Evolution and Biodiversity Laboratory
Activity: Constructing a Phylogeny

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Systematics is a two-part endeavor. When studying a group of related organisms, the systematist must

- Devise an hypothesis of their evolutionary relationships
- Devise a classification that faithfully reflects the hypothetical relationships

Different schools of thought in systematics have come and gone over the past decades. **Cladistics** is the modern survivor. We will be using the **cladistic** method of phylogenetic analysis. The Caminalcules will be our model organisms.

Examine the Caminalcules in Figure 1. Each one is an **Operational Taxonomic Unit (OTU).** We use this term to avoid assigning each to a specific taxon. (Think of them as biological species, and refer to them by number.)
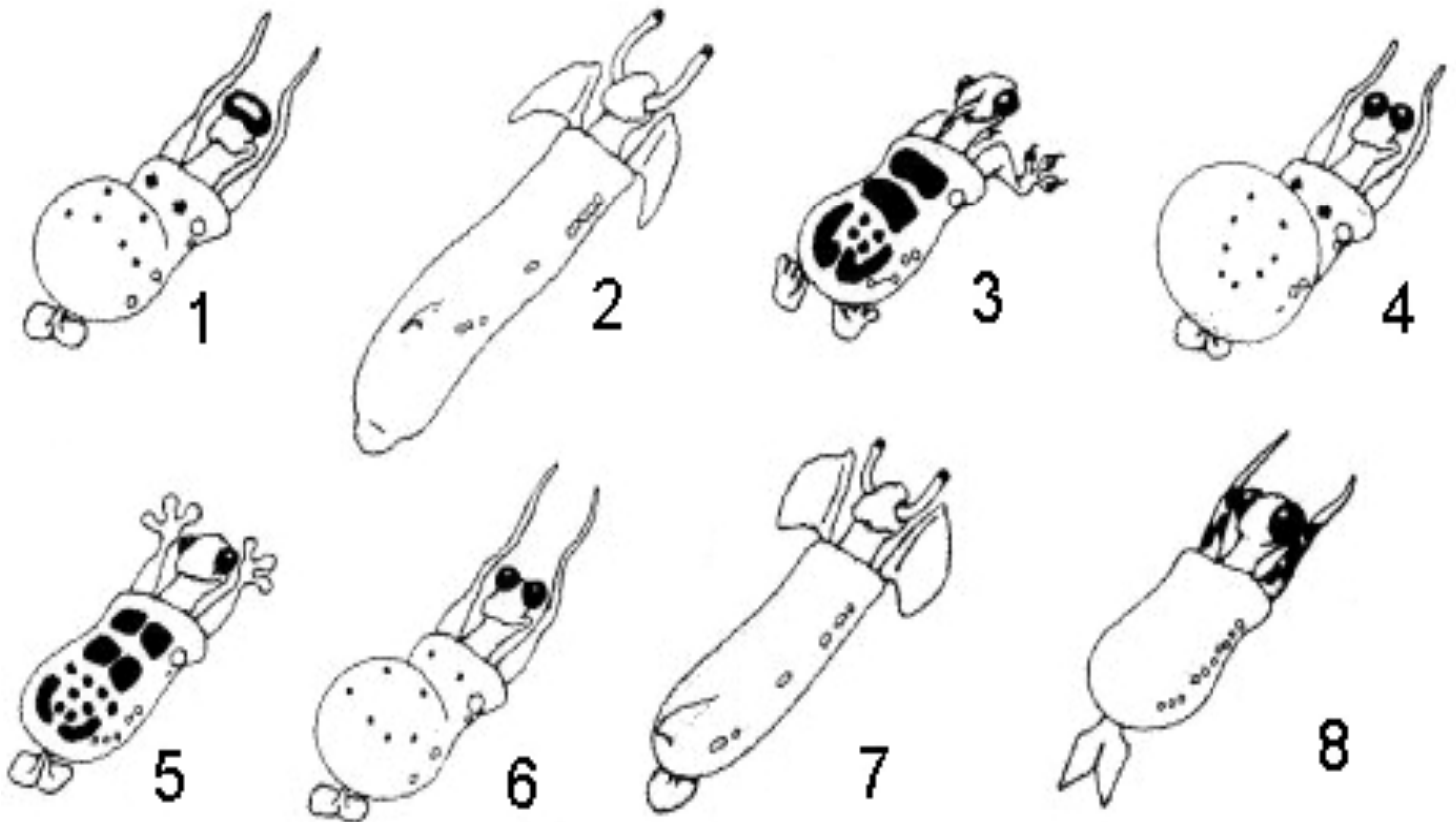

Figure 1. A variety of Caminalcules, arranged in no particular order.

I. Example: Using Synapomorphies to Construct a Phylogeny

OTUs are grouped together on the basis of **synapomorphies**. The presence (or absence) of a synapomorphy in two or more OTUs is inferred to be the result of common ancestry.

Results of a cladistic analysis are summarized in a phylogenetic tree called a **cladogram** (from the Greek *clad* meaning "branch"), an explicit hypothesis of evolutionary relationships. Following is an example of how to create a cladogram of our Figure 1 OTUs.

Step One. Select a series of binary (i.e., two-state) characters. For example:

character a: "eyes present" (+) versus "eyes absent" (-)
character b: "body mantle present" (+) versus "body mantle absent" (-)
character c: "paired, anterior non-jointed appendages present" (+) versus
                 "paired, anterior non-jointed appendages not present" (-)
character d: "anterior appendages flipperlike" (+) versus
                 "anterior appendages not flipperlike" (-)
character e: "eyes stalked" (+) versus "eyes not stalked" (-)
character f: "body mantle posterior bulbous" (+) versus
                 "body mantle posterior not bulbous" (-)
character g: "eyes fused into one" (+) versus "eyes separate" (-)
character h.   "forelimbs with digits" (+) versus "forelimbs without digits" (-)

Step Two. Examine each OTU and determine which character state (+ or -) it exhibits. Enter the data in a matrix like the one shown in Table 1.

In our example:
- **character *a*** is the same in all eight OTUs
- **character *b*** is the same in all eight OTUs
.

These two characters are **primitive** to the entire group.

Primitive characters are not informative for separating OTUs into unique taxa.

Also in our example:
- **character e** is shared only by **OTUs 2 and 7**
- **character f** is shared only by **OTUs 1, 4, and 6**
- **character h** is shared only by **OTUs 3 and 5**

Inferences:
- **character e** was inherited by **OTUs 2 and 7** from a common ancestor
- **character f** was inherited by **OTUs 1, 4, and 6** from a common ancestor
- **character h** was inherited by **OTUs 3 and 5** from a common ancestor

A cladogram consistent with the distribution of these eight characters among the OTUs is shown in Figure 2.

Table 1. Character states of characters a - h in Caminalcules in Figure 1.

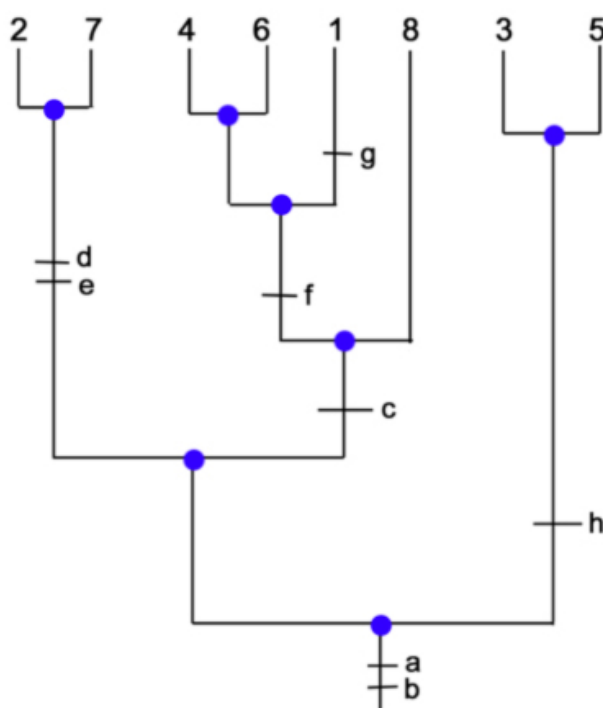| character	1	2	3	4	5	6	7	8
a	+	+	+	+	+	+	+	+
b	+	+	+	+	+	+	+	+
c	+	+	-	+	-	+	+	+
d	-	+	-	-	-	-	+	-
e	-	+	-	-	-	-	+	-
f	+	-	-	+	-	+	-	-
g	+	-	-	-	-	-	-	-
h	-	-	+	-	+	-	-	-

Figure 11. A cladogram based on synapomorphies of Caminalcules 1 - 8.
Blue circles at each node (branch point) designate (hypothetical) common ancestors of the OTUs above them on the tree.

Other phylogenies may be consistent with the character distributions among the OTUs. In practice, there are often several--or even many--cladograms consistent with the data.

The rule of <u>parsimony</u> states that when two or more competing hypotheses are equally consistent with the data, we provisionally accept the simplest hypothesis.

This is not to say that evolution is parsimonious, only that our hypotheses should be.

The most parsimonious cladogram is the one with the fewest steps.
For example...

We *could* hypothesize that OTU 6 is actually more closely related to OTU 1 than to OTU 4.

However, this would require that character g (fused eyes)
- evolved once in the ancestor of OTUs 1, 4, and 6
- then secondarily disappeared in both OTUs 4 and 6

This is less parsimonious than stating fused eyes evolved only once (in OTU 1).

A. Natural Taxa: Monophyly (and Linnaean Classification)

A classification should faithfully reflect evolutionary relationships. The hierarchical results of a cladistic analysis can be incorporated into the Linnaean hierarchy (Figure 3)

A **monophyletic taxon** consists of a (hypothetical) common ancestor and all its descendants. ***In cladistic analysis, all taxa must be monophyletic***.

In our cladogram
- OTUs 2 and 7 and their common ancestor comprise a monophyletic genus
- OTUs 1,4, 6 and 8 and their common ancestor comprise a monophyletic genus
- OTUs 3 and 5 and their common ancestor comprise a monophyletic genus

Order Caminalcula:
    Family 1
        Genus 1
            Species 2
            Species 7
        Genus 2
            Species 1
            Species 4
            Species 6
            Species 8
    Family 2
        Genus 3
            Species 3
            Species 5
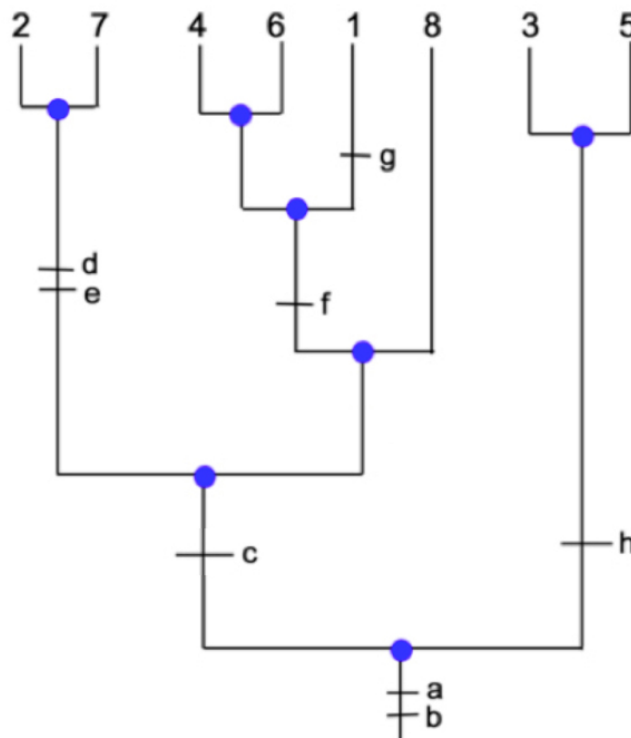
Figure 3. **Incorporated results of a cladistic analysis showing Linnaean relationships among the OTUs.**

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B. Artificial Taxa: Paraphyly and Polyphyly

A **paraphyletic taxon** contains _some, but not all,_ of a particular ancestor's descendants.

A Family consisting of only OTUs 2 and 7 would be considered **paraphyletic** because it does not include all the descendants of the most recent common ancestor.

A **polyphyletic taxon** consists of descendants of _more than one_ most recent common ancestor.

A Family consisting of _only_ [OTUs 2 and 7] and [OTUs 3 and 5] would be considered **polyphyletic** because it includes groups descended from both (1) the ancestor just below the appearance of character h and (2) the ancestor just below the appearance of characters c, d, and e.

Monophyletic, paraphyletic, and polyphyletic taxa examples are shown in **Figure 4**.
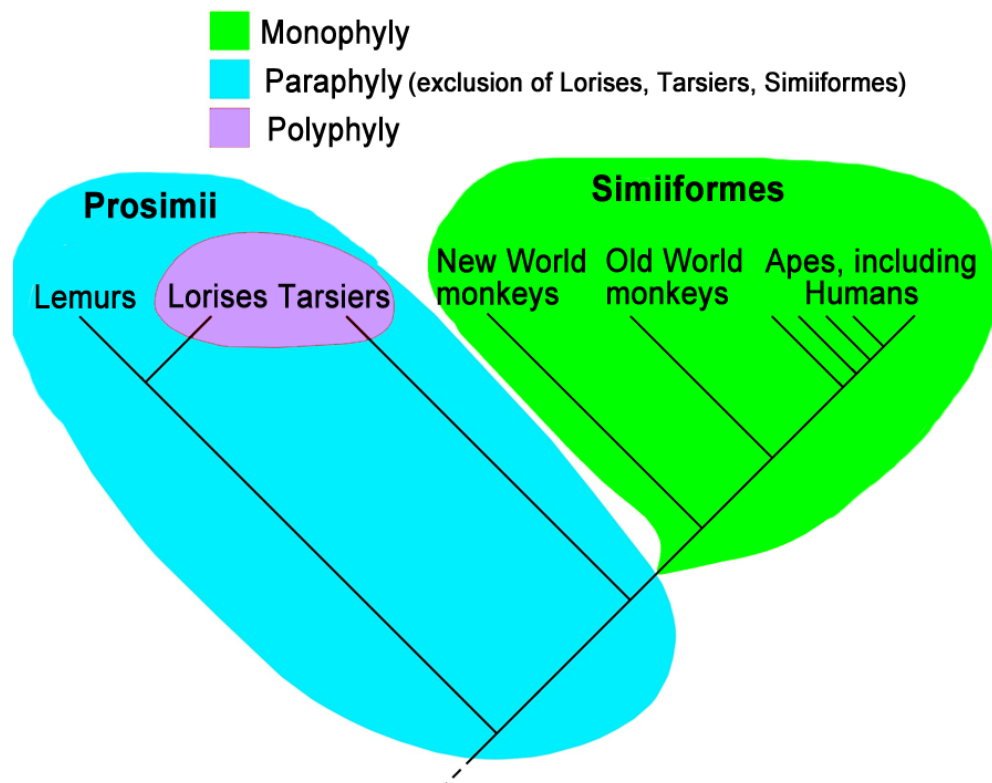

Figure 4. A phylogenetic tree showing hypothetical relationships of major primate groups. A monophyletic taxon consists of all the descendants of a single common ancestor. A paraphyletic group contains some, but not all, of a particular ancestor's descendants. A polyphyletic group consists of descendants of more than one most recent common ancestor. Paraphyletic and polyphyletic groups are artificial, and not considered valid in the cladistics system.
Original work by Petter Bøckman, revised by Peter Brown and Dana Krempels - http://commons.wikimedia.org/wiki/File:Monophyly-paraphyly-polyphyly.jpg

II. Activity: Using Synapomorphies to Construct a Phylogeny

Use the Caminalcules provided to do construct a phylogenetic tree with cladistics methods as shown in the example in Section I.

- If you are doing this **in person**, the caminalcules will be sets of **laminated cards**.
- If you are doing this **online**, your instructor will provide you with a **set of eight different OTUs plus another caminalcule that will serve as your outgroup**.

Use the Phylogenetic Tree Template linked to your online syllabus.

A. Determine OTU synapomorphies

1. Select binary characters (different from the ones in the example)
2. Assign character states to each OTU
3. Enter them in **Table A** (**Phylogenetic Tree Template**, online lab manual).
4. Determine which OTUs have synapomorphies
5. Enter them in **Table B** (**Phylogenetic Tree Template**, online lab manual).
6. In the space on the last page, draw a cladogram of your Caminalcules
7. Include the appearance of each character as shown in Figure 2

B. Outgroup Analysis: Rooting the Tree

Your phylogenetic tree of Caminalcules is **unrooted** because you have no way to know which character states are derived and which are primitive.

Do not assume that a more complex form is more derived.

There is always the chance that a taxon has *secondarily lost* an ancestral character.

To root your tree, you must consider the character states of an **outgroup**.

Your Caminalcule cards includes an outgroup on a light grey card. It represents a Caminalcule species related to your **ingroup** (the rest of the Caminalcules on your cards), but in a different taxon (e.g., a different Family).

Use the outgroup to determine which characters are primitive, and then root your tree. The rooted tree may be quite different from your unrooted one.