Ecol 483/583 – Herpetology
Lab 12: Reptile Anatomy
Spring 2010

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Lab objectives
The objectives of today's lab are to:
1. Learn the basic internal anatomy of the major reptilian groups.
2. Learn the differences in anatomy among these major groups.
3. Consolidate your knowledge of "reptile" diversity.

Today's lab is the final reptile lab. It will introduce you to some basic anatomy of reptilian groups, give you a chance to dissect a specimen, and compare anatomy among the "reptiles". As it is the last indoor lab that is devoted to "reptile" clades, you are encouraged to spend any extra time reviewing from the previous labs. Keep in mind that your lab final is quickly approaching, so make sure that you use your time wisely.

Tips for learning the material
Anatomy is a very detailed and precise field of science. Small differences matter and every structure has a name. Although we are not learning any new species during this lab period, there is a considerable amount of material to learn, so do not blow this lab off. You should already be familiar with the external anatomy of the "reptiles", so today's lab will focus on the internal anatomy. Some of this will be osteology (the study of bones), and some will soft tissues.

To learn the material, work through everything on display systematically. Pay attention to how bones and organs are arranged in each animal. Also be aware of how these animals' anatomies differ from one another. A turtle will have a different arrangement of organs from a snake. Although the organs are in similar positions, there are considerable changes that have occurred in the evolution of these different body shapes.

Use the concept of homology to help you throughout. Most of the anatomical structures that you will be learning are not novel to each clade, but homologous among clades. This means that a lizard liver and a turtle liver serve very similar functions, look similar (not the same), and are in a similar location within the body. This will help you to identify and learn structures, but is also useful in studying the evolution of the animals covered.

Exercise 1: Osteology

A. Cranial Anatomy

There are several skulls on display for you to look at: a sea turtle (*Chelonia mydas*), a small alligator (*Alligator mississippiensis)*, a Gila monster (*Heloderma suspectum*), a bearded dragon (*Pogona vitticeps*), a chuckwalla (*Sauromalus obesus*), and a cat (*Felis catus*). These skulls are instructive of many evolutionary patterns and include examples of anapsid, synapsid and diapsid amniotes.

What are the differences between a synapsid, anapsid and diapsid skull?

Which of the species listed above belongs to each group?

Anapsid:

Synapsid:

Diapsid:

Homology of the major fenestrae of the skull can be confirmed by noting which bones line each opening (fenestra). Instead of having you simply memorize all of the bones in these skulls, we will focus on several bones that are important in distinguishing them. The lower temporal fenestra is lined by three bones: **postorbital, squamosal** and **jugal**. The upper temporal fenestra is also lined by three bones: postorbital, squamosal and **parietal**. One other bone that is important to know is the **frontal**; it makes up a large portion of the roof of the skull and is a good landmark. The frontal is a single, medial bone that lies in between the orbits. The parietal is a large bone immediately posterior to the frontal. Beware that in the cat both the frontal and parietal are paired bones with a medial suture. The frontal and parietals can be paired or fused, depending on taxon.

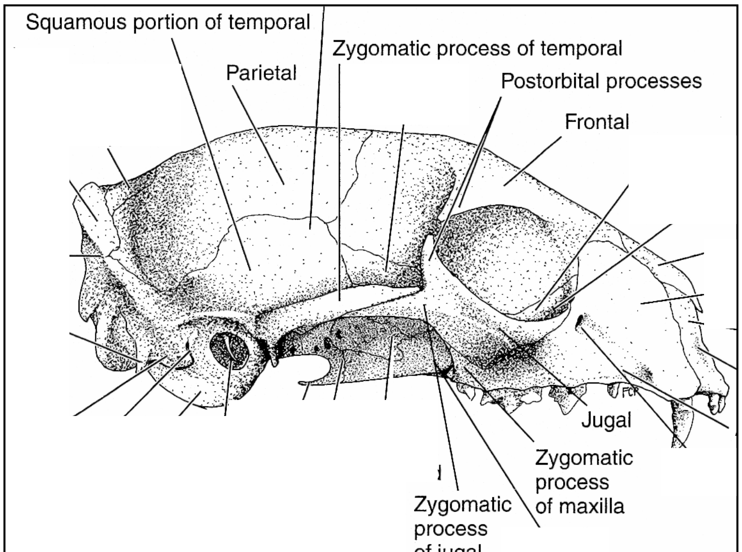
In the skulls on display, locate the frontals and parietals. In which of the skulls are the frontals paired? Fused? How about the parietals?

The postorbital generally shares a suture with the anterolateral edge of the parietal, and, as its name suggests, also lines the orbit posteriorly. The squamosal shares a suture with the posterolateral edge of the parietal, and a posterior one with the postorbital. The jugal lines the posteroventral edge of the orbit, and articulates with the postorbital. It sometimes also articulates with the squamosal, although at other times it articulates with the **quadratojugal**. A final bone that you should know is the one that suspends the lower jaw: the **quadrate**. The best way to identify the quadrate is that it articulates with the lower jaw and is in the posterior part of the skull.

Identify the postorbital, squamosal, parietal, jugal and quadrate in the skulls on display. Use the detailed anatomical descriptions in the preceding paragraphs to identify them. You will have to rely on the descriptions especially with the turtle skull because in has no temporal fenestrae to use as further landmarks. Find the orbits and go from there. Any and all of these might show up on an exam.

Although the alligator has a typical condition, most lizards have a modified diapsid condition. The bar formed by the jugal, connecting to the quadrate is absent. This means that there is only the dorsal-most fenestra present and the ventral fenestra is expressed simply as an open region of the skull. This allows the quadrate to move more freely and is what is known as a <u>streptostylic</u> condition. You can confirm that it is indeed the ventral fenestra that is absent, not the dorsal one, by identifying the bones lining it.

Note that the cat has a highly derived morphology so the homologous bones look very different and are called different things. The bones are labeled for you on the diagram of the cat skull below. Confirm them on the actual skull. Notice that in the cat, there is a temporal bone that has a zygomatic process and a squamous portion. It is formed from the fusion of several other bones. You will not need to know the bones in the cat skull, but you do need to know where its temporal fenestra is.



From Kardong & Zalisko 2002

Why is the cat skull included as part of a herpetology anatomy lab?

The skulls have many more features than a few fenestrae, and it is important to consider how they came to be the way they are. Not having any temporal fenestrae, space for muscles in turtles must be made in other ways. **Emargination** is the degree to which the skull is reduced in the posterodorsal region to accommodate jaw closing muscles. Compare the sea turtle skull with that attached to the turtle skeleton elsewhere in lab. Compare the level of emargination between the two species.

Which skull has a higher degree of emargination?

More emargination means more space for muscles that close the jaw and this has various ecological correlates.

What differences in diet would you expect between the two turtles on display, given the degree of emargination in each?

Move on to the alligator skull. Notice the high degree of fusion among the bones, resulting in a very solid structure. These are some adaptations to resist large forces that are exerted on the skull when the alligator is killing and eating large prey. On the dorsal side, find the **external nares**, or nostrils. Flip the skull over and look at its ventral side. Find the **choanae**, or internal nostrils. **Where are they (use anatomical terms)?**

The secondary palate is a bony division that separates the oral cavity from the nasal cavity. The choanae mark the posterior extent of the secondary palate. Mammals also have a secondary palate. **Touch the roof of your mouth with your tongue – you're feeling the secondary palate. Now locate the secondary palate on the alligator skull.**

Next consider the degree of **kinesis** in the skulls in lab, including that of the rattlesnake, which we have not considered yet. **Cranial kinesis** refers to the amount of movement possible within the skull. We humans have little to no cranial kinesis – our skull is a solid structure composed of multiple but closely fused bones. The only movement occurs at the jaw joint. This is the same situation as in the cat. **Take a look at the cat skull and confirm this for yourself.** Cranial kinesis increases with the number of discrete, unfused bones in the skull. In highly kinetic skulls, there is more connective tissue (particularly ligaments) that hold the bones together.

Examine the skulls on display: the sea turtle, lizards, snake, and alligator. Consider the degree of cranial kinesis in each. Where would you expect movement in each skull to occur?

What differences do you see between the skull of the bearded dragon (*Pogona vitticeps*) and the rattlesnake (*Crotalus* sp.) that would result in different amounts of cranial kinesis in each?

Also compare the skull of *Pogona vitticeps* to that of *Sauromalus obesus*. The former species has what is called **acrodont** dentition, while the latter has **pleurodont** dentition. The acrodont condition has teeth that are in a groove in the jaw, attached on both **lingual** and **labial** sides. Teeth are also fused to each other anteriorly and posteriorly, and no replacement of teeth occurs. The "Agamidae" and Chamaeleonidae are acrodont. All other lizards are pleurodont, where teeth are not fused to one another and teeth attach only on the labial side directly to the jaw. On the lingual side, the teeth are attached to a low bony ridge. Pleurodonts replace their teeth throughout their lives.

From examining the two skulls, describe the differences between these two forms of dentition in your own words.

Which dentition seems more robust? Why?

What is meant by the terms "labial" and "lingual"? (hint: they are anatomical positions, like "anterior" or "caudal".)

B. Postcranial skeleton

The postcranial skeleton consists of everything posterior to the skull and jaws. On display, we have several postcranial skeletons: a turtle, a bearded dragon (*Pogona vitticeps*), and a rattlesnake (*Crotalus* sp.). There are also **osteoderms** from a Gila monster (*Heloderma suspectum*).

Osteoderms form via **intramembranous ossification**, which means that they form from connective tissue in the dermis. They become ossified, so they become bone. Osteoderms cover the entire body and head of *H. suspectum*. They become larger and more closely associated as an individual ages.

Why might osteoderms evolve? What function do you think that they serve?

Examine the turtle skeleton and the turtle shells on display. There are many notable traits that appear nowhere else among vertebrates. Take note of the lack of teeth and the short tail, but pay particular attention to the shell. The shell has a dorsal **carapace** and a ventral **plastron**. These two halves of the shell are connected on either side by the **bridge**. The shell is made of bone and overlaid with keratinous **scutes**. Examine the shell with the scutes peeling off it. Notice that the edges of the scutes do not coincide with the edges of the bones. This is typical of a turtle shell and is one indication of different developmental origins of the shell bones and scutes.

Are there more bones or scutes on the turtle shell you are looking at?

The carapace of the shell is composed of **neural** bones that form the midline row of bones. Internally, **note that the vertebrae are fused to the neurals**. Immediately lateral to the neurals are the **pleural** bones. **Note that the ribs are fused to the pleurals.** The **peripheral** bones lie lateral to the pleurals and form the margin of the carapace. The scutes are arranged in much the same way, but have different names: **vertebral, costal and marginal**, in order from medial to lateral. In addition there is a **nuchal** bone and scute at the anterior midline, and a **pygal** bone and **supracaudal** scute at the posterior midline. **Identify all of these bones and scutes on the shells available for examination.**

The plastron is also composed of multiple scutes and bones, but they are arranged quite differently from the carapace. They are arranged in bilaterally symmetrical pairs that meet at the midline, and bones and scutes coincide. Anterior to posterior, the scutes are named as follows: **gular, humeral, pectoral, abdominal, femoral** and **anal**. **Identify all of these on the specimens on display.** Then take a closer look at the plastron of the box turtle (*Terrapene carolina*) on display. It has a **hinge** between the pectoral and abdominal scutes/bones. This hinge allows the animal to firmly close its shell to avoid a predator.

Finally, **notice that the limbs and limb girdles are enclosed within the shell**. This is a major difference between turtles and other vertebrates – the limbs and their girdles are internal to the ribs. It is a major question in the field of evolutionary development (evo-devo) how this internalization of the limb occurred.

Compare the situation that you see in the turtle with what you see in the lizard skeleton. Where are the pelvic and pectoral girdles placed in the lizard?

What is the pelvic girdle in the turtle and the lizard articulated with?

Although primates have five types of well-differentiated vertebrae (**cervical, thoracic, lumbar, sacral, and caudal**), many other vertebrates do not have such differentiation. Nevertheless, the squamates do have several types of vertebrae.

Examine the postcranial skeleton of the lizard, with particular emphasis on the vertebral column. How many different types of vertebrae do you see?

Squamates, for instance, lack lumbar vertebrae. How would you distinguish the other types of vertebrae on the lizard skeleton from one another?

Now look at the rattlesnake skeleton. Clearly it has more vertebrae than the lizard, but to what region of the vertebral column have all of these extra vertebrae been added? What kind of vertebra is there the most of in the snake?

Have the relative counts of vertebrae in the lizard and snake changed in other regions as well? Explain.

Exercise 2: Soft anatomy

During this exercise, you will be dissecting a snake in small groups and comparing your anatomical findings to what you see in pre-done dissections of a turtle and lizard. It is beyond the scope of this lab to do detailed dissections of the muscles, circulatory system and nervous system. We will be concentrating on the gross internal anatomy of the digestive and urogenital systems.

As you go through this material, and particularly as you compare the anatomy of the different animals on display, consider how the positions of the various organs differ. Keep in mind that all of the animals you will look at will have the same general set of organs due to homology, but their positions will change. The fact that these organs are homologous will help you identify them between animals, because although there will be some detailed instructions on finding the organs in the snake, you are expected to find the organs in the turtle and lizard demos on your own, or with the help of the instructors, if need be. Also, work in pairs, which will allow you to discuss and resolved uncertainties, ultimately allowing you to learn the material better.

A. Anatomy of the snake

From looking externally at the snake, its specialized morphology should be obvious – a very elongate body with no limbs. Such a body shape requires that organs be packed into it in different ways, when compared to an animals like a lizard, with a shorter body. As you dissect the snake, notice if bilateral structures, such as lungs and kidneys are the same size. Also take note of the length of these organs relative to their width. Finally, pay attention to whether any of these organs are staggered within the body cavity. Your kidneys, for example, are at the same anteroposterior level, but those of the snake are staggered, with one anterior to the other.

To dissect the snake, follow these directions:

1. Examine the snake before even touching the dissecting tools. Appreciate what it looks like as you try to identify it – there may be different species of snakes available for dissection. Turn the snake over so that the ventral side is facing up. Notice the large scales on the ventrum, and find the cloaca. The cloaca marks the beginning of the tail.

What is the difference between an anus and a cloaca?

2. Using scissors, preferably with one blade that has a dull point, make a small snip through the skin ventrally and just anterior to the cloaca. It may be easiest to pinch the skin first and the snip through the pinched up skin to make a small hole. Widen the hole anteriorly using small, superficial snips until it is easy to slide the **dull blade** of the scissors under the skin. **It is important to stay superficial so as not to damage the internal anatomy that you are trying to study.** If you point the internal, dull blade of the scissors slightly up/superficially, you will avoid snagging organs with them. Also make sure the dull blade is internal – this prevents

puncturing of organs. Cut carefully just to the side of the ventral midline from the cloaca to the neck. Once you are just posterior to the head, switch again to small snips and cut through the skin all the way to the tip of the jaw. Staying to the side of the ventral midline prevents you from cutting unpaired, medial structures.

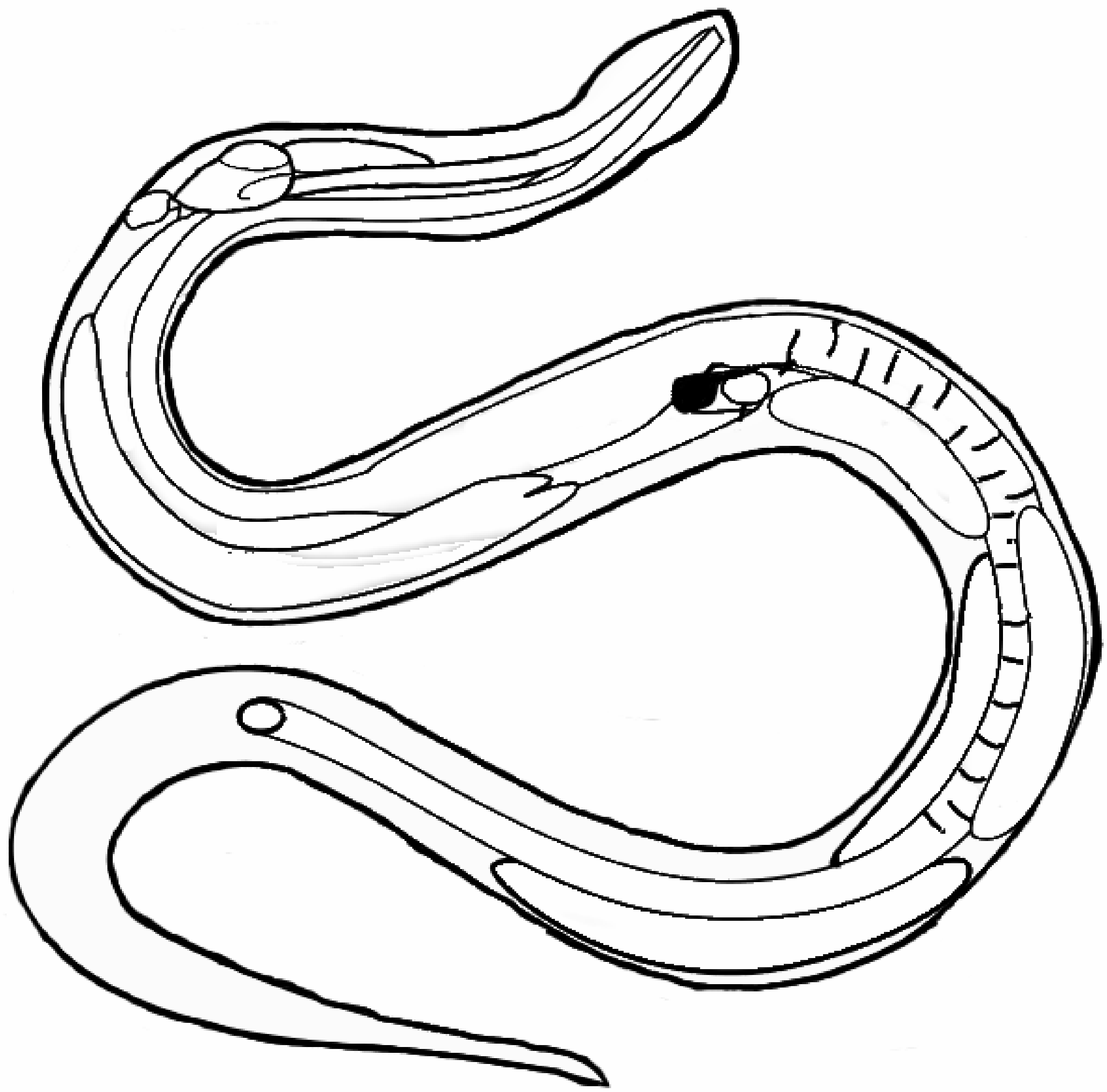
3. Close your scissors and, using your fingers, **gently** pull the skin away from the midline to reveal the internal structures. There are a few tips before moving on with the dissection. **First, do not cut anything unless it is in the instructions – cutting things during a dissection should only be done when needed , otherwise it is an easy way to destroy structures that you need to learn. In fact don't use the scalpel at all, and scissors only when required. Second, keep in mind that the specimen is ventral side up. This means that its left is on your right and vice versa. Dissection instructions always refer to the animal's left or right, not yours.**

4. You should now have a ventral view of many of the organs of the snake. On the next page you have a generalized diagram of the snake, with organs drawn in. As you proceed with the dissection, label the organs that you see on the diagram.

5. The first organs that you should find are the lungs. These are very delicate, so be careful. They will likely get destroyed during the dissection, so find them now, before proceeding. In snakes, as in most elongate tetrapods, the left lung is reduced. **Look for and label the left lung** – it should be on the snake's left side, projecting anterior to the heart. **The heart can be identified as a hard, dark tear-drop shaped structure in the anterior quarter of the snake's body. Label the heart as well.** The right lung is a much larger structure. It runs from approximately heart level (or a little anterior to the heart), posteriorly for almost a half of the snake's body. Both lungs are delicate and membranous. If you have troubles finding the lungs, find the **trachea** first. It will run from the head to the lungs. The trachea is easy to identify because it has thin transverse cartilaginous rings along its length to give it structural support.

What purpose would cartilaginous rings in the trachea serve? (Hint: feel the tranchea with your fingers to see how it behaves when pressure is exerted on it.)

Why do you think that the right lung of the snake is so long? Why do you think that the left lung is so much smaller?



6. Next, we will follow the digestive system from anterior to posterior. Find the trachea at the level of the neck. Use forceps to gently separate it from any connective tissue towards the head. Gently probe with the forceps in the neck to find a second tube that is next to the trachea. This is the **esophagus**. Note that it differs from the trachea in important ways – it lacks cartilaginous elements and it is not translucent, but colored more like a muscle. It will likely be collapsed, so will appear more like a piece of elastic band than a tube. **Label the esophagus.**

7. Now follow the esophagus posteriorly, separating it gently with your forceps from thin **mesentries** as you go. A mesentry is a thin sheet of connective tissue that suspends an organ in the body cavity (connecting it to the body wall). Posterior to the level of the heart, the esophagus widens into the **stomach**. To the right of the stomach is the dark **liver**. Both are quite elongate and may extend about a quarter to a third of the length of the body. **Locate both the stomach and liver, labeling them before moving on.**

8. Posterior to the stomach, the digestive tract begins to bend back and forth. This is the **small intestine**. The liver ends anterior to this point, but also has another structure for identification. The **gall bladder** appears as a deep green-colored sac near the posterior extent of the liver. The gall bladder stores **bile**, which is used to emulsify fats. Typically, the size of the gall bladder is proportional to the size of meals. For example, a cow, which eats constantly, has no gall bladder. The gall bladder empties via a **bile duct** into the anterior portion of the small intestine. **See if you can find the bile duct – it is quite small.** Also look for a small glandular mass near the gall bladder. This is the **pancreas**.

Why does the snake have a very large gall bladder?

9. As you continue to move **caudally** ("towards the tail"), you may notice yellowish, irregularly-shaped masses obscuring your view of the organs. These are fat bodies, so the quantity of these depends on the snakes' health before death. Some snakes may have lots of fat bodies, others may have none. **Remove the fat bodies as needed to see the organs. Be careful not to remove the organs along with the fat bodies.**

10. As you move caudally along the small intestine, its path will straighten once again and ultimately it will widen into the **rectum**, which in turn empties into the **cloaca**. **Find these structures to complete your trace of the digestive system.**

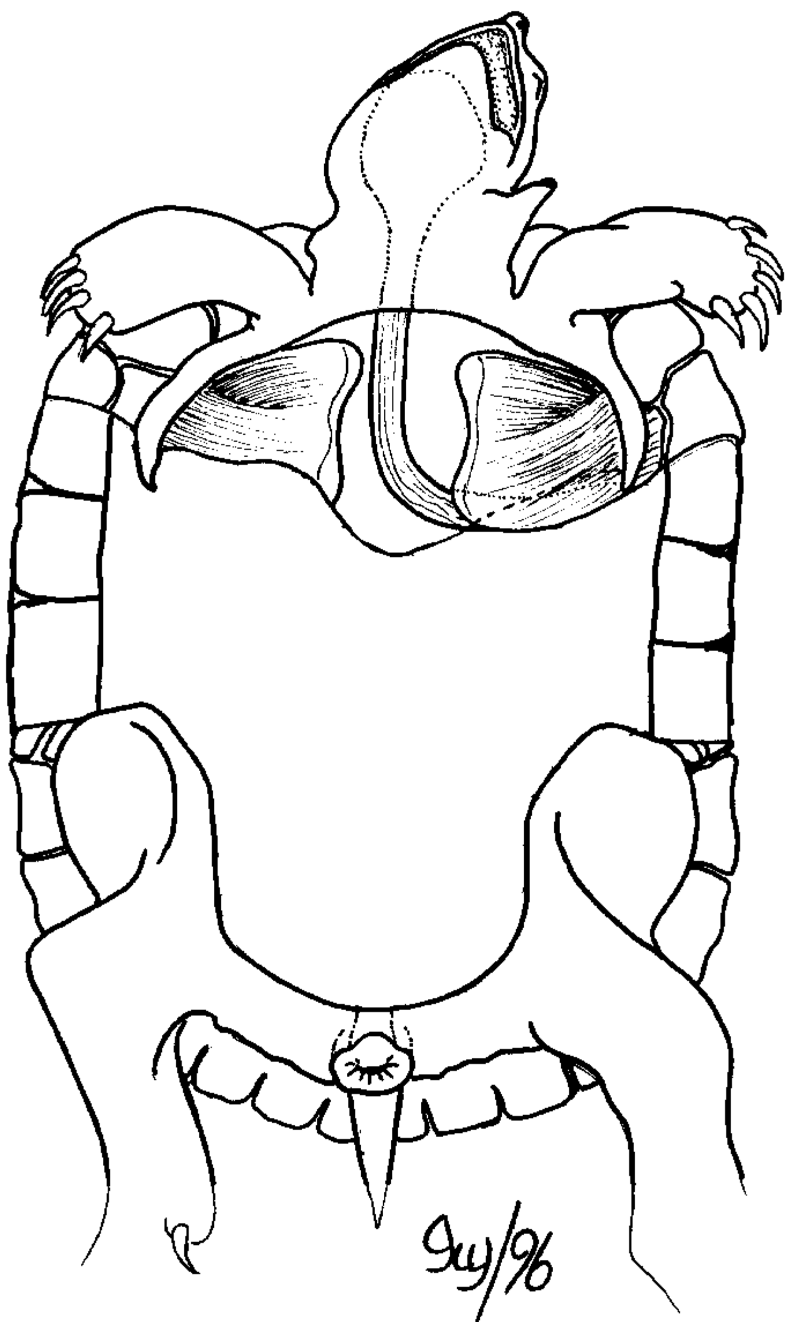
11. Several structures remain to be identified: the **gonads** and **kidneys**. Both are located in the posterior half of the body, and both are staggered, with the right one being anterior to the left one. The gonads are anterior to the kidneys, and may be more substantial. **Find both kidneys and both gonads, labeling them once you find them.** The appearance of the gonads will depend on whether you are dissecting a male or a female snake. Clearly, males will have **testes**, and females **ovaries**. If you have a female snake that is sexually mature, there may be developed follicles in the ovaries or eggs in the **oviducts**. Ovaries will probably look more lobular that testes, which will look more like an elongate bean. The oviducts may be quite substantial as well. **Try to determine the sex of your snake.**

12. Because there is sexual dimorphism in the internal anatomy of all vertebrates and because individuals differ from one another, take some time to examine other student's dissected specimens. Consider the variation that you see and the differences between what the male and female structures look like. **Just because you dissected a male snake doesn't mean that a female will not be on the lab exam – be familiar with both sexes.**

B. Anatomy of the lizard and turtle
On demo are already dissected specimens of a turtle and a lizard. Be careful with these specimens, but examine them in detail to compare them to what you saw in the snake. The relative positions of the organs are relatively similar in the turtle and lizard, but quite different from the snake.

Why are the positions of organs in the lizard and snake so different?

Start by identifying the turtle and lizard organs that you identified in the snake in the previous exercise. Draw what you see in the outlines provided below and label the structures. You should be able to identify the following: **trachea, left and right lung, esophagus, stomach, liver, small intestine, gall bladder, pancreas, rectum, cloaca, gonads and kidneys.** Use your knowledge of snake anatomy, mammalian anatomy from previous courses, and homology to identify these. Discuss uncertain structures amongst yourselves or ask the instructor. Work in pairs.



I. Livingstone © BIODIDAC

Why is the right lung of the snake reduced, but not that of the turtle or lizard?

As you trace the respiratory system of the turtle, consider how its position differs from that of the lizard. Where does the trachea lead? Where are the lungs positioned?

What implications does the position of the turtle's lungs have on ease of breathing in different positions, like up-side-down?

How might the shell constrain ventilation of the turtle's lungs?

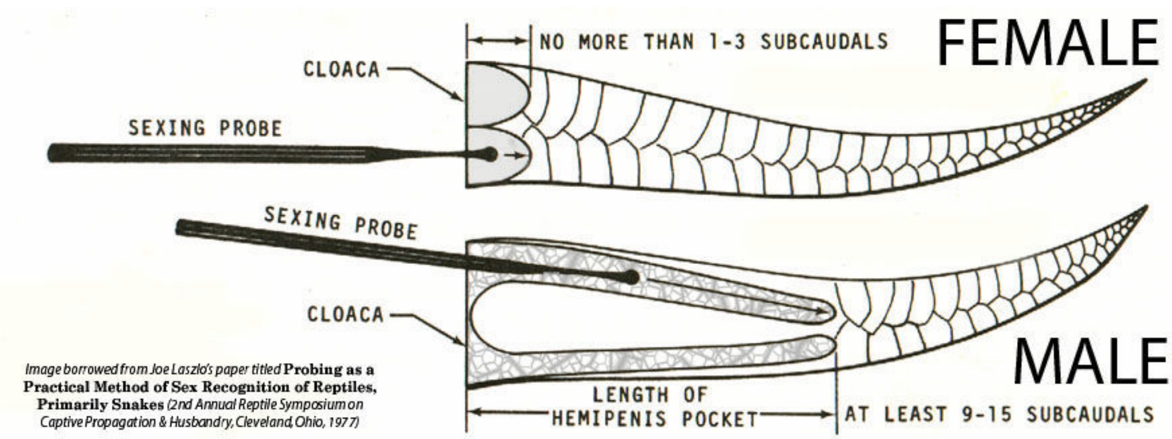
Exercise 3: Sexing Reptiles

On demo are several pairs of "reptiles" labeled A and B. For each, identify whether it is male or female and list characteristics that tell you this:

	A	B
Pond turtle		
Desert Tortoise		
Side-Blotched Lizard		
Western Banded Gecko		

Snakes, some lizards and many immature animals are much harder to differentiate externally. In order to tell the sex of these squamates, a metal probe must be inserted into the cloaca of the animal to see if it has hemipenes. On a live animal, the probe must be sterilized and lubricated between animals. You also need to be careful to choose the appropriately sized probe and to be gentle when inserting the probe so as not to injure the animal. Bend the tail slightly dorsally and insert the probe along one side of the cloaca under one of the larger cloacal scales, pointing the end of the probe (the end with the ball) towards the tail (as shown in the diagram below). Roll the probe between your fingers to find the opening. DO NOT force the probe! It should go in easily and you should feel it stop without applying pressure. Use a finger to mark the length that the probe has been inserted. If the probe goes in the length of 1-3 subcaudal scales, it is a female. If it goes in the length of 9 or more scales, you have a male. The number of subcaudals that sexes probe to differ somewhat between species, but a male will always probe further. Use the specimens provided to practice this technique.

How many are males and how many are females?



Exercise 4: Maturation and Modes of reproduction

Most reptiles are **oviparous**, but many are also **viviparous**. The former occurs when eggs are produced and laid by the female, the embryos are nourished by the yolk in the egg, and they hatch sometime considerably after being laid. The latter term refers to live birth, where the female retains the young, nourishes them from her reserves of energy directly (there is no yolk), and deposits them in a state where they are able to interact with the environment.

On display, we have examples of each of these modes of reproduction. Chameleons are all oviparous. Examine the gravid female with eggs of *Chamaeleo calyptratus*. Notice that the clutch is composed of many small eggs, something that is typical of chameleons. Also look at the gravid kingsnake, *Lampropeltis pyromelana*. In addition, we have new born boas (*Boa constrictor*), which are viviparous. There are also some unfertilized ova with the young. **Consider the implications of these two strategies.**

What do you think are advantages and disadvantages of each of these strategies?

How does the shape of the chameleon and kingsnake eggs differ? Why night this be?

A third, intermediate mode of reproduction is called ovoviviparity. Describe what this is.

As reptiles grow and age, they mature reproductively, just like most animals. Some reptiles change considerably as they age, gaining various secondary sexual characteristics. Some of these characteristics may be involved in communicating with other individuals of the species, both of the same sex and the opposite sex. As development concludes, some structures are easier to see than others and the sexes are easier to differentiate.

On display we have several **ontogenetic series** of various species, including *Aspidoscelis tigris*, *Sceloporus magister, Crotalus cerberus,* and *Gopherus agassizii*. All of these species have ontogenetic morphological changes that happen as they mature. In some species, the males will change dramatically, while the females will look more like large juveniles. In other species, both sexes change as they mature.

Examine each series on display and for each, note the key differences between juveniles and adults.

Aspidoscelis tigris

Sceloporus magister

Crotalus Cerberus

Gopherus agassizii