the mental is labeled). A postmental scale is immediately posterior to the mental. Such a scale can either be medial (at the midline) or not.*

7. *There are uniform keeled scales covering much of the body (1), or other types of scales (0).*

8. *There is a pair of dark, lateral bands on the body between the front and hind limbs (1), or not (0).*

9. *The interparietal scale is as large or larger than the ear opening (1), or smaller than the ear opening (0).*

10. *The cloacal opening is a transverse slit (1) or a longitudinal slit (0).*

For which species was it hardest to code the characters? Why?

Why might this species be a poor choice to include in the analysis?

Which species was most likely the outgroup? Why?

If this species were taken out of the analysis, which species would then be the outgroup?

Exercise 2: Building a phylogeny

Once we have a character matrix, we can go about producing a phylogeny. Before we do that, however, look at your character matrix to see how the characters are distributed. It should be obvious that different characters have different distributions across taxa. Think about them in the context of the types of characters introduced earlier and in lecture.

List the numbers of the characters that are plesiomorphies.

List the numbers of the characters that are synapomorphies.

List the numbers of the characters that are autapomorphies.

How many of the 10 characters are useful for reconstructing phylogeny using a parsimony approach?

Quite often characters are coded in such a way that zeros (0) are interpreted as ancestral and other character states are derived. The most popular way of determining **polarity** of characters (which states are ancestral vs. derived) is through the use of an outgroup.

Ignore the salamander for the rest of this exercise. From looking at your character matrix, which species is the outgroup (you already answered this part of the question on the previous page)? Explain.

Now that we have identified which characters are useful in reconstructing the phylogeny and which taxon is the outgroup, we can go about making a phylogeny. As mentioned earlier, synapomorphies are used in reconstructing phylogeny because they unite some but not all taxa. These are the characters we will be using today.

There are several ways to build phylogenies. Today we will use the algorithmic approach, which works well for a small matrix like the one you created. Go through the steps below to first make a **venn diagram**, and then to draw a phylogeny from your matrix (use the space provided on the next page and a pencil – you will probably have to erase things):

1. **Select all characters that unite only two OTUs and list these taxon pairs.**

2. **Are any OTUs represented in >1 pair?**
    a. **If yes, list all of the OTUs together.**
    b. **If no, go to #3**

3. **In the space provided, write the names of OTUs belonging to each pair/group together and draw a circle around each pair/group.**

4. **For each pair (or group of OTUs), search for a character that unites that pair with another single OTU or pair/group that you have already defined.**
    a. **If a single OTU is added, write its name just outside the circle of the pair/group it is allied with and draw another circle around the previous circle and the new taxon/taxa.**
    b. **If two of your already-defined pairs/groups are united by a character, then draw a larger circle that goes around both groups' circles.**

5. **Repeat steps 2-4, working from the least inclusive characters (those with fewest 1's) to most inclusive characters (those with most 1's) until you run out of synapomorphic characters.**

Use the space above to work on your venn diagram.

Congratulations, you have now made a venn diagram, which is another representation of a phylogeny. Each circle coincides with a node and associated clade on the phylogeny.

Use the space below to draw your phylogeny, using your venn diagram for guidance.

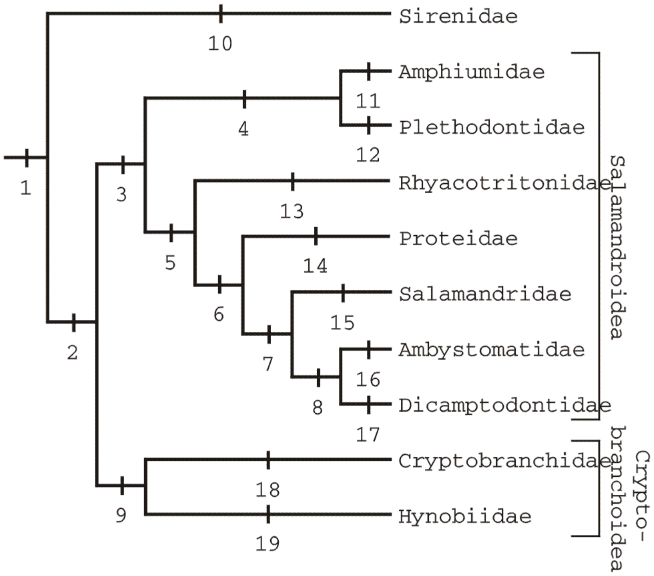
Now that you have a phylogeny, take some time to consider whether it makes intuitive sense. Are the species related to one another as you expected? As you will learn throughout the semester, phylogenies are not only useful, but necessary to study the evolution of any sort of trait you might be interested in (although studying the many uses of phylogenies is beyond the scope of this lab and is covered in classes entirely devoted to the subject. For now, consider your phylogeny.

Is the phylogeny fully resolved?

What is a 'polytomy'?

What approach would you take to resolving the polytomy?

Exercise 3: Urodela diversity



General information
Salamanders are a monophyletic group called Urodela. Some people call it Caudata, so you should be familiar with both terms (see p. 41 of Pough et al. 2004). Salamanders have been around at least since the Jurassic (~170 mya) and are relatively similar morphologically to the earliest amphibians. Currently, we recognize 61 extant genera and about 415 species that are placed into 10 major clades (see Pough et al. Fig. 3-1, to the right). The greatest diversity is in North America, with nine of those clades. They are also found in Eurasia, northern Africa, and Central and South America.

Generalized morphology
Salamanders typically have a tail in all stages of their life cycle (the names Urodela and Caudata both refer to a tail) and four limbs of approximately equal size set at right angles to the body (except Sirenidae). Many bones are absent from the skull. However, they do have ribs and true teeth on both jaws. Larvae usually have external gills and gill slits. The largest salamanders can reach 1.8 m, but most are fewer than 15 cm in total length. Some salamanders lack lungs, and rely on cutaneous respiration for gas exchange.

Generalized life history
Urodela are found in moist, cool habitats, and are often nocturnal. Typically they start out as aquatic larvae with external gills, lateral line systems, no eyelids, very permeable skin, tail fins, a non-adult tooth pattern, as well as other features. Then they metamorphose into terrestrial adults that breathe through lungs and/or skin. They return to ponds or streams for breeding. Fertilization is external in 3 clades (Sirenidae, Cryptobranchidae, and Hynobiidae), and internal in all others, which use a **spermatophore**. A spermatophore is a pile of jelly with a sperm cap on top that the male deposits on the ground. After an elaborate courtship ritual, the receptive female picks up the spermatophore with the lips of her cloaca. Her eggs are then fertilized as they pass through her cloaca and are usually deposited in water. Almost all salamanders are oviparous and parental care of eggs is fairly common. Salamanders are carnivorous in all life stages, primarily eating insects. Amphibians have two chemo-sensory organs in their nose; the olfactory epithelium and the vomeronasal (Jacobson's) organs. Chemoreception is well developed in most salamanders and may play an important role in courtship, species recognition, feeding, and territoriality.

Evolutionary trends
Two evolutionary trends found in salamanders are the loss of lungs and **paedomorphosis** (retention of larval or juvenile characteristics as adults). Salamanders can breathe through their skin and the loss of lungs may free up some muscles that facilitate tongue projection. Some

species always metamorphose from larvae into terrestrial adults, some always remain in their larval form, and some have no larval stage. In some species, only certain populations or individuals exhibit paedomorphosis. Paedomorphosis has evolved independently in several salamander lineages.

Miscellaneous facts
Salamanders have the largest genomes of any tetrapod. Genome size (amount of DNA) ranges from 5 to 30 times larger than the human genome. In fact, there is so much DNA in the nucleus in some species that cell size is constrained. The cells of these salamanders are large to accommodate the genetic material.

<u>North American Salamanders</u>

Ambystomidae: Mole Salamanders

Content and distribution: 1 genus, about 30 species. Canada, the U.S. and Mexico.

Morphology: Ambystomatids are robust salamanders that typically have a broad, short head; large limbs; small eyes; well-developed costal grooves; and a laterally flattened tail. The teeth form a row across the roof of the mouth. Larvae and non-metamorphosed adults also have broad heads, but exhibit long, filamentous external gills; 4 pairs of gill slits; and caudal fins.

Life history: All start off as aquatic larvae. Some species never metamorphose (these are called **axolotls**, an example of **paedomorphosis**), some metamorphose facultatively, some obligately. Metamorphosed adults are **fossorial** and mostly live under cover or in burrows that stay relatively moist, returning to ponds or slow streams in the spring to breed.

Miscellaneous facts: This family includes two all-female triploid species (*Ambystoma platineum* and *Ambystoma tremblayi*). Females of these species mate with males of two other species (*A. jeffersonianum* and *A. laterale*, respectively), but the males make no genetic contribution. *A. platineum* and *A. tremblayi* arose from hybridization between *A. jeffersonianum* and *A. laterale*.

Barred tiger salamanders: The larvae of the only salamander species in Arizona (*Ambystoma tigrinum*) are also known as waterdogs and often used as fish bait. As a result, *A. tigrinum* (including some subspecies which are probably not native to the area) have become established in cattle tanks and reservoirs throughout the state.

Some paedomorphic females can lay up to 5,000 eggs in one clutch, making *A. tigrinum* one of the most **fecund** salamanders. However, most females deposit far fewer eggs (< 200). Some larvae are cannibalistic. These larvae have larger heads, wider mouths, and more highly-developed teeth than non-cannibals. The cannibal morph may develop as a result of high larval densities.

Species in lab:

Ambystoma californiense (A. tigrinum) – California Tiger Salamander
A. mabeei – Mabee's Salamander
A. maculatum – Spotted Salamander
***A. tigrinum nebulosum (A. mavortium)* – Barred Tiger Salamander**

Examine the *Ambystoma* salamanders on display. How does the local *A. tigrinum nebulosum* differ from each of the other species?

What is meant by "paedomorphosis"?

Cryptobranchidae: Hellbenders

Content and distribution: 2 genera, 3 species. Eastern U.S., Japan, and central China.

Morphology: Cryptobranchids are elongate, paedomorphic, aquatic amphibians with four robust limbs. They have flat heads with small eyes, dorsoventrally compressed bodies, and thick folds of skin on their sides. Adults have a pair of gill slits, but no external gills.

Life history: Cryptobranchids live under rocks in cold, fast-flowing mountain streams. The males defend territories on the stream bottoms and construct nests under stones or logs. Fertilization is external and the male guards the eggs.

Miscellaneous facts: Cryptobranchids are the largest extant salamanders. One genus (*Andrias*) can reach 1.8 m in length! *Andrias*, which is restricted to Japan and China, is severely endangered due to the culinary demands of the human populace.

Species in lab:

Cryptobranchus alleganiensis – Hellbender

Examine the Hellbender on display. Given the habitat of this animal, described above, hypothesize why the body is dorsoventrally flattened and why it has the prominent folds of skin on its body.

Dicamptodontidae: Pacific Giant Salamanders
Content and distribution: 1 genus, 4 species. All found on the U.S. west coast.

Morphology: Large, robust salamanders with wide heads, prominent eyes, and laterally flattened tails.

Life history: Found in coniferous forests. Most species **metamorphose facultatively**, but one is paedomorphic. Larvae grow up in streams and springs, metamorphosed adults use cover in the forest and return to water to breed. Females guard eggs.

Miscellaneous facts: Dicamptodontids are very closely related to ambystomatids, which explains their morphological resemblance.

Species in lab:
Dicamptodon ensatus – California Giant Salamander

How would you distinguish between an Ambystomatid and a Dicamptodontid?

What is the difference between facultative and obligate metamorphosis?

Plethodontidae: Lungless salamanders
Content and distribution: 27 genera, about 360 species. Distributed in the New World and southern Europe.

Morphology: These salamanders lack lungs, so all gas exchange is through moist skin. Thanks to the lack of lungs, many species can project their tongues to capture prey. A characteristic of this family is forward facing eyes that probably allow them binocular vision. A **nasolabial groove**, used for chemoreception, is also a synapomorphy of the Plethodontidae. Female Red-backed Salamanders will "nose-tap" a male's feces to determine the quality of a male's food supply and show breeding preference for male's that have access to high-quality prey. They have well-defined **costal grooves**. Other external characteristics are variable, as this family includes arboreal species with prehensile tails, cave-dwelling paedomorphic aquatic species with external gills, and everything in between.

Life history: Plethodontids have good chemoreception and use pheromones for a variety of social interactions. All of the genera we will review in lab (except *Eurycea*) have **direct development**, meaning there is no larval stage and that eggs hatch directly into adults. These genera, like most plethodontids, are highly terrestrial and rarely enter water. However, *Eurycea* and some other plethodontids have an aquatic larval stage or are permanently aquatic. Usually females (and sometimes males) guard eggs.

Miscellaneous facts: This is the most diverse group of salamanders (including about 60% of all extant salamanders) and the only family to successfully exploit the tropics. Many of the rain forest-inhabiting plethodontids are surely becoming extinct before they are even described.

Species in lab:
Aneides flavipunctatus – Black Salamander
Aneides lugubris – Arboreal Salamander
Batrachoseps attenuatus – California Slender Salamander
Ensatina eschscholtzii – Ensatina
Eurycea bislineata – Northern Two-lined Salamander
Plethodon vehiculum – Western Red-backed Salamander

Examine the specimens on display. Find the nasolabial groove. How is it oriented? Describe it. How might it be involved in chemoreception?

Why would direct development be advantageous to a salamander?

Amphiumidae: Amphiumas
Content and distribution: 1 genus, 3 species. Distributed in the southeastern U.S.

Morphology: Amphiumas are elongate, paedomorphic, aquatic amphibians with 4 **vestigial limbs**, each ending in 1-3 toes (depending on species). Adults have gill slits, but no external gills. They can reach over 1 m in length. They have large, powerful heads that can deliver a painful bite.

Life history: Amphiumas spend most of their time in slow-moving or stationary water, including streams, lakes, marshes, and swamps. They surface periodically to breathe air because they lack gills. Amphiumas deviate from the usual mating procedure because males deposit a spermatophore directly into the female's cloaca. Eggs are laid in muddy water and are usually guarded by the female. Amphiumas are voracious predators.

Species in lab:
Amphiuma tridactylum – Three-toed Amphiuma

Since amphiumas have vestigial limbs, what do you think is their primary mode of locomotion?

How might depositing the spermatophore directly into the female's cloaca be an adaptation to an aquatic habitat?

Where do most other salamanders deposit their spermatophores?

Proteidae: Waterdogs and Mudpuppies

Content and distribution: 2 genera, 6 species. Eastern North America and southern Europe.

Morphology: Proteids are large, elongate, paedomorphic, aquatic amphibians with large external gills, caudal fins, and strongly compressed tails. Due to a lack of maxillary bones, they develop a long, pointed snout.

Life history: Proteids inhabit streams, rivers, and lakes. Some are cave-dwellers. After mating, females lay fertilized eggs under stones or logs on stream bottoms. Males or females may guard eggs.

Species in lab:
Necturus maculosus – Common Mudpuppy

Rhyacotritonidae: Torrent Salamanders

Content and distribution: 1 genus, 4 species. Found in the U.S. Pacific Northwest.

Morphology: These are small salamanders with short tails, small heads, and large eyes. Adult males have squared-off glands posterior to the vent. Lungs are present but reduced.

Life history: Rhyacotritonids live in and near cold, fast-flowing streams in old-growth conifer forests. They have a typical salamander life cycle involving aquatic larvae with gills and semi-terrestrial adults.

Miscellaneous facts: Rhyacotritonids are closely tied to old-growth forest and do not tolerate logging, making them good indicator species for forest restoration efforts.

Species in lab:
Rhyacotriton olympicus – Olympic Torrent Salamander

Salamandridae: Newts and "True Salamanders"
Content and distribution: 15 genera, 62 species. North America, Eurasia, and North Africa.

Morphology: These are small to moderate-sized salamanders with robust legs and indistinct costal grooves. The skin of newts is usually rough and keratinized.

Life history: Many are poisonous and have **aposematic coloration** and defensive displays. Most are oviparous. Some species, such as *Notophthalmus viridescens*, metamorphose twice, with aquatic larvae metamorphosing into terrestrial **efts**, which finally metamorphose into terrestrial adults. Most species have the more usual life cycle of an aquatic larval stage followed by a terrestrial adulthood.

Species in lab:
Notophthalmus viridescens – Eastern Newt
Taricha granulosa – Rough-skinned Newt
Taricha torosa – California Newt

What is "aposematic coloration"?

Sirenidae: Sirens
Content and distribution: 2 genera, 4 species. Southeastern U.S. and extreme northeast Mexico.

Morphology: Sirenids are elongate, paedomorphic, aquatic amphibians with two tiny fore limbs and no hind limbs. They have external gills, small eyes, and keratinized beaks.

Life history: Sirenids inhabit warm, shallow, stationary bodies of water including lakes, swamps, marshes, and roadside ditches. Fertilization is external. At least some species can burrow in mud and aestivate in mucus cocoons to survive droughts. Females guard eggs.

Miscellaneous facts: Sirenids are the sister group of all other salamanders.

Species in lab:
Pseudobranchus striatus – Northern Dwarf Siren
Siren intermedia – Lesser Siren

When we say that Sirenids are the sister group to all other salamanders, what does this mean? (Hint: look at the phylogeny at the beginning of the lab.)

Exercise 4: Gymnophiona diversity

General Information
The Gymniophiona is a monophyletic group of lissamphibians, commonly referred to as caecilians. There are 33 described genera with ~170 species, but not much is known about them because they are found in tropical areas and are quite secretive. There are no caecilians in Arizona now (except in some pet stores), but the oldest fossil caecilian was found here, dating from the early Jurassic (~190 ma).

What is the difference between the Lissamphibia and the Amphibia?

Generalized Morphology
Caecilians are elongate, legless, burrowing amphibians with **annulated bodies** that look like earthworms. They have small, highly reduced tails, and eyes which are often covered by skin or even bone. They have extremely rigid skulls for burrowing. The left lung is reduced or absent, and at least one species is lungless. They have a unique chemosensory organ called a **tentacle** that extrudes through an opening between the eyes and nostrils.

Generalized Life History
Adults are mostly fossorial, but some are aquatic. Fertilization is internal - males extrude part of their cloaca into the female to transfer sperm. Most species (~75%) are oviparous. The embryos of the viviparous species have gills, eyes, and cone-like teeth. These teeth are used to scrape the walls of the oviduct, stimulating the oviduct to secrete nutrients. The embryos eat these nutrients and sometimes part of the oviduct walls as well. They completely metamorphose inside the female, losing the gills, eyes, and scraping teeth and getting some regular adult teeth before birth.

Most of the oviparous caecilians lay their eggs on land. Some of these have direct development and hatch as morphological adults; others hatch as eel-like larvae with eyes and gills that have to travel to water to develop into adults.

Species in lab:
    Dermophis mexicanus (?)

Look at the caecilian specimen. What is meant by the term "annulated body"?

Why would a rigid skull be an adaptation to fossoriality?

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