Lab II. Lycopodiales: the Clubmosses

I. Lycopodiales: The Vegetative Features of the Sporophyte Phase

The clubmosses (traditionally classified species of the genus *Lycopodium*) are low, evergreen plants with tiny leaves (Fig. 1). They commonly, though not always, have their spore-producing structures in club-like structures that stick up in the air above the rest of the plant. The name comes to us from the Greek, *lykos* meaning *wolf* and *podos* meaning *foot*, in reference to these club-like structures' superficial similarity to the paws of these iconic mammals. Forest floors, alpine tundra, bogs, and barren ground are all habitat for various genera of clubmosses in Vermont, including *Dendrolycopodium*, *Diphasiastrum*, *Huperzia, Lycopodium, Lycopodiella,* and *Spinulum*.

A. Life Form

Clubmosses come in various life forms, which is another way of saying that they have a number of different branching designs. The fascinating thing is that the life forms are all variations on a basic theme of forked - or dichotomous - branching.

1. To keep things simple, let's contrast the life forms of just two species of clubmoss, both of which are common species of barren ground here in Vermont, *Dendrolycopodium hickeyi* (tree-clubmoss) and *Huperzia lucidula* (shining clubmoss).

2. Note that in the ground cedar, the main creeping stem axis, which is indeterminate, forks to yield both determinate aerial axes and new creeping stem axes. This kind of branching is called anisotomous (literally, "unequal cuts"). In the shining clubmoss, however, each branching event yields two equal forks. This is isotomous branching.

Figure 1. Plate 224, *Lycopodium clavatum*
English Botany, published in 1833

3. Clubmoss leaves, like most leaves, are appendicular (lateral on the stem), dorsiventral (have a top and bottom), and determinate (stop growing). The clubmoss is in addition an example of a primitive leaf type, the microphyll, thought to have evolved as a simple projection of a stem.

4. Notice that not all clubmosses have the same leaf designs and arrangements. Look at the herbarium specimens of the various genera of clubmosses found in Vermont for comparison. For example, *Huperzia lucidula* has many-ranked, needle-like microphylls that are arranged in a spiral around the stem axis, whereas *Diphasiastrum digitatum* has scale-like leaves in four ranks.

5. Clubmoss **roots** are adventitious only: note the roots growing out of the horizontal stems of the clubmosses you are diagramming – if present on your specimen be sure to label them in your diagram. Note whether they branch or not, and if so what type of branching do they show?

6. Finally, note the club-shaped structures called **strobili** (singular = **strobilus**). These are collections of **sporangia**, the **spore**-producing organs, and their associated microphylls, which we call **sporophylls**.

***S1: Sketch the living plant of the *Dendrolycopodium hickeyi*, and label with the features described above. Be sure that your plant has both reproductive and vegetative characters.**

B. The Interior of the Stem

Some of the most useful vegetative features are found in the stem interior, especially in the stele. Remember that the stem interior of all vascular plants is divided into three regions or "tissue systems": the epidermis (an outermost, protective layer), the vascular cylinder (center for stem support and transport of water, sugars, and nutrients), and the ground tissue, usually parenchyma cells (for storage, including a cortex outside the vascular cylinder and sometimes a pith inside the vascular cylinder).

1. <u>A *Lycopodium* stem</u>

Look at a transverse (cross) section of a *Lycopodium* stem (= rhizome) in a prepared slide; start with the 10x objective and observe the following:

a. <u>Dermal Tissues</u>

Note that there is a single epidermis layer with a protective covering, the **cuticle**, made of wax. Inside of the **epidermis** is a large **cortex** layer, made of numerous layers of loosely packed, unspecialized cells (remember, these are called **parenchyma** cells.) In addition, you may see some other things, depending on which slide you have. Some stems have leaves visible to the outside of the stem, each with a vascular bundle or not. You may also be able to see **leaf traces**: the stele and the leaf connect through these vascular bundles.

b. <u>The Stele</u>

A stele is an independent column of vascular tissue in the stem (or root); in clubmoss, the vascular cylinder includes a single stele. Our interest is in the design of the water and nutrientconducting tissues of the stem. In this course, we emphasize the design and layout of the **xylem**; the **phloem** receives less attention, largely because there is so much more variation in the xylem. Look at the stele of *Lycopodium* under high magnification:

i. There are two colors of cells in your stele; red and turquoise. Recall that the red color is produced by **safranin**, which is here staining the lignin present in the secondary walls of the tracheary elements. Take a careful look at them: of the two types of tracheary elements, *Lycopodium* has only **tracheids**. Tracheids are a relatively unspecialized tracheary element that lack large perforations connecting adjacent cells. Note that the largest tracheids are near the middle of the red zones, whereas the smallest are toward the outside of the stele. The small cells are **protoxylem**, and the large ones are **metaxylem**. Location of the protoxylem is an important character, since it is constant within large groups of plants: for instance, in the division Lycophyta, to which the Lycopodiales belong, the protoxylem is exarch (outside the metaxylem). These two tissues also differ in the period during development that they function. The protoxylem begins to function earlier in development, when the individual cells are closer to the tip of the stem axis, than do the metaxylem cells.

ii. Now consider the layout of the zones of red cells. These zones are actually connected together in the *Lycopodium* stele, but in any one section, they look separate. You would have to look along the stele, down or up, to see that each of these zones merges with other zones to form a single continuous - but highly invaginated - xylem mass, without a central pith. Your section may include a red zone that is just separating or two that are just merging. This sort of a stele, invaginated but without a pith, is called an **invaginated protostele**, or, more simply, a **plectostele**.

iii. Note: there is no vascular cambium and no secondary growth in the Lycopodiales.

iv. Where is the phloem? Check between the red zones. The phloem layer, comprised of a mixture of **sieve cells** and parenchyma, separates the cells of the xylem by a layer of parenchyma cells.

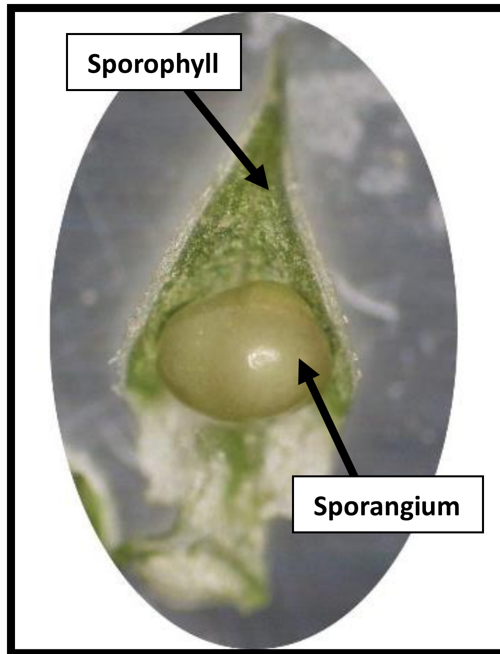
v. Finally, look once again at a **leaf trace**, either in the cortex or in a leaf. See how simple the layout of the leaf trace is: a few tracheids and a bit of accompanying phloem, which is hard to pick out.

***S2: Make a sketch of the *Lycopodium* stem cross-section. Label all of the features discussed above. Make sure to draw big enough that you can capture the xylem arrangement in the stele, OR do another zoomed in sketch of just the stele.**

II. Lycopodiales: The Reproductive Features of the Sporophyte Phase

Lycopodium produces spores in sporangia, like all vascular plants. The location, structure, and dehiscence of these sporangia are critical characters, so we should look at them. In addition, the prepared slides we have of Lycopodium sporangia are excellent for looking at tracheid structure and the development at an apical meristem, as well as seeing the stages in the development of a sporangium. We'll try to see all three things without spending too long on one slide.

A. Take a look at the living *Dendrolycopodium* strobilus to get a feeling for the layout of the sporophylls. Remember that strobili are by definition determinate sporangium-bearing axes. In clubmosses, the strobili are made up of modified leaves (**sporophylls**), each of which bears a sporangium on its adaxial (upper). Look for the sporangia on the adaxial surface of the leaves surface (Fig. 2). Also look closely at the sporangia, and see if they are open and the spores have been released. It's important to note that the sporangia are dehiscing (splitting open) along a line transverse to the axis of the sporangium stalk.



obscurum on the adaxial (top) surface of the sporophyll.

1. Now look at the slide of the *Lycopodium* strobilus under low power. You should see in this slide an axis with elongate red cells in its interior. Attached to the axis are leaves, which bear sporangia on their upper surfaces. Begin by looking at the basic structures of the strobilus.

Note that each **sporangium** is a hollow capsule bound by one to three layers of cells, the **sporangium** wall. Inside are spores in various stages of development. These are **trilete** spores: they are **tetrahedral** and have strongly marked ridges along three of the edges of the tetrahedron. Spore shape is related to packing in the meiotic tetrad. The sporangium is on a short, massive **stalk**, attached to the leaf surface. The leaf itself has a **leaf trace** and sometimes a mucilage body. Note that the sporangium-bearing of the leaves. Remember that strobili are by definition determinate sporangium-bearing axes. In *Lycopodium*, the strobili are made up of modified leaves (sporophylls), each of which bears a sporangium on its adaxial (upper) surface (Fig. 2). Look for the sporangia on the adaxial surface of the leaves. Also look closely at the sporangia; they are open and the spores have been released. It is important to note that the sporangia are dehiscing (splitting open) along a line transverse to the axis of the sporangium stalk. Leaves are attached to the axis of the strobilus.

***S3: Sketch a whole strobilus with these structures and label the features described above.**

2. Now look more closely at the interior of the strobilus axis. Here you should see elongate cells that have odd, red-staining rings and helices in them. These cells are tracheids - in fact they are fantastic examples of tracheids. Remember the three characteristics of tracheids:

i. Elongate

ii. Dead at functional maturity

iii. Secondary wall thickenings

You can see all of these features here. The tracheids nearest the center of the xylem mass mostly have scalariform-pitted secondary wall thickenings; those near the outside have helical thickenings. Why do you think this so?

3. But there is something even more important to see here. You are looking at a point near the apex of an axis, and in fact you can see tracheids in various stages of differentiation right in this

slide. Start at the very tip. There is a small meristem, with cells that are small and densely granular, at the tip. At the very top is the small group of initials typical of *Lycopodium*. Follow down from there to see the cells in various stages of differentiation, until you see recognizable tracheids. Then look for undifferentiated tracheids above: elongate cells still without fully developed secondary thickenings, and probably still alive (until a botanist came along and killed this plant).

***S4: Sketch an up-close tracheid with helical secondary wall thickenings, and one with scalariform-pitted thickenings. Look up and down the strobilus axis to find the different kinds; indicate where each is found in your sketch.**

B. Finally, if you look at all of the sporangia on one side of an axis from the top to the bottom, you can get an idea of the whole sequence of events in sporangium development, since the youngest are at the top and the oldest are at the bottom. At the top the sporangia are small, the wall is of three layers, and the spherical cells inside are sporocytes in various stages of meiosis. As you go down the axis, look for the following things:

1. A decrease in the number of sporangium-wall layers, first to two, then to one - the first to decompose is the **tapetum**, which provides the material for building spore walls

2. The development of secondary thickenings in the cells of the outermost **sporangium-wall** layer, presumably there to function in the opening of the sporangium.

3. The accumulation of a red-staining **exospore** coat on each of the spores

4. Spores falling apart from their original groups of four (**tetrads**)

***S5: Make a sketch of two sporangia in place on the strobilus axis, one from the base and one from the tip. Label the features discussed above in both your sketches.**

III. Lycopodiales: Gametophyte Phase

People find *Lycopodium* gametophytes in nature by looking for tiny sporophytes, usually in disturbed habitats like treefalls, sandpits, and old streambeds. Then they dig up the gametophytes by carefully following the young stem down into the earth. Our last efforts for this lab are to look at some of these odd gametophytes whole and at some mounted on prepared slides to see the gametangia.

1. Look at a gametophyte in a test tube. As you can see, they are reminiscent of an old piece of chewing gum attached to a young *Lycopodium* sporophyte. The roots that you see belong to the young sporophyte; gametophytes have no roots.

2. Now look at a prepared slide under low power. You should be able to discern the following characters.

a. The cells of the gametophyte are unspecialized parenchyma.

b. Large **antheridia** containing reddish-staining **spermatozoans** are buried in the parenchyma of the gametophyte.

c. Also present are **archegonia**, the female reproductive structures. Each flask-shaped archegonium contains a single **egg cell** (Fig. 3). Regrettably none of our slides show these interesting structures.

d. There is in addition a special meristem, the **marginal meristem**, which encircles the periphery of the gametophyte like a belt.

*S6: Sketch a gametophyte with antheridia, and sketch the marginal meristem or archegonia, if visible.

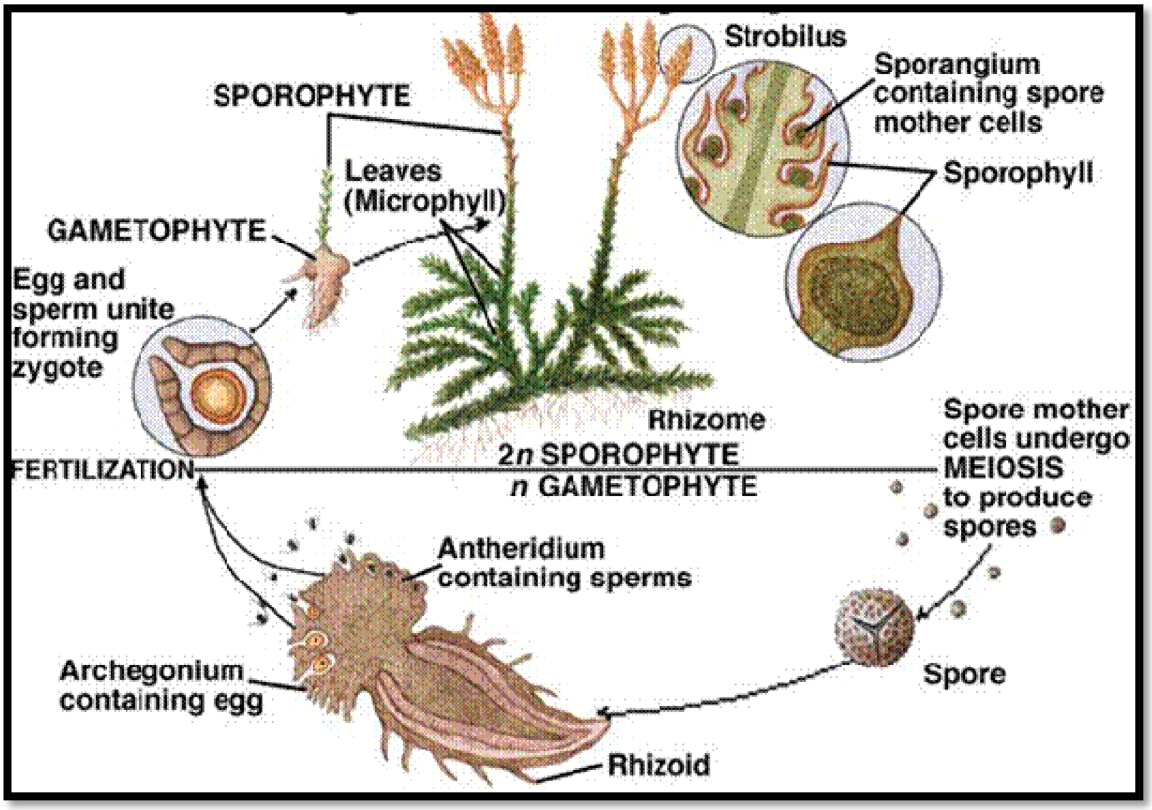


Figure 3. Lycopod life cycle. Lycopods – like all the rest of the lycophytes, have a hetero-morphic alternation of generations as a gametophyte and sporophyte with different morphology.