Biology 337   *NAME:* _____   **Lab worksheet**
Biology of Invertebrates   *LAB TIME:* _____   **Dr. Bob Podolsky**

LAB A. PHYLOGENETICS of the BOLTOZOA

In this lab exercise you will use traits first to infer evolutionary relationships and then to examine patterns of trait evolution. It is important that you learn the difference between these two uses of traits. (1) In *phylogenetic inference*, the goal is to distinguish among a set of hypotheses for how groups are related. Traits are used to choose the best hypothesis. (2) If one already has a well-supported phylogenetic hypothesis in hand, the goal of *trait mapping* is to look at how traits most likely evolved by locating where changes in the past would most likely explain the distribution of traits among taxa in the present. *Note: using the same set of traits for both goals can lead to circularity in your conclusions (more below).*

I. Phylogenetic inference (using a set of traits to infer evolutionary relationships)

In this exercise you will reconstruct the evolutionary history of a group by choosing among a set of alternative hypotheses (phylogenies). You will be given a set of "species"—members of the phylum Boltozoa—and will use characteristics of these species to deduce their most likely "evolutionary" relationships (imagining, of course, that their morphologies resulted from a process of evolution during descent from a common ancestor). Be sure to follow the recommended steps in order:

→ **Step 1:** Based on their characteristics, sort your individuals into different species and place the species on a piece of paper.

→ **Step 2:** Looking over your species, identify all the characters that can be used to distinguish among the species. **Fill in** the following table as you identify each character (more than 7 are possible). Also, **fill in** the two "states" that you can identify for each character (for example, "eye color" is a character name, and two of its character states are "blue" and "brown"). **For this exercise, distinguish each character into only two possible character states.**

Character name	Character state #1	Character state #2
a.		
b.		
c.		
d.		
e.		
f.		
g.		

TQ: Identify any cases of "covariation" between characters (the same character states for those characters *always* occur together). Considering each case separately, is there a <u>necessary</u> reason why these traits must covary, or could they evolve independently?   In cases where they must covary, should they be counted as one or two evolutionary events? Why?

→ **Step 3:** Can you tell which species is the **outgroup**? (Why not?) An outgroup is assumed to be close to, but outside of, the group whose phylogenetic relationships are of interest (for example, reptiles would be a good outgroup for reconstructing relationships among the orders of mammals). An outgroup helps to figure out which character states are *ancestral* and which are *derived*.

Ask me which species, in this constructed example, will be the outgroup. Make this species into species "O," and assign numbers 1-4 to the remainder of the species. In the table above, **circle** the character states of the outgroup—we will assume these are *ancestral*.

→ **Step 4: Fill in** the "character state matrix" below. In each space put either a "0" (ancestral state) or "1" (derived state). Note that the outgroup is assumed to possess the *ancestral* character states— those that existed before the common ancestor of the other four species.

| Character	Species O	Species 1	Species 2	Species 3	Species 4
a	0				
b	0				
c	0				
d	0				
e	0				
f	0				
g	0				

→ **Step 5:** *(For this step, ignore what you've done so far. Trust me!)* Assume that your five species are related according to a tree with the branching pattern (= **topology**) shown below. First, **place the number of your outgroup** on the branch of each tree that makes it the **sister group** to the clade that includes all the rest of the species. Then, **place the numbers of the other species** on the branch tips in a way that creates three distinct phylogenetic hypotheses. *Remember: for a hypothesis to be distinct, the sister species relationships must be different.*
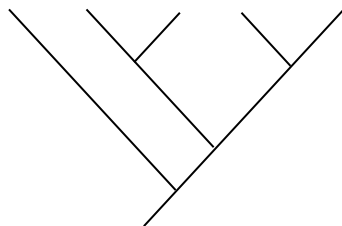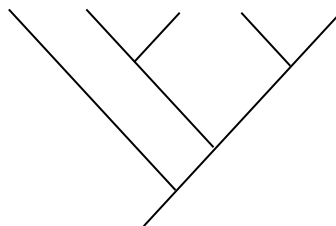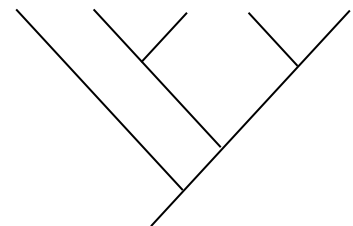
| **Tree A** | **Tree B** | **Tree C** |

TQ: Notice that all three trees above have the same **topology** (= branching pattern, ignoring species location). Assuming that the relationship of the outgroup to the other species remains the same, how many other **topologies** are possible? Draw them here, and **be sure to verify that they are different**.

TQ: For what practical reason did I exclude any other topologies from this exercise? (*Hint: how many other phylogenetic hypotheses would be possible among the other topologies?*)

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→ **Step 6:** If you haven't filled in the species numbers on <u>all</u> of the branch tips, go back to step 5! Now, using the character state matrix from step 4, **deduce where** (on which branches) changes in character state would have had to occur to account for the distribution of character states on the branch tips. For each state change, **place a bar** across the appropriate branch, and label the bar with the character number.

Tip: work on one character for all three trees before moving on to the next character. For each character, ask yourself, what was the ancestral state? Then, where must it have changed?

→ **Step 7:** In the boxes, record the minimum number of character state transitions that are necessary:

Tree A: [ ]   Tree B: [ ]   Tree C: [ ]

TQ: Is one of your phylogenetic hypotheses better supported than the others? If so, circle it. Explain how and why you reached your conclusion.

TQ: Not all characters help to determine which hypothesis has the best support. Those that do are considered "phylogenetically informative." Which characters (identified by letter) are phylogenetically informative? What distinguishes those that are from those that are not?

II. Trait mapping (using a well-supported phylogeny to deduce patterns of evolutionary change in traits)

For this exercise, assume that the phylogeny shown to the right is well supported using a set of characters *different* from those you used in the first exercise. (Imagine, for example, that these species carry molecular information that evolves). You will use this phylogeny to determine the number of times that particular phenotypic characters evolved.
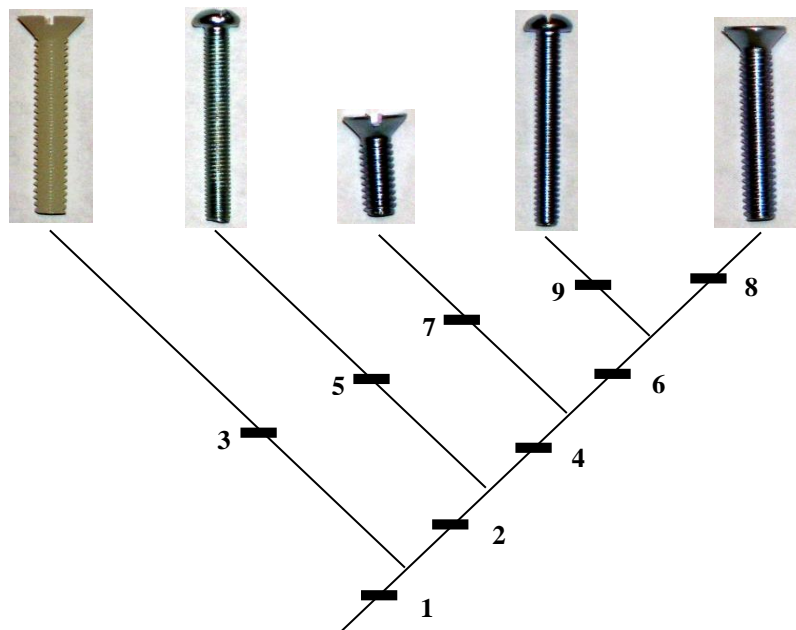
Assuming this phylogeny represents the true evolutionary history of the group, record the point(s) in evolutionary history

where the following traits evolved, based on simple parsimony. **Assume for this exercise that traits can change from ancestral to derived, but cannot change back from derived to ancestral**. (*Note that <u>not</u> all blanks must be filled in, and numbers can be used more than once since they just mark branches.*)

  I.   narrow body   _____   _____   _____

  II. short body   _____   _____   _____

  III. metal body   _____   _____   _____

  IV. rounded head   _____   _____   _____

  V.   narrow threads   _____   _____   _____

TQ: According to the phylogeny, which of these five traits, when found in more than one member of the Boltozoa, show evidence of **homoplasy** and which show evidence of **homology**?

TQ: In this exercise you were interested in learning the number of times a certain phenotypic character (like thread number) evolved. Why is it necessary to use a different set of traits that does not include thread number to infer the best-supported phylogeny?

III. Drawing as an aid to observation

While observing animals this semester, you should use drawings and annotations to develop your observational skills. In light of digital photography, it may seem pointless to labor over drawings and seek out details that have been described countless times. But remember: the *process* of taking a photograph does not replace the *process* of drawing. Your observations and their representation on paper are part of a learning process that forces you to pay attention to how individual parts relate to one another and how they differ from one organism to the next. If focused, your brain sees and stores more useful information than does taking a detailed and more "accurate" photograph.

The value of drawing for observation is no more apparent than when observing an object that is boringly familiar. The final in-class exercise is to choose two members of the Boltozoa that differ in several characteristics and to do an informational laboratory drawing of them. Use this opportunity to observe detail and to practice the following essential skills—your drawing should include each of these types of information. **Remember to make use of these skills in future weeks**.

1. Record collection information. If possible, record information about whether the specimen is live or preserved, and if live, where it was collected from if available (in this case, Lowes Home Improvement).

2. Record scale information. To serve as a useful reminder for studying, always provide some estimate of size, either a scale bar or a measure of the whole organism or structure or a bracketed part.

3. Label important parts. An unlabelled diagram is nearly useless. Assume that the bolt is an organism new to you and label the important features so that you could make use of the diagram several months later.

4. Include descriptive information. If your drawing does not convey all the important information that you can see, add some information in words. For example, the threads on a bolt are not just lines, they run in a particular helical direction.

5. Include functional explanations. Write little notes to yourself about what you observe and how you think something works. There are different parts of a bolt that do particular things, so include some piece of information that would help you to remember later what you saw. For example, the "male" threads on a bolt engage with the "female" threads on a nut that run in the same helical direction.

6. Use "blow-ups" to record detail and to avoid drawing repeated structures. One of the most important skills to learn. Details are small, organisms are large. It's not worth your time to sketch every small detail, or to repeat details. This scaling problem can be overcome by making a simple outline sketch of the animal and then making little "blow-up" drawings that show the important details. For example, you can more easily show exactly which way the helical threads wind in an enlarged drawing of a small part of the shank. For this example, use this technique to highlight differences between your two chosen specimens.

V. Definitions and concepts. Phylogenetic relationships will be used throughout the semester to understand the pattern of evolutionary change in key traits. Talk with your partner to figure out the definition of each of the following terms. Do not look them up—base your answer on the extent of your understanding.

- **Taxon** (pl. taxa):

- **Sister taxon**:

- **Taxonomy vs. Phylogeny:**

- **Outgroup**:

- **Clade** or **monophyletic group** (compare to **Paraphyletic** and **Polyphyletic**):

- **Homology** (compare to **Homoplasy):**

- **Shared, derived character** (= **synapomorphy**):

- **Invertebrata** (addressing whether this is a valid grouping for taxonomy):