Ecol 483/583 – Herpetology
Lab 3: Amphibian Diversity 2: Anura
Spring 2010

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(Modified from Bonine & Foldi 2008)

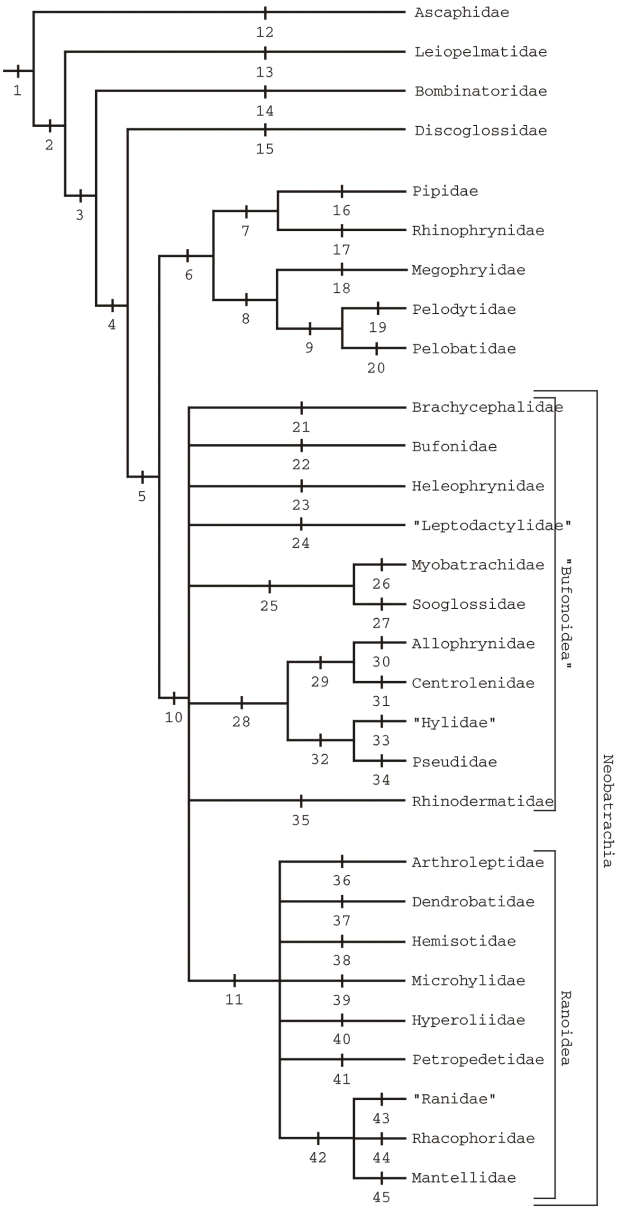
Lab objectives
The objectives of today's lab are to:
1. Familiarize yourself with Anuran diversity.
2. Learn to identify local frogs and toads.
3. Learn to use a taxonomic key.

Today's lab is the second in which you will learn about amphibian diversity. We will cover the Anura, or frogs and toads, the third and final clade of Lissamphibia.

Tips for learning the material
Continue what you have been doing in previous weeks. Examine all of the specimens on display, taking notes, drawings and photos of what you see. Attempt to identify the local species to species and the others to their higher clades. Quiz each other to see which taxa are easy for you and which ones give you troubles, and then revisit the difficult ones.

Although the Anura has a conserved body plan – all are rather short and rigid bodied, with well-developed limbs, there is an incredible amount of diversity. Pay close attention to some of the special external anatomical traits that characterize the groups of frogs you see today. You will also learn to use a taxonomic key today. This is an important tool for correctly identifying species, especially when they are very difficult to distinguish from other species.

Exercise 1: Anura diversity



General Information

Frogs are a monophyletic group comprising the order Anura. Salientia includes both extant and extinct frogs. Frogs have been around since the Triassic (~230 ma). Currently, we recognize over 300 extant genera and ~4,800 species that are placed into 29 major clades (See Fig 3-20 from Pough et al. 2004, left), making frogs the largest group of amphibians. Several clades of frogs are found on all continents except Antarctica. They reach their greatest diversity in tropical areas, particularly in South America and Africa. Eight major clades are found in North America, six of which are native to Arizona. More than half of the world's frogs belong to the Hylidae, Leptodactylidae, and Ranidae, all of which may not be monophyletic. Although relationships among some of the more basal clades of frogs are well resolved, those of more derived groups are ambiguous (see to left).

Generalized morphology

Frogs have four limbs, a short body, a large head, an internalized tail, very short ribs, 9 or fewer presacral vertebrae, hind limbs larger than front limbs, a fused radius and ulna, a fused tibia and fibula, elongate ankle bones, and the ability to project their tongue. Many of these traits are specializations for salutatory locomotion.

Generalized life history

The eggs of all amphibians lack a shell and extraembryonic membranes that arecharacteristic of amniotes. As a result, amphibian eggs are not resistant to water loss and are laid either directly in water or closely tied to water. Most amphibians also lack internal fertilization. Anuran mating typically starts with males calling from a selected location announcing their presence to receptive females. Generally males congregate at a source of water during the spring and call. These aggregations are known as **choruses**. A female approaches a selected male and the male mounts the female's back and grasps her in a mating hold known as **amplexus**. The pair enters the water and either the female lays eggs in the water at the same time the male deposits his sperm over the egg mass, fertilizing the eggs, or the female takes up a quantity of water for her to include in the egg jelly for deposition at a terrestrial location overhanging water. As she lays the egg mass over the water the male deposits his sperm over the egg mass. A modification of the

chorus, that is common in Arizona, is the **explosive mating aggregation**, where both males and females converge in large numbers in a small area for a short period of time. Eggs develop into tadpoles and either hatch directly in the water or drop into the water from the overhanging egg mass. Most frogs start off as larvae called tadpoles, which are typically aquatic and eat algae. Larvae in anurans are *very* different ecologically, behaviorally, and morphologically from adults and are often under entirely different selective pressures. After about two weeks to well over a year, depending on the species, the tadpoles undergo major changes as they metamorphose into frogs. They develop limbs, lungs, bones, teeth, true jaws, a tongue, and a digestive system designed for carnivory. They also absorb the tail.

Why would explosive mating aggregations be common in Arizona?

Evolutionary trends
There is an evolutionary trend toward fewer presacral vertebrae.

Some reproductive vocabulary
Frogs have incredible reproductive diversity, and here are three terms to know:
Oviparous – eggs that are deposited by the female and develop outside the body.
Viviparous – young develop inside the female and obtain nutrients from her.
Ovoviviparous – eggs develop inside the female and the female gives birth to live young. However, the young obtain no nutrients directly from the female other than those initially in the egg.

Some notes on anuran identification
The following characteristics can be used to identify different species by determining if they are present or not, and by noting their attributes if present.

Toads and Spadefoot Toads:
- Size and shape of parotoid glands
- Size and shape of cranial crests
- Size and shape of tubercles
- Size and shape of spots/botches
- Number, "color", and location of warts

Frogs:
- Length and "location" of dorsolateral fold
- Size and location of spots
- Eye and mouth stripes
- Toe pads
- Relative size of tympanum/ear drum
- Tubercles in skin
- "Coloring" under hind limbs

North American Taxa

Ascaphidae: Tailed Frogs

Content and distribution: 1 genus, 1 species. Found in NW U.S. and SW Canada.

Morphology: Ascaphus truei is the only extant frog with a tail-like protrusion. This "tail" is actually the copulatory organ of the male and is used to direct sperm into the female's cloaca, facilitating internal fertilization; it is unique among frogs. It is not a true tail, but a highly vascularized extension of the cloaca which is supported by cartilaginous rods. These frogs also lack external ears. *Ascaphus* has 9 presacral vertebrae and tail-wagging muscles, both primitive features in frogs.

Life history: This species inhabits cold streams in forests. Tadpoles live in torrents or quiet waters, clinging to rocks with large sucker-like mouths. Larvae may take 2-4 years to transform and may not be reproductively active until 7-8 years old. Unlike most frogs, males do not call to attract females.

Miscellaneous facts: Ascaphidae is the most evolutionarily ancestral extant Anuran clade.

Species in lab:

Ascaphus truei – Tailed Frog

Why might these frogs not use calls to communicate?

Bufonidae: True Toads

Content and distribution: 33 genera, 455 species. Almost everywhere except extreme northern latitudes, NW Africa, and Australia (where one species is introduced).

Morphology: Most have numerous wart-like glands that secrete sticky white poison, which can paralyze or kill other species. The most conspicuous of these glands are the **parotoid** glands, which are useful for identifying species. All bufonids lack teeth. Many have cranial crests which are also useful for species identification. Color may change from light to dark in response to temperature.

Life history: Most are fairly terrestrial, but return to ponds and streams to mate. Most bufonids are oviparous, but a few are ovoviviparous.

Miscellaneous facts: Bufo marinus, the cane toad, has been widely introduced around the world and has wreaked much ecological havoc, especially in Australia. An Arizona species, *Bufo alvarius*, has parotoid glands that contain one of the most powerful hallucinogens in the natural world. Toads demonstrate both explosive and chorus breeding behavior.

Species in lab:

***Bufo alvarius* – Sonoran Desert Toad**
Bufoi americanus – American Toad
Bufo boreas – Western Toad
***Bufo cognatus* – Great Plains Toad**
***Bufo debilis* – Green Toad**
Bufo fowleri – Fowler's Toad
Bufo hemiophrys – Canadian Toad
Bufo marinus – Cane Toad
Bufo mazatlanensis – Mazatlan Toad
***Bufo microscaphus* – Arizona Toad**
***Bufo punctatus* – Red-spotted Toad**
***Bufo retiformis* – Sonoran Green Toad**
Bufo speciosus – Texas Toad
Bufo valliceps – Gulf Coast Toad
***Bufo woodhousii* – Woodhouse's Toad**

For each *Bufo* species listed above in BOLD, list one autapomorphy that you can use to distinguish it from all of the other local species. Use the space above.

Hylidae: Treefrogs

Content and distribution: 40 genera, 835 species. North, Central, and South America, Europe, north Africa, Middle East, China, and Australasia.

Morphology: Most hylids have well-developed toe disks that work like suction cups. Some have skulls modified for burrowing. They have slim waists and long legs.

Life history: As the common name suggests, most hylids are arboreal. However, most return to water to mate. Some species are burrowers. Look at the toes and head shape to determine whether a hylid is a climber or burrower. Hylids are generally chorus breeders.

Arizona Species not in Lab:

Smilisca fodiena – Lowland Burrowing Treefrog

Species in lab:

Acris crepitans – Northern Cricket Frog
***Hyla arenicolor* – Canyon Treefrog**
Hyla cinerea – Green Treefrog
***Hyla eximia* (*Hyla wrightorum*) – Mountain Treefrog**
Hyla femoralis – Pine Woods Treefrog
Hyla versicolor – Gray Treefrog
Osteopilus septentrionalis – Cuban Treefrog
Pseudacris crucifer – Spring Peeper
***Pseudacris regilla (Hyla regilla)* – Pacific Treefrog**
***Pseudacris triseriata* – Western Chorus Frog**

Leptodactylidae: Leptodactylid Frogs

Content and distribution: 50 genera, over 1,100 species. Extreme southern U.S., Mexico, Central America, and South America.

Morphology: Very variable, ranging in size from 12 mm to 250 mm.

Life history: Also highly variable. Some are arboreal, some are terrestrial, and some are aquatic. The majority of species have direct development and do not lay eggs in water, but guard them, including the leptodactylid found in Arizona. At least one species is viviparous, and a couple has internal fertilization. They are predominantly chorus breeders.

Miscellaneous facts: This group is probably polyphyletic, which may explain why it is so variable in morphology and life history. The genus *Eleutherodactylus* has over 600 species and has radiated extensively in the Caribbean.

Species in lab:

***Eleutherodactylus augusti* (*Coagulator augusti / Hylactophryne augusti*) – Barking Frog**

What is meant by "polyphyletic"? Why would this explain their variability?

Microhylidae: Narrow-mouthed Toads

Content and distribution: 69 genera, 350 species. Southern U.S., Central and South America, sub-Saharan Africa, SE Asia, northern Australia.

Morphology: Most are relatively small. New World species generally have very narrow mouths, pointed heads, teardrop-shaped bodies, and little or no webbing between their digits. They have a fold of skin across the back of the head, and a distinct pattern of **palatal folds** in the mouth.

Life history: Most are burrowers that feed on ants and termites, emerging after heavy rains. Amplexus in *Gastrophryne olivacea* is termed "glued" because the males possess specialized secretory cells on their ventral side that produce an adhesive substance for attachment to the back of the female.

Miscellaneous facts: This family contains more genera than any other Anuran family. Chorus breeders generally.

Species in lab:

***Gastrophryne olivacea* – Great Plains Narrow-mouthed Toad**

Pelobatidae – Spade Foot Toads
Content and distribution: 3 genera, 11 species. North America, Europe, western and SE Asia.

Morphology: Pelobatids have a well-developed keratinous metatarsal tubercle, known as a **spade**, on each hind foot. Some have glandular skin, including parotoid glands. They are fairly squat animals, with short limbs, large eyes, and vertical pupils.

Examine some pelobatid specimens and identify the spade.

Life history: Arizona's pelobatids are fossorial, emerging during the summer rains en masse to quickly eat and reproduce, not necessarily in that order. They are explosive breeders, responding quickly to heavy rainfall and thunder. Tadpoles metamorphose very quickly (in just over a week in *Scaphiopus couchii*). These frogs use their spades to dig burrows in which they **aestivate** most of the year. Although spadefoots are called "toads" because of their appearance, they are really fossorial frogs.

Species in lab:
***Scaphiopus couchii* – Couch's Spadefoot**
Scaphiopus holbrookii – Eastern Spadefoot
***Spea bombifrons* (*Scaphiopus bombifrons*) – Plains Spadefoot**
***Spea intermontana* (*Scaphiopus intermontanus*) – Great Basin Spadefoot**
***Spea multiplicata* (*Scaphiopus multiplicatus*) – Mexican Spadefoot**

What does "aestivate" mean? Why would it be useful for amphibians that live in Arizona to aestivate?

How would being fossorial help with aestivation?

For each of the four local species of pelobatids listed above, list one autapomorphy.

Pipidae: Tongueless Frogs
Content and distribution: 5 genera, 30 species. South America, sub-Saharan Africa.

Morphology: These frogs have no tongues. Their bodies are typically compressed dorsoventrally. Most have keratinous, clawlike fingertips and lateral line organs.

Life history: These frogs are fully aquatic, which explains the loss of the tongue. Some even call underwater. In some species, such as *Pipa*, there are elaborate courtship rituals which result in eggs sticking to the female's back and ultimately they get enveloped by swelling of the skin. The eggs hatch in the female's back either into tadpoles, or directly into adult form.

Miscellaneous facts: This family is not native to North America. However, *Xenopus laevis* is widely used in lab experiments as a model organism, and sometimes sold as a pet. Apparently there are breeding populations of this species in ponds at some of Tucson's golf courses.

Species in lab:
***Xenopus laevis* – African Clawed Frog**

"Ranidae": True Frogs
*Content and distribution:*38 genera, 600+ species. Everywhere except southern South America, the Sahara Desert, and most of Australia.

Morphology: Ranids typically have long legs, smooth skin, and pronounced webbing between the toes. However, there are no unequivocal synapomorphies because they are likely paraphyletic.

Life history: These frogs typically live in and near lakes and ponds. Most lay eggs in water.

Miscellaneous facts: Many populations of ranids have been mysteriously declining in recent years. In Arizona, *Rana tarahumarae* was extirpated in the 1980's, possibly due to airborne pollution from copper smelters. Reintroduction of *R. tarahumarae* in isolated Arizona localities was begun in summer 2004 by AZGF using individuals originally brought from Sonora, Mexico. Some of our leopard frog populations are speculated to be negatively impacted by a disease caused by chytrid fungus, but we do not know whether this fungus is an ultimate or proximal cause of death in the affected populations. One important threat to native ranids in Arizona is the non-native *Rana catesbieana;* intentionally introduced by the Arizona Game & Fish Department to promote a frog meat industry. *R. catesbeiana* eats just about anything, including the smaller native leopard frogs. Another major cause of ranid frog declines in Arizona is loss of wetland environments due to agricultural and urban development. However, some populations of ranids are using manmade habitats such as cattle tanks and irrigation canals. The loss of habitat has become so severe that one species, *Rana chiricahuansis*, has been listed as federally threatened. Ranids are chorus breeders.

Arizona species not in lab:

Rana berlandieri – Rio Grande Leopard Frog
Rana blairi – Plains Leopard Frog
Rana chiricahuensis – Chiricahua Leopard Frog
Rana onca – Relict Leopard Frog

Species in lab:

Rana aurora – Red-legged Frog
Rana boylii – Foothill Yellow-legged Frog
***Rana catesbeiana* – American Bullfrog**
***Rana pipiens* complex – Northern Leopard Frog complex**
Rana pipiens – Northern Leopard Frog
Rana sylvatica – Wood Frog
***Rana tarahumarae* – Tarahumara Frog**
***Rana yavapaiensis* – Lowland Leopard Frog**

For each species of *Rana* listed above in bold font, list one autapomorphy.

Look at the specimens of *Rana catesbiana* on display in the lab. Why would you expect this species to be such a threat to Arizona's native ranids?

Rhinophrynidae: Burrowing Toads

Content and distribution: 1 genus, 1 species. Southern Texas to Central America.

Morphology: Rhinophrynids have short limbs, well-developed spades, small eyes, and a reinforced skull. The head is pointed and has thick skin at the tip.

Life history: Highly fossorial. Eat termites and ants underground. Eggs are laid in temporary puddles.

Species in lab:

Rhinophrynus dorsalis – Mexican Burrowing Toad

Anuran Clades not Found in North America

Bombinatoridae: Fire-bellied Toads
Content and distribution: 2 genera, 10 species. Distributed in Europe and Asia.

Morphology: Warty glandular skin with toxic secretions. Many species have bright aposematic coloration on their bellies.

Life history: They use **inguinal amplexus** during mating, and lay eggs singly. They are mostly aquatic.

Miscellaneous facts: They display their aposematic coloration (bright ventral orange, red, or yellow) using the unken reflex when threatened. During the unken reflex, the animal twists its body to reveal the bright coloration, which is normally hidden.

Species in lab:
Bombina orientalis – Fire-bellied Toad

Define "inguinal amplexus". Name one other type of amplexus.

Dendrobatidae: Poison Arrow Frogs
Content and distribution: 6 genera, 185 species. Distributed in Central and South America.

Morphology: Pair of dermal scutes on dorsal side of fingers. Most dendrobatids have aposematic coloration to warn predators of their toxicity.

Life history: They use **cephalic amplexus**, if they amplex. They are diurnal and terrestrial. Parental care includes egg attendance and transport of larvae by adults. Dendrobatids may provide unfertilized eggs to their developing tadpoles as a food resource.

Miscellaneous facts: A single individual can contain 2 milligrams of batrachotoxin, enough to kill 10-12 humans. The toxins that these frogs have are dietary in origin – evidence shows that when they are fed a non-natural diet, such as in captivity, they become harmless.

Species in lab:
Dendrobates auratus

Discoglossidae: Midwife Toads and Painted Frogs
Content and distribution: 2 genera, 10 species. Found in western and central Europe, and NW Africa.

Morphology: Small to moderate sized frogs (30-70 mm SVL).

Life history: Inguinal amplexus. In *Alytes*, amplexus takes place on land. Male maneuvers fertilized eggs onto its back and legs. He then carries and moistens eggs when necessary until deposited in water just before hatching.

Species in lab:
Alytes spp.

Exercise 3: Using a taxonomic key

An important tool for identifying organisms from many groups is a taxonomic key. A taxonomic key is something that uses a series of nested questions that, if answered correctly for an unknown organism, will identify it. Most keys are dichotomous in that there are only two options/answers at each step/question. Thus, such a key will divide a group of organisms into two smaller groups at each step, which ultimately leads to a single taxon that is correctly identified. Taxonomic keys are especially useful when taxa of interest are very similar, perhaps only differing in a few very subtle characters.

Although one could potentially draw a phylogenetic tree from a taxonomic key, this wouldn't necessarily be a good idea. The characters chosen for a taxonomic key are chosen to best discriminate between taxa, not elucidate evolutionary history. Many of the characters are not synapomorphies (for example, red stripes on two species of snakes may be good characters in a key to identify these species easily, but because they both have red stripes does not mean they are closely related). It is also important to keep in mind that answering a question incorrectly *will* lead to the wrong answer, so be careful when using a key. One strategy to avoid this is to key a specimen out several times. **Another strategy is to get a partner to key a specimen out independently and compare answers.** When using a key, you will find that some answers are obvious for a particular specimen, while others are not. Pay particularly close attention to instances when you are not sure how to answer a question. This could indicate that you are dealing with a difficult character to score, or that you made an error earlier on.

In this lab, you have at least two taxonomic keys at your disposal. On pages 25-34 of the Peterson Field Guide, there is a taxonomic key that you can use if you are stuck, particularly in the field. Note that it is a general guide that doesn't identify species, just refers you to a page of plates for precise identification. The other taxonomic key available in lab is more traditional in form (Powell, Collins & Hooper 1998). It is a key to all of the amphibians and reptiles of the U.S. and Canada, and will be used during this exercise. **Use it through out the semester when you are unsure how to distinguish species.**

We will reinforce your learning of frog taxonomy today by keying some of these animals out. Anurans are good for this activity because many may look very similar, at least at first, and so

may be confusing to identify on their own. Keying some out will help you to notice differences between some of the local anurans, allowing you to identify them more easily in the future.

Throughout this lab and future labs, you should feel free to key out specimens that give you problems. This will both give you practice using a taxonomic key and identifying local species.

There are many "Ranids", Bufonids, and Pelobatids on display today, so that is where we will concentrate. There are several specimens on display today that have no names associated with them, but have capital letters. Identify them using the taxonomic keys provided. Work with a partner; this allows you to confer with someone about which option is most likely at a given step and is a more effective way to learn. If you have questions or problems, make sure that you ask the instructor.

For each specimen, start at the beginning of the anuran section. Start by determining the "family" of each specimen, then work down to genus, and finally to species. Even if it is easy for you to identify the specimens to their higher taxa, key out at least a few to see what characteristics are used. Everyone should use the species level keys. Start at step 1 and work your way through the numbered steps until you arrive at a positive identification.

Identify the specimens on display and write down the steps that you took to make the ID (for example: 1b → 4b → 5b → 6a are the step taken on the *Bufo* key to arrive at *Bufo alvarius*):

A. Steps: _____
Species: _____

B. Steps: _____
Species: _____

C. Steps: _____
Species: _____

D. Steps: _____
Species: _____

E. Steps: _____
Species: _____

F. Steps: _____
Species: _____

Exercise 4: Make your own key

During lab you will receive a handout with a take-home assignment that will allow you to make your own key for a number of local frog species. The key will be due at the beginning of next lab, so be ready to hand it in. This exercise will give you a better understanding of how taxonomic keys work and will be useful for identification of local frogs because it will get you to notice subtle differences between the species. Refer to the handout for details.

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