III. Extant Heterosporous Lycophytes: Selaginellales and Isoëtales

I. Selaginellales: the Spikemosses

The plants in this part of the lab all belong to a single large genus, *Selaginella*: they are known in English as the spikemosses. Like the clubmosses (Lycopdiales) we've just studied, spikemosses are low, evergreen plants with microphylls only. In Vermont, we have two species of spikemosses, each representing one of the two basic life forms in the genus. One is a cushion-like plant that grows on dry ledges in old pastures and along the lakeshore (*Selaginella rupestris*); the other is a small spreading species, easy to overlook, that grows mostly in wet, low, grassy areas like damp cow pastures (*Selaginella apoda*).

Figure 1. Body plan of the typical spreading life from of Selaginellales.

A. The Living Plant: Spreading Life Form

Take a moment to look over the living plants of *Selaginella* in the lab. These are members of the spreading life form (Figure 1). Identify the microphylls and strobili. Now look at the shoots near the surface in the pot to find the peculiar roots of *Selaginella*. These roots appear to be exogenous, not endogenous like most roots; they arise from angle meristems, not from the pericycle of the vascular cylinder. These are called rhizophores. There has been considerable argument about the homology of rhizophores, since they do not arise in the normal way.

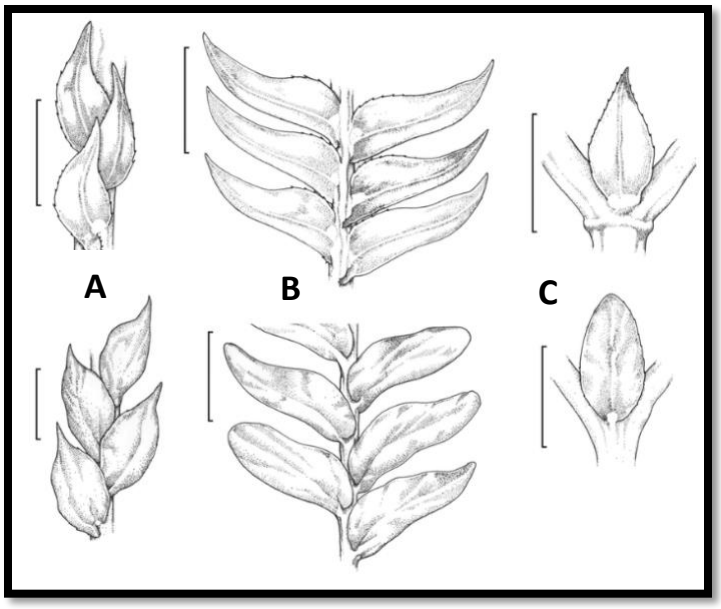

Figure 2. The three forms of microphylls found in the Sellaginales. (A) dorsal, (B) ventral or lateral, and (C) axillary.

1. Compare the diversity of the spreading form of *Selaginella* exemplified by the living plants and herbarium specimens in the lab. Identify the type of branching present and find the microphylls along the stem. Remind yourself that microphylls in the spreading species of *Selaginella* are not the same (heterophyllous) and are arranged in four ranks (longitudinal rows along the stem): two upper dorsal microphyll rows that are smaller, and two lower lateral microphyll rows that are larger. A third type of microphyll, called an **axillary microphyll,** is found at each branching point (Figure 2).

*S1: Sketch of a whole plant of *Selaginella* AND a close up of the different types of microphylls as shown in Figure 2. Label the features described above.

2. Next, try to find the quadrangular strobili found at the tips of some stems and make a dissection of a single **strobilus** from the living plant. Remove one that looks particularly handsome, and work with two probes under the dissecting microscope.

a. Look carefully at the leaf surface just distal to the sporangium. (Distal means away from the point of origin or attachment.) There is a very small, narrow, pale tab of tissue right near the sporangium. When you locate it, you've seen your first **ligule**. Ligules are characteristic of most Lycophytes (Lycopodiales is an exception). If you don't find ligules here, check a vegetative leaf, where they may show up better

b. Push back a microphyll to reveal the **sporangium**: note that the sporangium dehisces along a clear transverse seam. In *Selaginella*, and other heterosporous plants, there is are two different types of sporangia: **megasporangia** and **microsporagnia**. Within a single strobilus both mega and microsporangia are found (Figure 3).

c. These two different sporangia produce two different types of spores: **megaspores** and **microspores.** Look for each type of sporangium in a strobilus and mount them in water on a slide with a cover slip. Look at the spores under the microscope - these spores are typical **trilete** spores (remember trilete is the term referring to spores that are tetrahedral in shape.) Most of the spores you see are probably all microspores, which are less than 0.1 mm in diameter: you may also see much larger megaspores.

*S2: Look at the spores under the compound scope from the slide you made or the prepared slide. Look for both the mega and microspores. Draw one or several microspores, and/or megaspores.

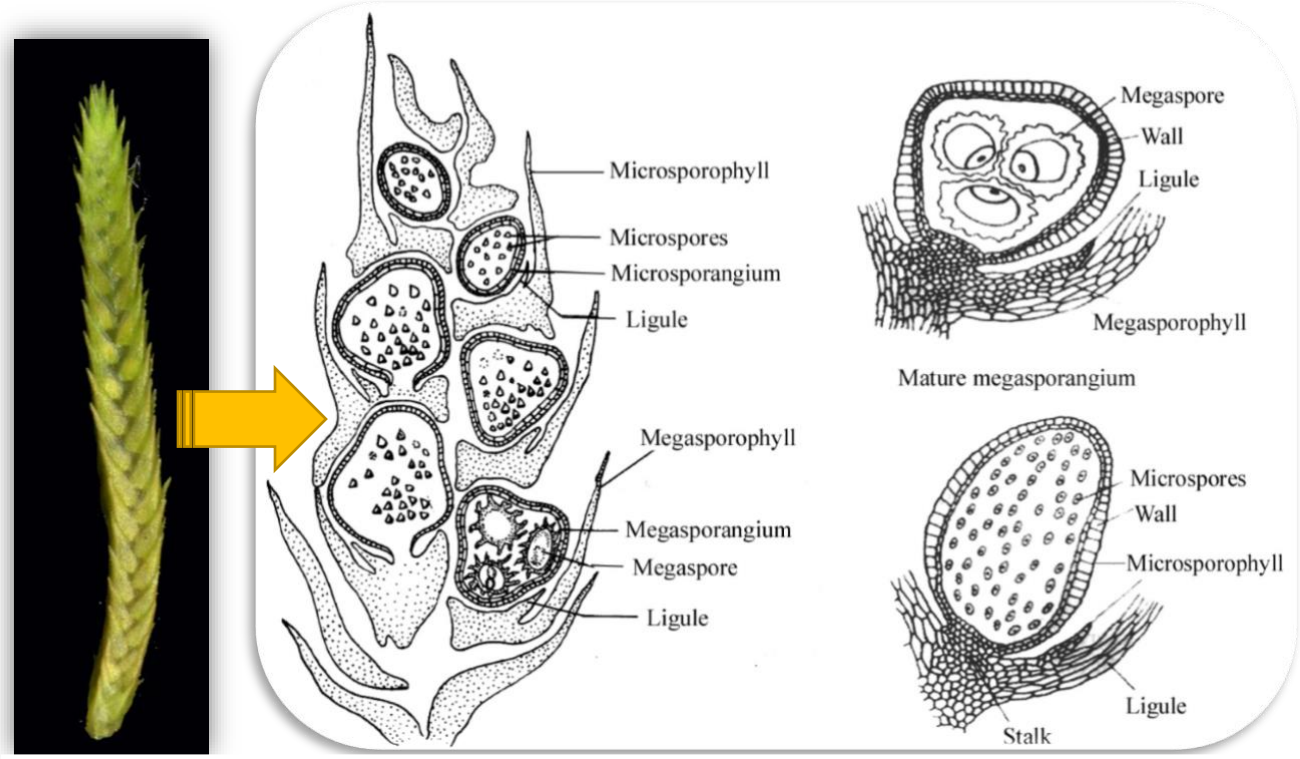

Figure 3. A *Selaginella* strobilus. Found at the tip of an axis, they are quadrangular in shape and composed of sporophylls. Attached to the adaxial surface are ligules and either mega- and microsporangia.

B. The Living Plant: Cushion Life Form

Look at the herbarium specimens of *Selaginella* on display in the lab. Note that there are a number of cushion plants represented. These are usually from drier habitats or regions with drier climates, such as *Selaginella ruprestris* found here in Vermont (Figure 4). They all share the cushion form, have microphylls that are helically arranged and all the same in shape (isophyllous). Be sure that you see the contrast between these helically arranged leaves isophyllous and those that are in four-ranks found in many other members of *Selaginella*, found in more wet habitats from tropical climates.

Figure 4. Habit of *Selaginella ruprestris* with its cushion form. Note that the microphylls are helically arranged and not in four ranks.

C. Features of the Stem Interior

Begin by making a cross section of the living *Selaginella* stem in the lab. Then look at some of the different prepared stem sections available as each is a little different from the next.

1. Start by looking at the outer regions of the stem. Note the **epidermis**; internal to it is a red-staining region of **sclerenchyma** cells providing structural support and protection to the stem.

2. Now look at the **vascular cylinder**, in which several stele segments may be visible. The segments appear separate but are actually connected, as in the Lycopodiales. Some may be bilaterally symmetrical (that is, flat).

3. A prominent cavity surrounds the stele, unique to *Selaginella*. Notice that the stele is suspended in the cavity by a few thin cells - these are called **trabeculae** (Latin for 'little beams'). Between these trabeculae are cavities of **air spaces**.

4. Check out the **protoxylem** location. You should see that the smallest-diameter **tracheids** are near the outside the xylem and at the ends of the flat steles (i.e. exarch, just as in Lycopodium).

5. A band of **phloem** and **parenchyma** surrounds the **xylem** within the cavity.

6. **Leaf traces** can be seen in some sections. They are either in the **cortex** (isolated, red-staining tracheid groups) or near the ends of the flat steles in the vascular cylinder.

*S3: Make one or more sketches using the various slides and your fresh section, sketch a *Selaginella* stem interior. Be sure to sketch the stele with cellular detail, and label the features mentioned above - not all are in a single section. Be sure to examine more than one slide!

D. The Strobilus in a Longitudinal Section

Get yourself a slide of the *Selaginella* **strobilus** longitudinal section. These sections are not all medial (cut through the center of the stem); make sure your slide has at least some vascular tissue showing in the strobilus axis.

1. The most prominent feature of these strobili is the two kinds of sporangia, each with a different size of spores. Some have a lot of spores that are about the same size as those you saw in *Lycopodium*. Others have a very small number of enormous spores, which are also trilete. These two spore types are called **microspores** and **megaspores** respectively; they differ in their size and in the structure and sexuality of the gametophytes that develop from them.

2. Notice that the **sporangium wall** is similar to that of *Lycopodium*. In younger sporangia, you can once again see three different layers: two **jacket layers**, and the **tapetum**. The innermost wall is once again the tapetum. The cells of the tapetum gradually break down to provide the material for the spore coat, just as in the clubmosses.

3. The **ligule** is typical of this order (and all other heterosporous lycophytes) is clearly visible in many of the slides of the strobili. It lies just distal to the sporangium on the adaxial surface of a leaf. Notice that there is an expanded base and a thin tip, each with different shapes and sizes of

cells (Figure 5). Ligules are precocious; they develop faster than their accompanying organs during early development - however they stop growing long before leaf and sporangium. You can see that the ligules develop to most of their mature size before the leaves do by looking at the tip of the strobilus. Consensus has not yet been reached on the function of the ligule in this lineage, but it is interesting that heterospory and the ligule are consistently found together. What are your thoughts?

4. Finally, you may again be able to see the **stele** suspended in its cavity in this longitudinal section. From this, you can gain good perspective on the three-dimensional layout of the stele.

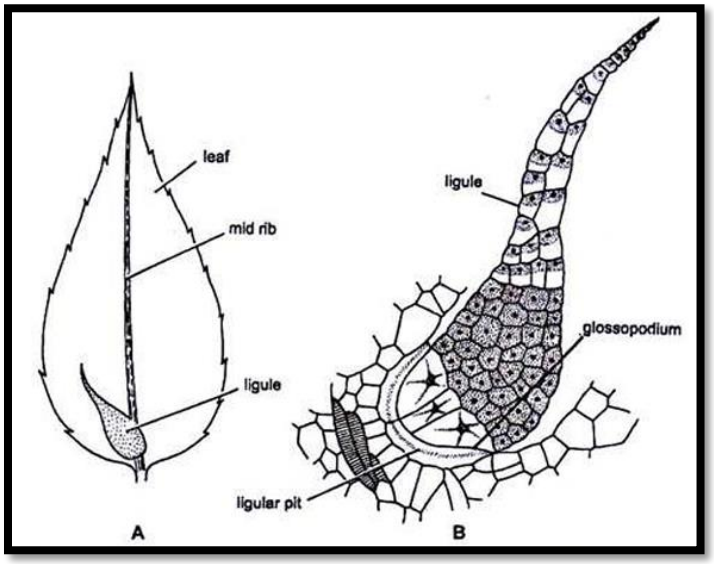

Figure 5. Diagram of a *Selaginella* ligule on a microphyll (A) and cellular detail of the the ligule in cross-section (B). Note the specialized cells which form the ligular pit, and glossopodium.

*S4: Sketch a strobilus in longitudinal section with at least one micro- and one megasporangium. Draw and label with the features described above.

II. The bipolar Lycophytes: Isoëtales

The Isoëtales (quillworts) include plants of streams, ponds, lakeshores, and sometimes bogs. They are alike in general life form – their parts above ground parts growing away from the below ground parts on opposite poles of the plant hence the term bipolar is often used to describe them. These this extant lineage of plants are bipolar like the extinct lineage of scale trees, known as Lepidodendron, but their stem axis does not acquire any height and Lepidodendron were as tall as modern day trees (Figure 6). However, this large growth form is no longer represented in the extant lineages of Isoetales and the living plants all resemble a squat little bulbs covered with a crown of leaves that sits in the water or mud.

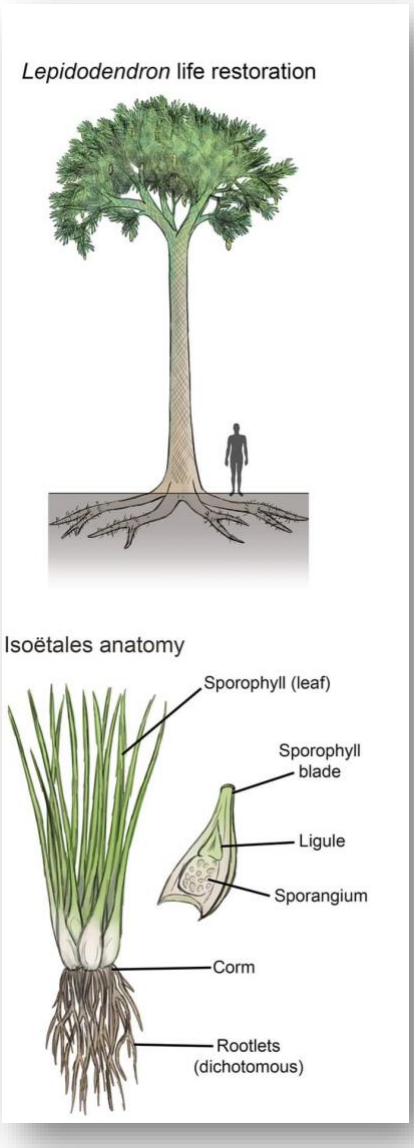

Figure 6. Comparison of the bipolar growth forms of the extinct *Lepidodendron* and extant members of the Isoetales.

A. General Features of the Sporophyte

Look at the pressed plants in lab. There is a squat corm (= stem without length) with microphylls attached above and roots attached below. All of the microphylls have sporangia; all but a few have spores.

*S5: Make a quick sketch of the overall life form. Include a detailed cross-section and a longitudinal-section of the microphyll in your sketch.

B. Stem Interior Features

Look at a longitudinal section of *Isoëtes* under the microscope. These sections are confusing, so just getting a simple grip on basic features will have to do.

1. Look for a region with few cells and lots of space in the center of the stem. This is the original vascular cylinder, comprising the mixed parenchyma and tracheids of the stele. The tracheids have been torn apart by the further growth of the plant.

2. Around the loosely-packed cells of the plant's center are several layers of squat cells in rows; this is the area of the lateral meristem (a vascular cambium). The lateral meristem is more or less cup-shaped, but in these sections it appears as a whole ellipse. This meristem gives rise to increased girth of the corm and to the roots that you should be able to see down at the bottom of the plant. The portion giving rise to the roots is often called the basal meristem.

3. You should also be able to see leaf traces up towards the top of the plant, but these are not visibly arising from the vascular cylinder. In fact, they arise from an apical meristem, which is not visible in these slides.

C. Roots

Look briefly at the prepared slide of the *Isoëtes* root cross section. Numerous adventitious roots are developed from the basal part of the stele (Figure 7). The roots are dichotomously branched, bearing numerous root hairs. On the slide, you should see a large central cavity and a single vascular bundle, with just a little xylem and phloem, is attached to the inner margin of cortex on one side of this cavity. This root anatomy is remarkably similar to the Stigmarian rootlets of *Lepidodendon*, an extinct relative of living quillworts, which we will look at in detail next week.

*S6: Sketch the longitudinal section of *Isoëtes* AND a close up of the cross-section of a root from the prepared slides. Label the features described in B. and C. above.

Figure 6. Numerous adventitious roots extending from a basal meristem on the corm of *Isoetes*.

D. Reproductive Features

Isoëtes is heterosporous, like all of the Lycophytes except the clubmosses. Quillworts are technically bisporangiate: each corm produces both microspores and megaspores (in different sporangia, as usual). But there is no specialized strobilus - in a way the *whole plant* is a strobilus sitting on a rhizophore.

1. Look at the herbarium specimens again. You should see that on at least some of them there are sporangia with spores visible at the bases of the outermost leaves. These leaves, formed first in a season, have megaspores. In fact, all of the leaves have sporangia.

2. Now look at a prepared slide of the *Isoëtes* sporophyll (spore bearing leaf). There are five basic features to note the following: a megasporangium with four megaspores, a microsporangium with many tiny microspores, partial interior partitions called trabeculae, a ligule just distal to the sporangium, a velum, which is a flap of leaf tissue covering the distal part of the sporangium. Look closely at a sporangium with microspores: notice that they are monolete, not trilete: these are the only monolete spores in the division Lycophyta. In fact, *Isoëtes* has trilete megaspores and monolete microspores: it's the only plant in which the two are shaped differently!

*S7: Sketch of the proximal portion of the leaf from the prepared slide. Label the features described above. You can do this sketch from either the stem *l.s.* or the sporophyll *l.s.*