Chapter 13

Phylogenetic Systematics: Developing an Hypothesis of Amniote Relationships

Daniel R. Brooks, Deborah A. McLennan, Joseph P. Carney
Michael D. Dennison, and Corey A. Goldman

Department of Zoology
University of Toronto
Toronto, Ontario M5S 1A1

Dan received his B.S. and M.S. from the University of Nebraska–Lincoln and Ph.D. from the University of Mississippi. He is presently a Professor in the Department of Zoology, U of T. His research interests include systematics and historical ecology using parasitic helminths as model systems, and neotropical biodiversity.

Deborah received her B.Sc. from Simon Fraser University and M.Sc. from the University of British Columbia. She is currently finishing her Ph.D. in the Department of Zoology, U of T. Her research interests include experimental and phylogenetic studies of the evolution of reproductive behaviour in fish.

Joe received his B.Sc. from the University of Lethbridge and M.Sc. with Dan Brooks from the U of T, and is currently pursuing his doctoral studies in parasitology at the University of Winnipeg.

Mike received his B.Sc.(Hons.) from Massey University, New Zealand and his Ph.D. from the University of Toronto. Since 1992 he has been a Senior Tutor in the Introductory Biology program at the University of Toronto. In addition to assisting in the coordination of the introductory biology course, he teaches the evolution section of the course in the summer. His research interest include the evolution of body size in vertebrates.

Corey received his B.Sc. and M.Sc. degrees from the University of Toronto and has been a faculty member at the U of T since 1983. He is the Course and Laboratory Coordinator for the large (1,500 students) introductory biology course at the U of T. He has edited seven past volumes of *Tested Studies for Laboratory Teaching* and hosted the 15th annual ABLE workshop/conference. He received the Faculty of Arts and Science's Outstanding Teaching Award for 1992/93. His research interests include mammalian systematic and taxonomy.

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Introduction

The Darwinian revolution was founded on the concept that biological diversity evolved through a combination of genealogical and environmental processes. Darwin (1872:346) wrote "community of descent is the hidden bond which naturalists have been unconsciously seeking." Systematics is the part of biology charged with uncovering that community of descent. The objective of this laboratory exercise is to give students hands-on experience with systematic biology by teaching them the basics of modern phylogenetic reconstruction. The lab begins with a *question*: What are the genealogical relationships among the major amniote groups (turtles, mammals, snakes, lizards, birds, and crocodilians)? In order to answer this question, students are asked (1) to **collect data**, in this case, descriptions of morphological characters from skeletal material for representatives from each amniote group and an outgroup (amphibians); (2) to polarize each character against the outgroup, and **construct a data matrix**; and (3) to follow the steps for Hennigian argumentation outlined in the Student Outline to **reconstruct the phylogenetic tree** for the amniotes. At least half of the lab time is taken up with data collection. This is a critical part of the lab because it teaches students that the robustness of a phylogenetic reconstruction is based upon careful and painstaking observations, not upon sophisticated computer programs for analyzing the data.

This laboratory exercise works best when accompanied by lecture material. We would suggest lectures presenting the phylogenetic component of evolution and why it is important. This would emphasize the fact that the patterns of evolution can be presented in tree form, and that such patterns can be used as templates for a wide variety of evolutionary explanations. There are currently no introductory textbooks that handle this material adequately, so we suggest referring to Wiley et al. (1991) and Brooks and McLennan (1991) for material. Lecture material can be supplemented with the video by Maurakis and Woolcott (1993).

This laboratory exercise has been used for two successive years at the University of Toronto in an introductory biology course with 1,500 students enroled per year; the content of the course is evolution, ecology, and behavior. This exercise can be completed in a 3-hour period. Before our students begin this exercise they will have completed a 3-hour comparative morphology exercise in which they have studied some of the characters used in this exercise in more detail.

Student Outline

Introduction

One of the most profound implications of Darwin's Theory of Evolution was that all life on this planet can be traced back to a common origin. This means there is one tree of life. Reconstructing the tree of life has proved difficult, and although we will never resolve it completely, in the last 30 years there have been advances in many fields which have greatly enhanced our view of the history of life.

In a previous lab on comparative morphology you surveyed examples of the groups of land vertebrate — by now you will have noticed that taxa share some characteristics in common and differ in others. Is there a pattern to these similarities and differences? We can use the features of present-day vertebrates to postulate their evolutionary history, given the key assumption that if two taxa share a given characteristic then they inherited the character from a common ancestor somewhere in the past before the two present taxa speciated and evolved differences in other characteristics. The more new characters that are shared by two taxa, the more closely related they are.

Your *objective* in this lab is to assume the role of a phylogeneticist or taxonomist, and to erect a hypothesis for the evolution of the land vertebrates. You will have an array of bones from different tetrapods in front of you and you must analyze information derived from this skeletal variation (and physiological variation) in a specific way in order to construct a geneology of the organisms. The geneology will be in the form of a branching tree, and the pattern of branching will depend on the characters which are shared among taxa. In the lab representatives of all the main living taxa of land vertebrates will be available: amphibians, birds, mammals, crocodiles, lizards, snakes, and turtles.

Preparation: Before this lab read Appendix A on how to construct a phylogenetic tree.

Step 1:
Collect Information on the Characters

What characters should you use for the analysis? For this exercise, we have suggested 10 skeletal and physiological characters for you to use. We have done this, in part, to simplify your analysis. Part of the skill of a taxonomist is in choosing relevant characters to use in the analysis, and this partly depends on an understanding of comparative and functional anatomy. Not all potential characters are homologous, easy to characterise, or unambiguous to code.

Below we list the characters, and we suggest the character states which you should record for each taxa. Record your original data in Table 13.1. For instance, Character 1, skull articulation (or number of occipital condyles) has two states, 1 or 2. You must record either a 1 or a 2 for each taxa in your table.

Skull Articulation (1)

The **number** of occipital condyles. The number and position of condyles is functionally related to an animal's ability to move its head. A single point of articulation with the vertebrate, for example, allows the animal a lot of downward and lateral movement relative to an animal with two condyles.

Number of Digits on Hind Limb (2)

Collect your information from diagrams and specimens in the lab.

Digestive System (3)

Presence or absence of a specialised gizzard. Some vertebrate groups have evolved a gizzard which is a very muscular portion of the stomach. The muscle action, together with a tough lining of cuticle, and especially grit that is ingested, aid in the grinding of fibrous foods such as seeds; refer also to page 947 in Purves et al. (1992). Collect your information from diagrams in the lab.

Urogenital System (4)

Presence or absence of a urinary bladder. A new structure evolved in the tetrapods — a bladder (distensible sac) in which urine is stored before being excreted. Urine storage is likely beneficial on land for sanitary reasons and it is also useful in water conservation. For instance, some vertebrates can reabsorb water from the urine in the bladder. Refer to diagrams in the lab of the urogenital systems of each of the taxa.

Nitrogenous Waste (5)

The **type** of nitrogenous waste. As discussed on page 972 in Purves et al. (1992), the excretion of ammonia has posed a major evolutionary challenge to the land vertebrates. Where water is not limiting, such as for freshwater fishes, the ammonia wastes can be excreted directly. Land vertebrates, however, either convert ammonia to urea or uric acid before excretion.

Metabolism (6)

The **type** of metabolism. The maintenance of a steady internal state (homeostasis) is critical to normal functioning of an animal. In vertebrates, body temperature is controlled either internally by metabolic processes (**endothermy**) or is more dependent on external environment (**ectothermy** or poikilothermy). The evolution of these metabolic strategies among the vertebrates is discussed in more detail on page 750 in Purves et al. (1992).

Reproductive System (7)

The **type** of egg. Does the egg have extra-embryonic membranes? A recurring theme for the land vertebrates was water conservation. There are various stages at which structures and physiologies must be evolved to combat desiccation and the egg stage is a particularly vulnerable stage. The types of vertebrate eggs are discussed on page 597 in Purves et al. (1992).

Male Genitalia (8)

The **presence or absence** of a hemipenis, or split penis. Snakes and lizards are unusual in that they have a double penis, whereas other vertebrate groups have a single penis.

Fusion of Quadrate Bone (9)

Is the quadrate bone ankylosed (fused in an immovable articulation) with the jugal and quadrato-jugal bones? Collect information from the specimens and diagrams in the lab.

Temporal Fenestrae (10)

The **number** of temporal holes in the skull. The evolution of temporal fenestrae (fenestrae = windows) in the skull to accommodate the temporal muscles has been a major theme in vertebrate diversification. The evolution of holes in the skull roof allowed more space for jaw-closure muscles and so enhanced the biting strengths of the vertebrates.

Table 13.1. Original character states for the vertebrate taxa.

Taxa	Skull joint (1)	Hind digits (2)	Gizzard (3)	Bladder (4)	Waste type (5)	Metab-olism (6)	Egg (7)	Hemi-penis (8)	Quadrate fusion (9)	Temporal holes (10)
Amphibian										
Mammal										
Bird										
Lizard										
Snake										
Turtle										
Alligator										

Step 2:
Recode the Characters as Ancestral or Derived

Next, you re-express each of the original character states for each ingroup in terms of its ancestry relative to the outgroup. We recommend you use amphibians as the outgroup.

Ancestral character states are coded "0" and derived states "1", "2", etc. A complete description of the coding is given in Appendix A. Recode your character states and complete Table 13.2.

Table 13.2. Recoded characters.

Taxa	1	2	3	4	5	6	7	8	9	10
Amphibian										
Mammal										
Bird										
Lizard										
Snake										
Turtle										
Alligator										

Step 3:
Group by Synapomorphies and Construct the Tree

You build your tree character by character, successively finding groups of taxa which are defined by synapomorphies (shared, derived characters). Initially, of course, you have one group consisting of all taxa plus the outgroup. Next, find a character which is derived for all the ingroup — you have now defined two groups, the ancestor and the ingroup. Proceed from there, gradually defining subsets of the taxa which define monophyletic groups (that is groups of taxa which share the same most recent ancestor). A step-by-step guide to building a tree is given in Appendix A.

We suggest that you first do a rough draft of the tree on scrap paper. Draw your final phylogenetic tree in your laboratory notebook. The taxa should be clearly labelled, and the position of characters shown (see Appendix A for style).

Step 4:
Classify the Taxa Based on Their Phylogenetic Relationships

Once you have your phylogeny, you have a hypothesis of the history of the taxa. The phylogeny represents a starting point for many different investigations in biology. For example, many taxonomists working in museums use a phylogeny to classify taxa into natural groups. Biologists studying evolution of plants or animals can use a phylogeny to separate historical influences from current influences on present-day patterns of variation.

Questions

1. Based on your phylogenetic tree, and in your own words, describe the main features of land vertebrate evolution.

2. What, if any, evidence is there from your phylogeny for convergent evolution of characters?

3. How would you classify the taxa into groups, based on your tree?

4. Traditional classifications put crocodiles and alligators together with turtles, lizards, and snakes into a group called "reptiles." Is this classification consistent with the one implied by your tree?

5. Your phylogeny is an hypothesis of evolutionary relationships. How might you now test your hypothesis?

Notes for the Instructor

Materials

Diagrams of hind limbs, digestive and urogenital systems, and skull bones
Skulls of turtles, lizards, snakes, mammals, birds, frogs, and alligators
Blunt probes (to use as pointers)
Magnifying glasses
Mounted skeletons (optional) of turtles, lizards, snakes, mammals, birds, frogs, and alligators
Human skulls or mounted skeletons (optional)
Introductory biology textbook with information on nitrogenous waste, type of metabolism, and type of egg (we use Purves et al., 1992)
At the University of Toronto we have been extremely fortunate to have had the opportunity to purchase turtle, lizard, snake, and bird specimens (skulls and hind limbs) from the Royal Ontario Museum (ROM). Over several years we have amassed a large collection of common and exotic

species. None of these specimens were collected from living specimens for our purposes, they were obtained from captive specimens donated to the ROM from zoos and private collections. Mammal skulls were purchased from biological supply companies.

Phylogenetics of Land Vertebrates

This exercise was developed from a phylogenetic study of relationships among the amniotes conducted by Gauthier et al. (1985). Their proposed family tree was based on a cladistic analysis of many morphological characters of fossil and extant specimens. From the original 207 characters, a subset of 10 characters was chosen for this exercise. (The resulting tree nevertheless agrees well with Gauthier's tree.) Students examine representatives of each taxa and collect data on these characters. Results are provided in Tables 13.3 (original character states) and 13.4 (recoded characters). A reconstructed phylogeny of the amniotes is given in Figure 13.1.

The Characters

Skull Articulation (1)

Students count the number of occipital condyles. Mammals and amphibians have **two** occipital condyles. All other taxa have just **one** occipital condyle. The double condyle is considered to be the plesiomorphic state, and the single condyle represents the apomorphic state.

Number of Digits on Hind Limb (2)

Specimens of hind limbs are not available for most taxa, thus diagrams are provided instead (see Appendix B). Amphibians, turtles, lizards, and mammals **have** a fifth digit. Birds and alligators **do not** have a fifth digit. Snakes have no hind limbs, and thus have no digits on the hind limb. In coding the character states, students might be tempted to order the states in sequence $5 \rightarrow 4 \rightarrow 0$ implying that the condition in snakes is directly derived from that in alligators and birds. Instead, it is recommended that the functional outgroup method be used on the character (see Appendix A). The presence of five digits is considered plesiomorphic. The presence of four digits is apomorphic for birds and alligators, and of no digits is apomorphic for snakes.

Digestive System (3)

Labeled diagrams of the gastro-intestinal system of each taxon are available in the lab (see Appendix B). Students must score each taxon for the presence or absence of a gizzard (specialized part of the stomach). Only birds and alligators **have** a gizzard, so the absence of a gizzard is considered to be the ancestral condition.

Urogenital System (4)

Labeled diagrams of the urinogenital systems for each taxon are available in the lab (see Appendix B). Students must score each taxon for presence or absence of a urinary bladder. Only birds and alligators **do not** have a urinary bladder, so the presence of a bladder is considered ancestral.

Nitrogenous Waste (5)

Students obtain information on the type of nitrogenous waste for each taxon from page 966 in their textbook (Purves et al., 1992). Although there is variation within taxa, by and large we can say that amphibians and mammals excrete nitrogen as **urea** and this is considered the plesiomorphic state. Most squamates, alligators, turtles, and birds excrete **uric acid**, and this is considered apomorphic.

Metabolism (6)

Students find out what type of metabolism each taxon has from page 750 of Purves et al. (1992). Amphibians, turtles, squamates, and alligators are **poikilotherms**, while birds and mammals are **endotherms**. In the final analysis, endothermy will be **homoplasious**, convergently evolved in birds and mammals.

Reproductive System (7)

Students obtain information on the presence of extraembryonic membranes in the eggs from pages 594–597 in Purves et al. (1992). Amphibian eggs have **no** extraembryonic membranes, whereas the eggs of all other taxa **do have** membranes surrounding the embryo (the amnion, chorion, and allantois). This character represents a synapomorphy that diagnosis the in-group as a monophyletic lineage separate from the outgroup.

Male Genitalia (8)

Students examine diagrams of the genitalia of male snakes and lizards (see Appendix B). Squamates are unique in that they have a double or split penis. Presence of a hemipenis is therefore considered apomorphic.

Fusion of Quadrate Bone (9)

Students examine diagrams and/or skulls. In amphibians, mammals, turtles, alligators, and birds, the quadrate bone is **ankylosed** (fused in an immovable articulation) with the jugal and quadrato-jugal bones. In mammals, the quadrate is one of the bones of the middle ear. In snakes and lizards the jugal and quadrato-jugal bones are reduced in size and the quadrate bone is **not ankylosed**. Being ankylosed is thus pleisomorphic and not ankylosed is apomorphic.

Temporal Fenestrae (10)

Students count the number of temporal holes for each taxon. They should count one side only (skulls are bilaterally symmetrical). Amphibians and turtles **do not** have temporal fenestrae (anapsid condition). The obvious hole in the back of the turtle skull is not considered homologous to temporal fenestrae, although it does function in a similar way (i.e., it is an analogous character). Mammals have a **single** temporal hole (synapsid). Birds, squamates (lizards and snakes), and alligators have **two** temporal fenestrae (diapsid). The holes are clear in alligators, less clear in squamates, and in birds are present only as troughs and here it takes an expert to recognize them.

Amphibians are used as the outgroup, so the absence of temporal holes is considered plesiomorphic (= ancestral character state), while the synapsid and diapsid states are apomorphic (descendent character state). In coding the character states, students might be tempted to order the states in sequence $0 \rightarrow 1 \rightarrow 2$, implying that the diapsid condition is more derived than the synapsid. Instead, it is recommended that the functional outgroup method be used on this character (see Appendix A).

Table 13.3. Original character states for the vertebrate taxa.

Taxa	Skull joint (1)	Hind digits (2)	Gizzard (3)	Bladder (4)	Waste type (5)	Metab-olism (6)	Egg (7)	Hemi-penis (8)	Quadrate fusion (9)	Temporal holes (10)
Amphibian	2	5	no	yes	urea	ecto	no	no	yes	0
Mammal	2	5	no	yes	urea	endo	yes	no	yes	1
Bird	1	4	yes	no	uric	endo	yes	no	yes	2
Lizard	1	5	no	yes	uric	ecto	yes	yes	no	2
Snake	1	0	no	yes	uric	ecto	yes	yes	no	2
Turtle	1	5	no	yes	uric	ecto	yes	no	yes	0
Alligator	1	4	yes	no	uric	ecto	yes	no	yes	2

Table 13.4. Recoded characters.

Taxa	1	2	3	4	5	6	7	8	9	10
Amphibian	0	0	0	0	0	0	0	0	0	0
Mammal	0	0	0	0	0	1	1	0	0	1
Bird	1	1	1	1	1	1	1	0	0	2
Lizard	1	0	0	0	1	0	1	1	1	2
Snake	1	2	0	0	1	0	1	1	1	2
Turtle	1	0	0	0	1	0	1	0	0	0
Alligator	1	1	1	1	1	0	1	0	0	2
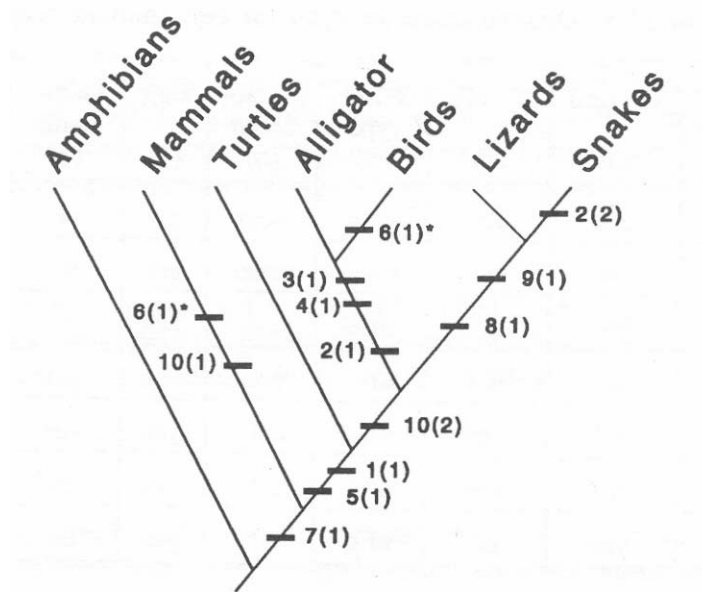
Figure 13.1. Phylogenetic tree for the major amniote groups.

Sample Answers to Questions in Step 4

1. Based on your phylogenetic tree, and in your own words, describe the main features of land vertebrate evolution.

    • A single synapomorphy, the amniotic egg, unites the ingroup into a monophyletic group.
    • Mammals as a lineage evolved early, and are characterized by only one unique character: a single temporal hole.
    • Two new character states evolved in the common ancestor of the remaining taxa: uric acid excretion and a single occipital condyle. Turtles, archosaurs (birds and alligators), and squamates (snakes and lizards) can thus be placed in a group within the amniota. With this dataset, there are no unique characters possessed by turtles. Turtles are an old lineage.
    • The ancestors of birds and alligators and squamates evolved two temporal holes.
    • Alligators and birds are most closely related to each other. They share an ancestor which had a gizzard but lost the urinary bladder.
    • Birds differ from alligators for endothermy. Endothermy evolved twice, independently in birds and mammals.
    • Snakes and lizards are most closely related to each other and differ from alligators and birds based on two synapomorphies: the presence of hemipenis and having a quadrate bone that does not articulate with the jugal and quadrato-jugal bones.
    • Snakes differ from lizards only in the loss of digits.

2. What, if any, evidence is there from your phylogeny for convergent evolution of characters?

    • Endothermy is postulated to have evolved independently twice: in birds and in mammals separately.

3. How would you classify the taxa into groups, based on your tree?

    Lizards + Snakes
    Birds + Alligator
    Lizards + Snakes + Birds + Alligator
    Lizards + Snakes + Birds + Alligator + Turtles
    Lizards + Snakes + Birds + Alligator + Turtles + Mammals

4. Traditional classifications put crocodiles and alligators together with turtles, lizards, and snakes into a group called "reptiles." Is this classification consistent with the one implied by your tree?

    • The "reptiles" represent a paraphyletic group because they exclude birds and so they are not a correct group based on this phylogenetic analysis.

5. Your phylogeny is an hypothesis of evolutionary relationships. How might you now test your hypotheses?

    • New, and hopefully fairly independent, data can be used to construct another phylogeny and then the congruence of the two trees can be compared. New evidence from the fossil record of possible ancestors and transitional types may help to test the hypothesis.

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APPENDIX A
Constructing a Phylogenetic Tree

Introduction

Perhaps the most common phrase used to describe evolution is "descent with modification." Descent entails an ancestor and its descendants: a genealogy. Modification involves a change in a characteristic or attribute in the descendant relative to the ancestor. Genealogical relationships among taxa cannot be directly observed. However, characteristics of living (or fossilized, when available) descendants can be observed. Since not all characters change at the same rate or to the same degree, descendants will be mosaics of unmodified (**ancestral**) and modified (descendant or **derived**) character states. It is the derived character states that you will identify and use to reconstruct the ancestor-descendant relationships, or phylogeny, of the tetrapods.

Because we can never directly observe these relationships, the tree that you develop is an hypothesis that can be tested by the examination of more characters. As a consequence, earlier phylogenetic hypotheses may be rejected with the inclusion of more data.

There are four basic steps in constructing a family tree:

1. Identify homologous characters.
2. Outgroup comparison: determine the order and polarity of the characters.
3. Code the characters and construct a matrix.
4. Group by synapomorphies: analyze the matrix to produce a phylogenetic hypothesis.

Homologous Characters

A **character** is an observable trait of an organism. It may be morphological, physiological, behaviourial, molecular, or ecological. A character may be passed on from an ancestor to its descendant either in unmodified or in some modified **character state**. For instance, if the character is eye colour, then character states might be brown eyes and blue eyes. If your parents have brown eyes which is the ancestral state, and you have blue eyes, then you have the derived character state.

Characters used for analysis must be **homologous**. Homologous characters in two or more species are derived from the same structure in a common ancestor. This definition presents a problem, since we would need to have some estimate of relationships in order to determine homology. If we then attempt to determine relationships among taxa using characters whose homology has been determined by reference to some estimate of phylogeny we will be confounded in a endlessly circular argument. In order to avoid this circularity, we recognize homology by developmental, structural, or positional similarity. If it looks the same and is found in the same place then we will assume it is homologous.

An example of homology is the **vertebrate forelimb**. The basic forelimb plan is retained throughout the vertebrates: there is a humerus, a radius and ulna, carpals, metacarpals, and digits comprising phalanges. However, there is variation among taxa in the exact morphology; frogs have one lower arm bone since the radius and ulna are fused; birds have a carpometacarpus which consists of fused carpals and metacarpals. How do we know which bones are homologous? The answer is that studies have shown that the bones develop in the same way and in the same position in the different taxa. During early development in the frog, for example, both radius and ulna bones can be distinguished and only later on do they fuse to look like a single bone. A similar pattern has been found for bird forelimb development.

Types of Characters

Apomorphy = a new or descendant character state. When an apomorphy (apo = derived, morphy = form) is found in two or more taxa it is called a **synapomorphy** (syn = shared) which is a shared derived character. It is the synapomorphies which are used to infer phylogenetic relationships. Phylogenetic reconstruction may be viewed as a search for synapomorphies. A character can be a synapomorphy for a group only if no other organisms outside the group under study have the same character state. For instance, the presence of feathers may be considered a synapomorphy for different species of birds.

Plesiomorphy = ancestral character state (plesio = old). A **symplesiomorphy** is a shared ancestral character state. These characters provide no information in resolving phylogenetic relationships. However, what is a symplesiomorphy at one level may become a synapomorphy at a higher level. For instance, the presence of feathers will provide no information if you are trying to reconstruct relationships among species of birds, because all birds have feathers of some form. However, if you are undertaking an analysis at the level of the tetrapods, then the presence of feathers becomes synapomorphic for birds. This illustrates an important point. What is derived at one level of analysis may be ancestral at another level of analysis, and vice versa.

Outgroup Comparison

Once you have identified potential characters for analysis, how do you determine which are plesiomorphic and which are synapomorphic? Several methods have been proposed to address this problem, with outgroup comparison being the system most commonly used.

Outgroup comparison works on the following two assumptions. The first is that the group being studied, termed the ingroup, is **monophyletic** (all members of the group share the same, most recent ancestor). Second, the outgroup, used to polarize characters, is not part of the ingroup. Based on these assumptions, any homologous character state found in the outgroup and in the ingroup is considered plesiomorphic for the ingroup. States found in the ingroup and not in the outgroup are considered synapomorphic for the ingroup. This technique works readily when there are just two states of a character and one is shared with the outgroup.

Functional Outgroups

What happens if there is no state shared between the outgroup and the ingroup, or, if there is more than one derived state (i.e., character states 1, 2,..) found in the ingroup? In this instance you create a tree based on the characters which can be polarized unambiguously. Once you have used these characters you will have resolved some of the relationships among the ingroup taxa. By using the character state found in the basal most members of the ingroup as the plesiomorphic condition, you may polarize the remaining characters to further resolve ingroup relationships. The technical term for this is "functional ingroup/functional outgroup analysis."

After you have completed polarizing the characters, you will construct a data matrix. This is a summary of the character states found in each taxon. Typically, the ancestral state is coded "0" and derived states are coded "1".

Some characters are not binary (i.e., present/absent) and instead exist in more than two different states. Such **multistate characters** may be coded using other numbers, but it is important to understand that a code of "1", "2", etc., used to represent the states in a multistate character **does not** necessarily imply a sequence of change for the character, only that there is more than one apomorphic state. There are several ways to deal with multistate characters. In this lab, you will use one technique, and it will be performed by hand. This technique is known as Hennigian argumentation, after its originator Willi Hennig. Note that this proceeds by consideration of one character at a time.

Classification

Once you have developed an hypothesis of phylogeny you can create a natural classification for the groups being analyzed. A natural classification is composed of only monophyletic groups and directly corresponds to the hypothesis of phylogeny upon which it is based. Such a classification is based on genealogical relationships among monophyletic taxa. This will permit the inferred phylogenetic relationships to be recovered from the classification scheme.

A Worked Example

Assume that you want to construct an hypothesis of phylogeny for a group of seven species which we will label A, B, C, D, E, F, and G for convenience. In addition, you use another species (X) — which has some similarities with A to G but which is not part of that group — as an outgroup to polarize the characters you have chosen for analysis. Table 13.5 contains a list of the presumed homologous character states found in each taxon.

Table 13.5. Character states for seven species (A–G) and an ancestral species (X).

Species	Legs (1)	Type of reproduction (2)	Body covering (3)	Feet webbing (4)	Tail (5)	Eyes (6)	Beak (7)	Horn or antler (8)	Teeth (9)
A	4	eggs	spines	yes	no	yes	no	antler	yes
B	4	eggs	feather	yes	no	yes	duck	antler	yes
C	4	eggs	feather	yes	yes	no	duck	horn	yes
D	4	eggs	feather	yes	yes	no	duck	horn	no
E	4	live	feather	no	no	yes	duck	horn	yes
F	4	live	feather	no	no	yes	raptor	horn	yes
G	4	live	feather	no	no	yes	raptor	horn	no
X	0	eggs	spines	yes	no	yes	no	no	yes

Outgroup Comparison

Now that you have arranged the original data, you can use outgroup comparison to polarize the characters. By definition, the ancestral taxa, X, is coded "0" for each character. Any character state of an ingroup taxa which is the same as the outgroup is thus coded "0", and if it is different from the outgroup it is a derived character and is coded "1" or "2". Based on the characteristics exhibited by each taxon, the data matrix in Table 13.6 can be constructed.

Table 13.6. Character states recoded with respect to the ancestor.

Species	1	2	3	4	5	6	7	8	9
X	0	0	0	0	0	0	0	0	0
A	1	0	0	0	0	0	0	1	0
B	1	0	1	0	0	0	1	1	0
C	1	0	1	0	1	1	1	2	0
D	1	0	1	0	1	1	1	2	1
E	1	1	1	1	0	0	1	2	0
F	1	1	1	1	0	0	2	2	0
G	1	1	1	1	0	0	2	2	1
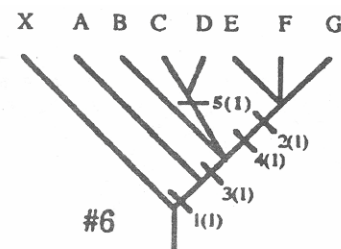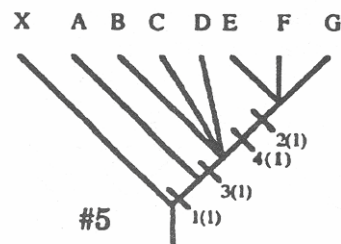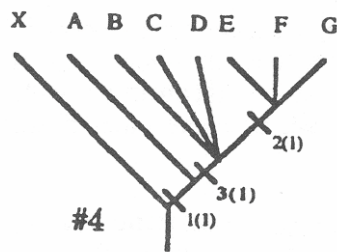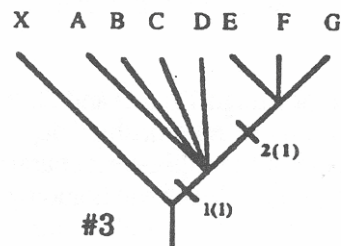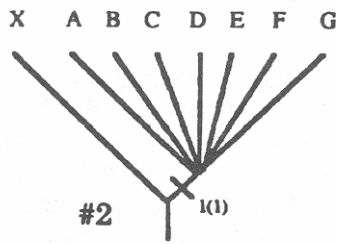
Building the Tree

Initially, there are no relationships known among the ingroup and outgroup taxa. Thus, if you were to draw a tree representing what you know of their relationships it would look like **Tree #1**.

We build the tree character by character, by successively finding groups which share new characters (group by synapomorphies). Remember that a primary requirement of phylogenetic analysis is that the ingroup be monophyletic and that the outgroup is not part of the ingroup. Therefore you must have a synapomorphy that is found in all members of the ingroup and not in the outgroup. Character 1 is such a character. (*Note:* For the purposes of this example, we made Character 1 a synapomorphy for the whole ingroup. In your own analysis, it may be any one of the characters). All of the ingroup taxa have legs while the outgroup does not. Therefore, having legs may be hypothesized to be a synapomorphy that defines the ingroup. Adding this character to Tree #1 produces **Tree #2**.

By adding this character you have separated the outgroup from the ingroup and provided a basis for your decision that the ingroup forms a monophyletic group. However, there are still no relationships resolved among the ingroup taxa. Adding Character 2 (type of reproduction) to Tree #2 produces **Tree #3**.

This still does not provide much in the way of resolution of ingroup relationships. Therefore proceed to add Character 3 (body covering) to the preceding tree to result in **Tree #4**.

Based on these three characters you can now make the following observation: Taxon A is the sister group to a group consisting of Taxa B, C, D, E, F, and G. Now include Character 4 (feet webbing) in the analysis to produce **Tree #5**.

Note that adding Character 4 to the previous arrangement did not bring any further resolution to the developing hypothesis of relationships. It did, however, strengthen the hypothesis that Taxa E, F, and G share a most recent common ancestor. Continue by adding Character 5 (tail) to produce **Tree #6**.

Adding Character 5 produces the hypothesis that Taxa C and D are sister taxa. This is strengthened when Character 6 (eyes) is added, producing **Tree #7**.

Character 7 (beak type) is a challenge because it has three states, one of which is shared with the outgroup. The condition found in the outgroup is the plesiomorphic condition. The question is, How did the character change from the plesiomorphic condition? Was it $0 \rightarrow 1 \rightarrow 2$; $0 \rightarrow 2 \rightarrow 1$; or $1 \leftarrow 0 \rightarrow 2$? This is where functional outgroups may be used to determine the order and polarity of the transformation series. Taxa EFG share a most recent common ancestor with each other that is not shared with any other of the ingroup members. Therefore, the rest of the ingroup (ABCD) may be considered to be the outgroup to EFG. By so doing, EFG functions as an ingroup (functional ingroup) and ABCD functions as an outgroup (functional outgroup}. As a consequence, the state found in the functional outgroup can be considered to plesiomorphic to the state found in the functional ingroup.

#7

#8

#9

#10
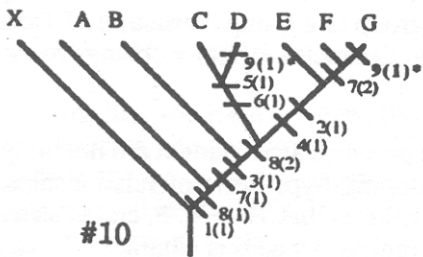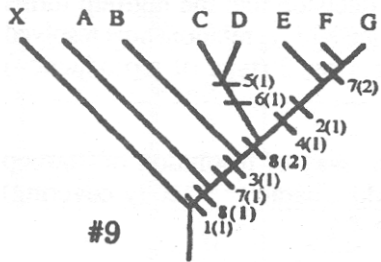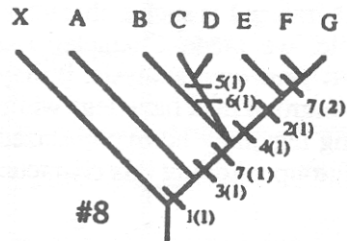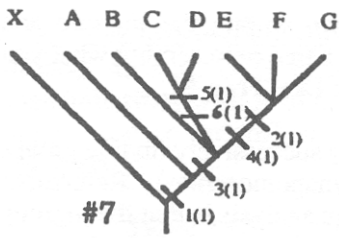
This also illustrates that what may be apomorphic at one level can be plesiomorphic at another. By applying functional outgroup analysis to Character 7, **Tree #8** is generated.

Character 8 (horns and antlers) also presents a challenge since there is no state shared with the outgroup, so it appears initially that this character cannot be used since there is no means to order or polarize its transformation. Using a similar argument to that used in the analysis of Character 7, **Tree #9** can be generated. In this instance, the state found in Taxa AB is plesiomorphic and the state found in Taxa CDEFG is derived. Note that in order to use functional outgroups you must first have some resolution of ingroup relationships and that this is dependant on binary characters.

The final character for analysis (9, teeth) occurs in Taxa D and G. This character is incongruent with the hypothesis of relationships depicted in Tree #9 and so we hypothesize that this is a **homoplasious** character. This demonstrates the distinction between homoplasies and homologous characters. Homologies are assumed before you begin your tree. Homoplasies are identified after you have completed your tree. There is no change in relationships with Character 9 included, as shown by **Tree #10**. However, there is not complete consistency between the depicted hypothesis of relationships and the characters used in the analysis. Therefore the characters that are inconsistent with the hypothesis are indicated with an asterisk or some other means of recognition.

It is important to realize that the resulting phylogenetic tree depicts relationships among taxa. There are many ways in which these relationships are represented. It is important that you be able to look at a tree and recognize the relationships that are being indicated. For example, all of the following trees represent the same set of relationships:
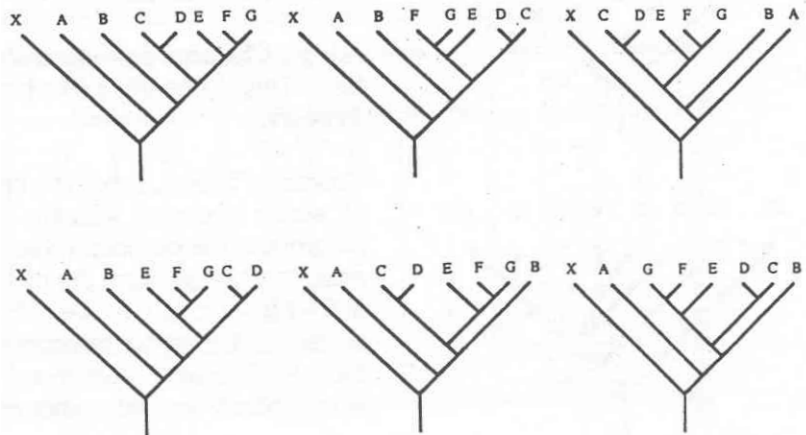
A Take-Home Example

Complete the following exercise in building a phylogenetic tree using the cladistics method at home. Answers will be discussed in class.

☐ You have the following information about four plant species. Plant X is the outgroup. Recode the characters as ancestral or derived and then build a phylogeny. There may be more than one possible tree.

Plant	Reproductive body	Type of leaves	Type of stem	Rhizomes	Plant height
A	seeds	compound	smooth	absent	short
B	seeds	compound	smooth	present	tall
C	seeds	compound	hairy	present	tall
D	seeds	simple	hairy	present	short
X	spores	simple	hairy	present	short

Plant	Recoded characters				
	1	2	3	4	5
A					
B					
C					
D					
X					

For the instructor: Two possible trees are provide below. Remember that the clades can be rotated among several different axes and yet still reveal the same set of relationships.
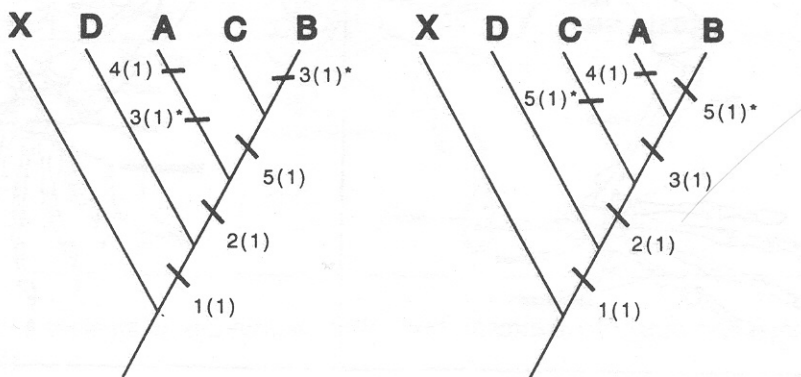
APPENDIX B
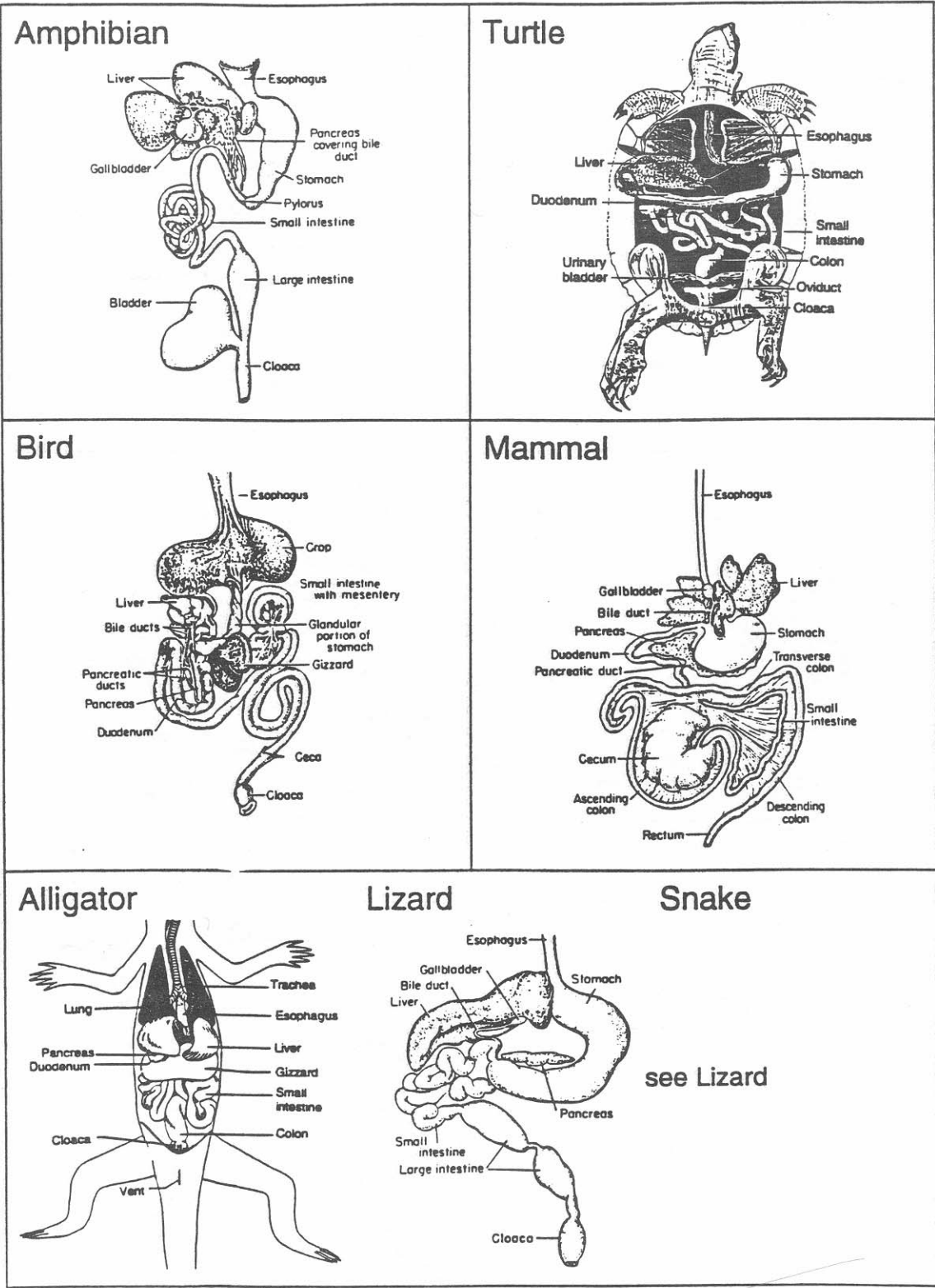*Diagrams of Hind Limbs and Digestive and Urogenital Systems*
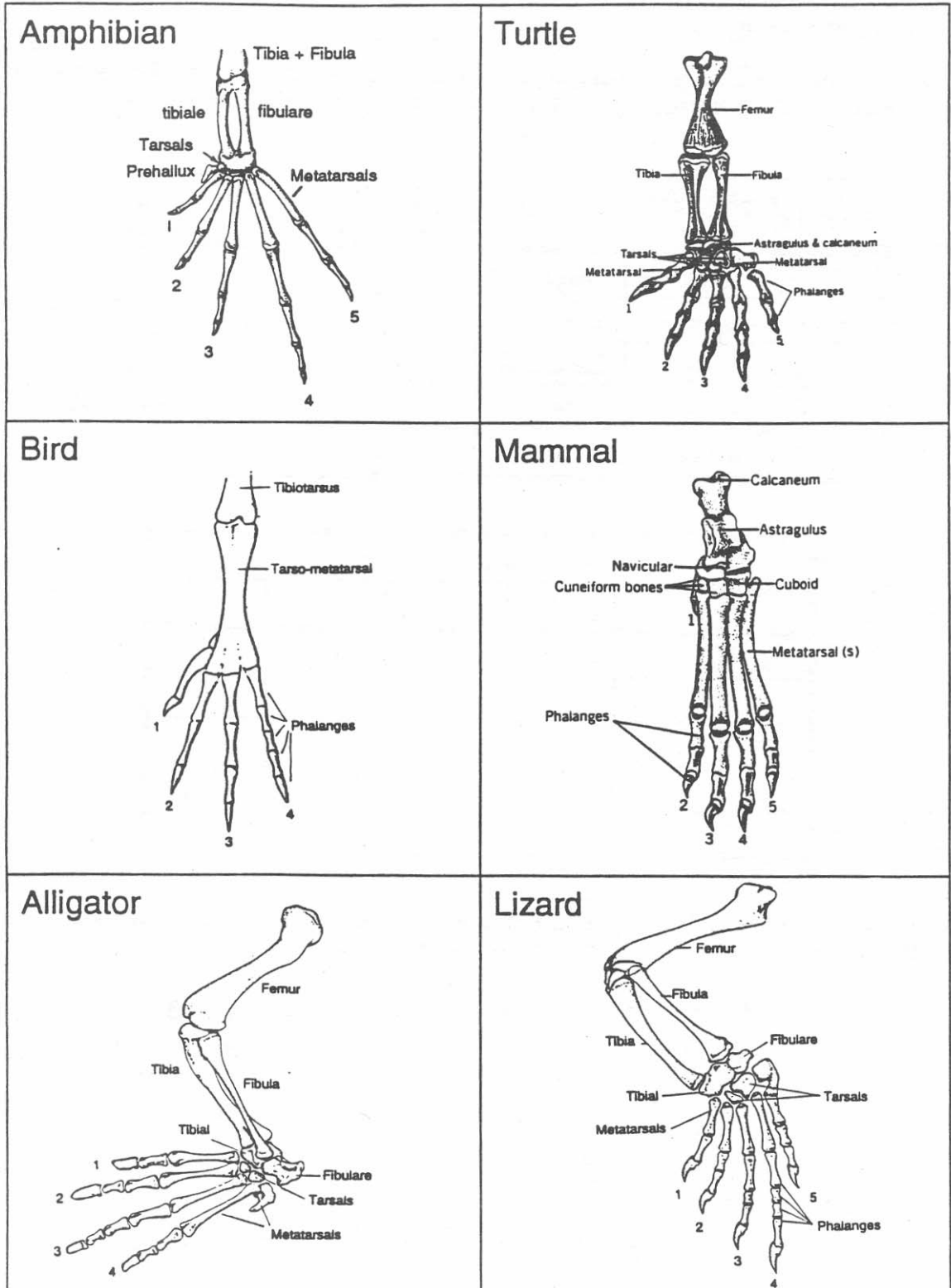
Figure 13.3. Digestive systems of amphibian, turtle, bird, mammal, alligator, and lizard (for snake see diagram of lizard).
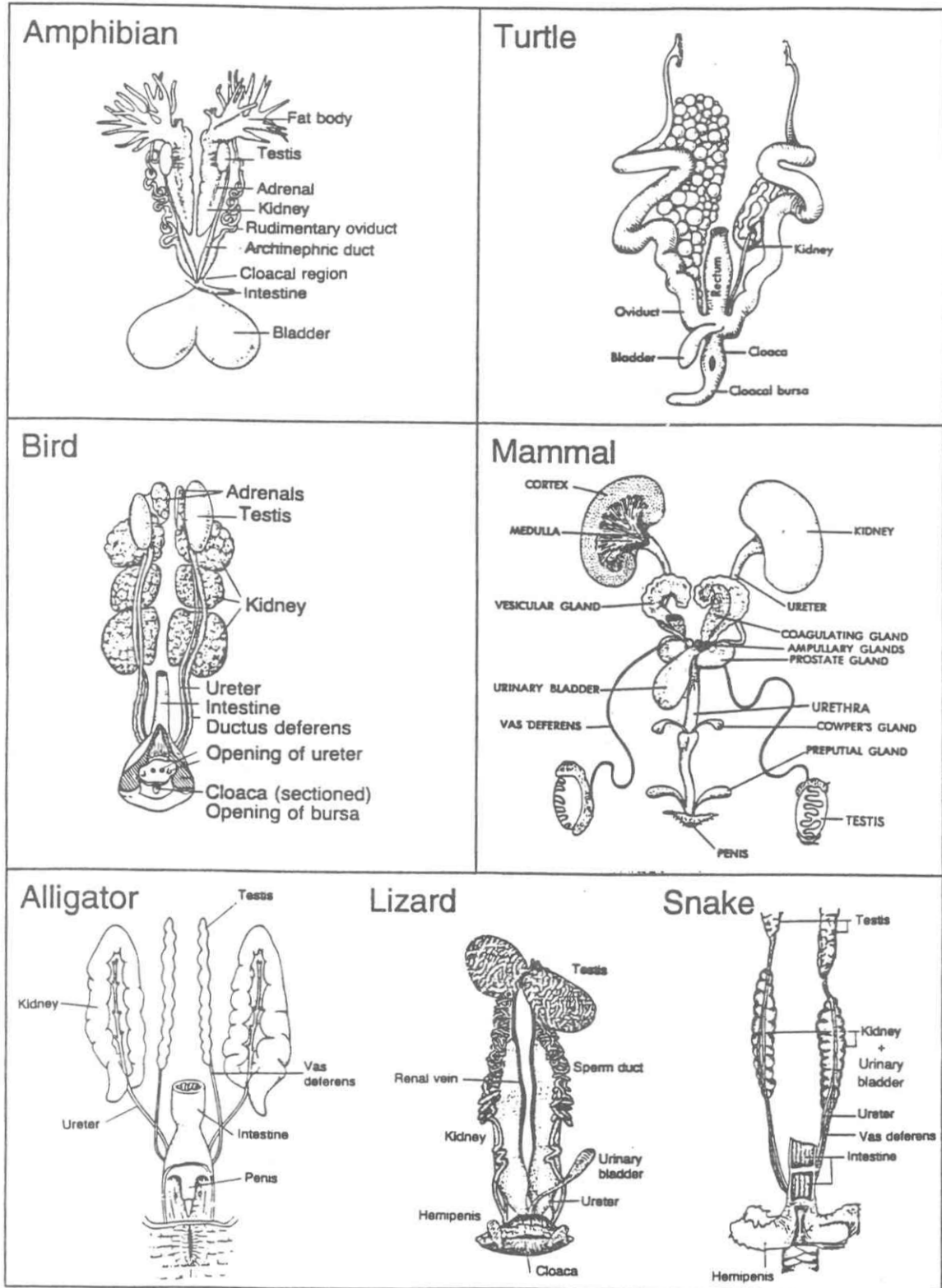
Figure 13.4. Urogenital systems of amphibian, turtle, bird, mammal, alligator, lizard, and snake.