VI. Polypodiidae, the Leptosporangiate Ferns

Now that you have gotten a feeling for the eusporangiate ferns, it's time to focus on the leptosporangiate ferns, subclass Polypodiidae. Polypodiids, the largest and most diverse clade of ferns, includes about 10,000 species in seven orders, the largest and most diverse of which is Polypodiales. The group has a fossil record going back at least to the Permian period, but its living representatives offer ample evidence that present-day speciation is continually yielding new evolutionary entities.

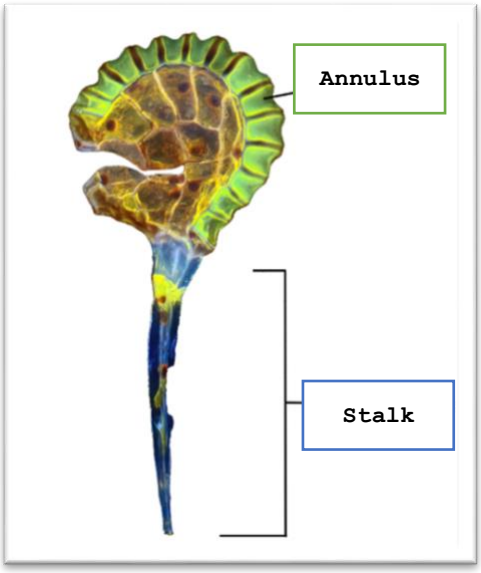


Figure 1. The leptosporangium, a syn-apomorphy for subclass Polypodiidae.

FEATURES SHARED BY THE MARATTIALES AND THE LEPTOSPORANGIATE FERNS (POLYPODIIDS):

megaphylls, circinate vernation, sporangia abaxial on megaphyll (or a transformation of this feature), no secondary growth, spores dispersal, homospory

FEATURES UNIQUE TO THE LEPTOSPORANGIATE FERNS:

leptosporangia (tapetum plus one wall layer, versus two wall layers) with few spores per sporangium, stalk long and slender, antheridia few celled, protruding (not sunken in the tissues of the gametophyte).

A. Sporophyte phase (Part 1): Vegetative Features of the Polypodiids

You are nearly surrounded by a diversity of megaphyllous, non-woody, leptosporangiate plants in the subclass Polypodiidae. Look at the leaf design of the various types to appreciate their variety

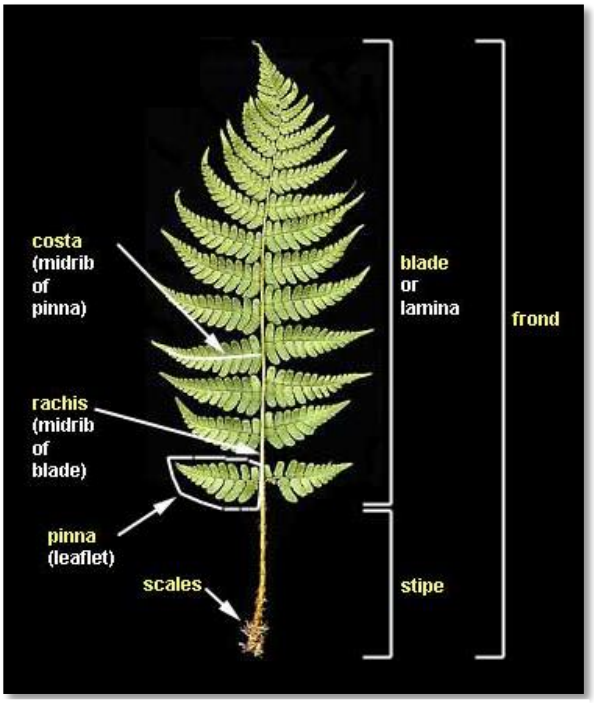


Figure 2. Diagram of *Dryopteris marginalis* leaf morphology,

1. *Phlebodium aureum*

Use this plant to focus on basic leaf design and the boundary between stem and leaf.

a. The stem of this plant is covered with papery, tan scales, which are thought to have a role in the retention of water around the stem axis. This creeping stem, called a rhizome, gives rise to leaves and adventitious roots as it grows.

b. The leaves have green stalks called petioles or stipes. The petioles may at first appear like stems, however their vasculature is bilaterally symmetrical, like leaves, not radially symmetrical, like stems.

c. The leaf blade itself, called a lamina, is deeply dissected in this species; we speak of this leaf design in *Phlebodium* as once-pinnate pinnatifid. Note that the venation of the lamina is anastomosing: the veins diverge and then come together again in a net-like pattern.

2. Compare Polypodiid leaf diversity

Now compare *Phlebodium* with several other ferns in the lab to gain an idea of the diversity of leaf design within the limits apparently imposed on ferns.

a. *Cyrtomium*: once pinnate, the pinnae or leaflets are broad and their veins anastomosing like *Phlebodium*, not free veins like *Pteris*.

b. *Pteris*: once-pinnate pinnatisect (note the pinnae are completely separated from the midrib of the leaf only at the base); basal segments of the pinnae strongly developed, resembling the rest of the pinna

c. *Microsorum*: leaves simple (undivided), with an entire margin.

3. Asexual vegetative propagation

Many ferns have specialized means of producing new plants asexually, that is without meiosis and syngamy. Look at three examples in the lab:

a. *Asplenium*: proliferous buds give rise to plantlets on the leaf axes.

b. *Tectaria*: also has proliferous buds on its leaves.

c. *Nephrolepis*: stolons, thin leafless stems that grow above ground, extend away from the parent plant to establish new sporophytes as 'offshoots'.

*S1. Sketch one leaf from any two genera listed above. Label the terms outlined above present on your plants and any other plant parts you recognize. Indicate the dissection and venation type as well.

4. Stem and stele anatomy

Now look at prepared slides of *Polypodium* rhizome transverse sections, in order to see cell detail in one of these stems. Focus on a single vascular bundle in the stem at medium or high power.

a. Identify the tracheids: angular cells that have red staining, lignin rich cell walls.

b. Surrounding this xylem is a complete ring of phloem: these bundles are amphiphloic.

c. An endodermis is clearly visible around each vascular bundle.

d. Locate the leaf gaps and leaf traces at a lower magnification.

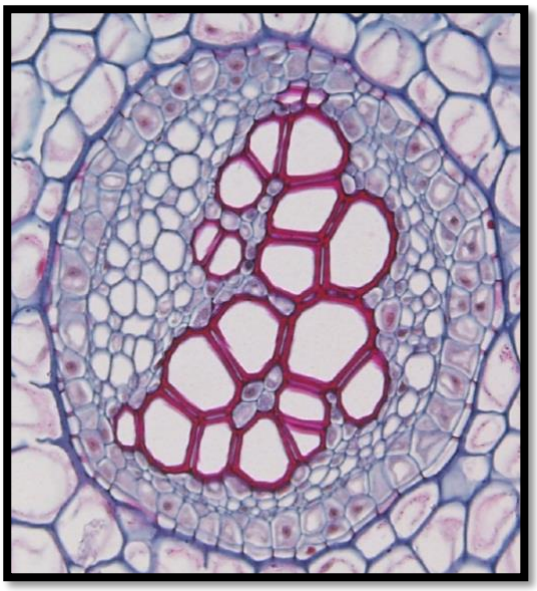


Figure 3. A single *Polypodium* vascular bundle. Notice the blue stained phloem surrounding the red stained xylem (i.e. amphiphloic).

*S2. Sketch the *Polypodium* stem in transverse section. Label terms listed above leaf traces. Include a close-up of a single vascular bundle.

B. Sporophyte Phase (Part 2): Reproductive Features of the Polypodiids

Almost all of the Polypodiids have sporangia abaxial on the megaphylls and organized into small-to-large groups called sori (singular sorus). The sori vary in shape and position, and the sporangia vary in structure. These sorts of variation have been traditionally used to distinguish the major groups within the Polypodiids.

1. *Cyrtomium falcatum* in focus.

We will begin by studying the **sorus** and **sporangia** of one species, *Cyrtomium falcatum* (the holly fern), carefully. Then we will go on to compare its morphology to other ferns in the Polypodiids. Look closely at a fertile leaf of one of the *Cyrtomium* plants in the lab. The sori are **abaxial** in several rows on the undersurface of the leaf and protected, at least when young, by a tiny umbrella-shaped outgrowth of the leaf epidermis, called an **indusium**. The indusium can shrivel up when totally mature. Look for both mature and immature sori to compare the morphology of the indusium.

2. Sections of the *Cyrtomium* leaf with sori.

Be sure to choose a slide that shows a good medial section through the stalk of the indusium.

a. Identify the upper and lower leaf **epidermis** and the **spongy** and **palisade mesophyll** (typical of most leaves). The palisade layer of thin, close-packed cells is adaxial in the leaf; the spongy layer - of **isodiametric**, loosely packed cells - is abaxial in the leaf. **Stomates** should be visible leading from the spaces among the spongy layer cells out to the leaf exterior.

b. The **sori** are on the abaxial side of the leaf, as you can see from their position relative to the spongy layer.

c. Note that the **indusium** has a **stalk** (a supporting axis like the handle of an umbrella) and a **flange** (that is the protective part comparable to the umbrella's canopy). Both the indusium and sporangia are attached to a little mound of tissue containing **tracheids,** called the **receptacle**.

d. The **sporangia** themselves are of different ages. Some are large and old and contain **mature spores**.

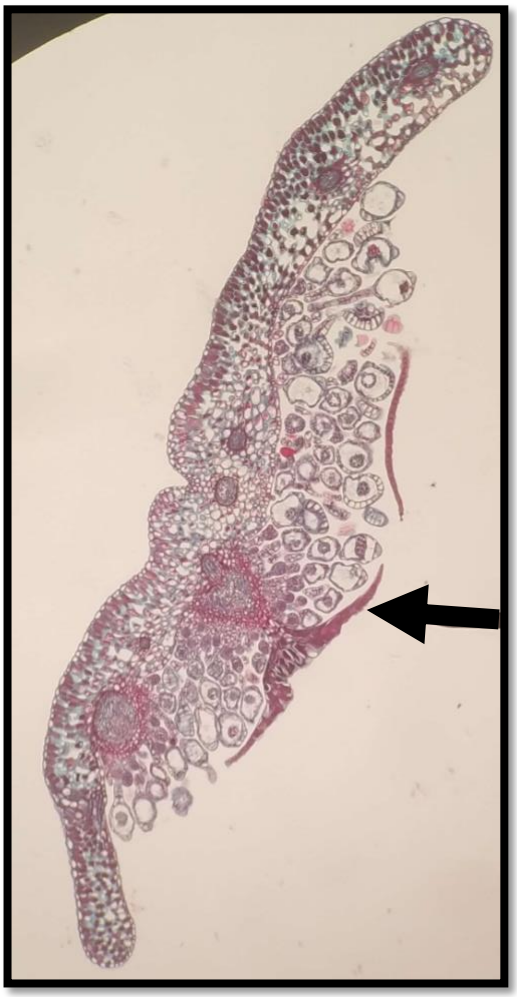


Figure 4. Transverse section of a *Polystichum* leaf and sorus. Black arrow pointing to indusium with developing sporangia underneath.

e. Others are small and hardly developed with only the developing **sporocytes** inside. The mature sporangium has prominent red-staining cells in a single row that make up what's called an **annulus**, which responds to drying by forcing the sporangium open, then snapping it shut, to disperse the spores. In the genus *Cyrtomium*, the mature sporangia contain **monolete** spores.

f. Remember this is a leptosporangiate fern, which are characterized by a sporangium with a stalk and a **sporangium wall** that is one cell thick, not counting the **tapetum**. Unlike eusporangiate ferns, the sporangium also has a small number of spores per sporagnium, and develops from a single initial, not from a large group of cells on the epidermis.

*S3. Sketch a leaf in transverse section that includes a sorus. (The sorus itself is in longitudinal section.) Label the terms indicated above. Sketch the leaf section at a lower magnification and then a close-up of the sorus at a higher magnification to highlight its features.

3. The submarginal sorus and false indusium of *Pteris* and *Pellaea*

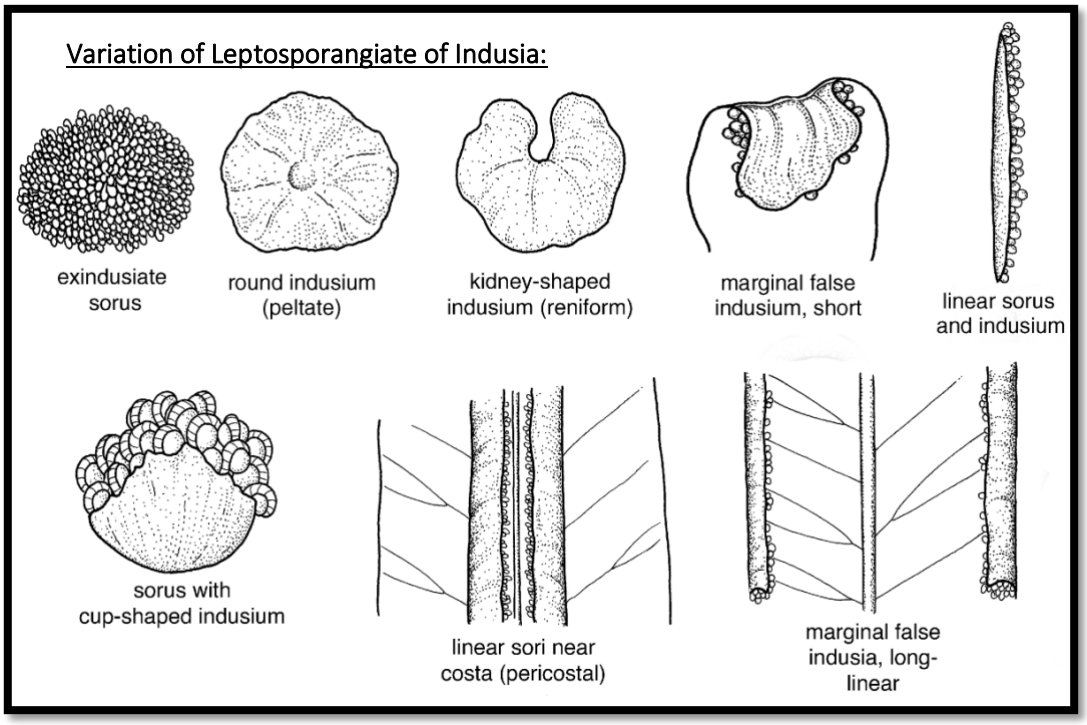
Pteris, another greenhouse fern, has an entirely different sorus from *Cyrtomium*, though the structure of the sporangium is virtually the same.

a. Look at the living plants of *Pteris* in the lab. Notice that the **sorus**, though **abaxial**, is near the margin (**submarginal**), and that the only protection for the sorus is the incurved **leaf margin** called a **false indusium**. Use a dissecting scope to determine where the sporangia are attached. Is it the abaxial surface of the leaf or on the false indusium itself?

*S4. Sketch the abaxial side of one or several pinnae of *Pteris*, highlighting the features of the false indusia. In your sketch indicate where the sporangia are attached in detail.

b. Look at prepared slides of *Pellaea*, a genus with sori very much like those you just observed in *Pteris*. You can see that there is a **false indusium**; it's only an extension of the **leaf margin**. Find the **sporangial wall** and the **tapetum**. Notice the **trilete spores**.

*S5. Sketch the *Pteris* leaf with sorus in transverse section from the prepared slide. Label the section with the features described above.



C. Gametophyte phase: Polypodiidae

Most leptosporangiate ferns have a surface-dwelling, thin, green gametophyte with a meristem located in an apical notch. According to the classical (but not necessarily common) story of gametophyte development, the antheridia are produced among the rhizoids soon after spore germination, and the archegonia are produced near the notch later in development.

1. To the Greenhouse!

Go to the greenhouse with a small petri dish and a pair of forceps and find a living fern gametophyte. Upon your return to the lab:

i. Mount it in water, with the rhizoids up on a slide and drop a coverslip on top.

i. Add a little more water to the side of the coverslip from a dropping bottle if there is air under it.

ii. Look under the dissecting scope and at low power of the compound microscope.

2. General gametophyte form.

First, use the dissecting scope to view the macroscopic features of these multicellular haploid organisms.

a. One end of the gametophyte is covered by the hair-like rhizoids, which are water absorbing cells. Why aren't they roots?

b. These are older gametophytes, so they have a well-organized notch meristem at the end way from the rhizoids.

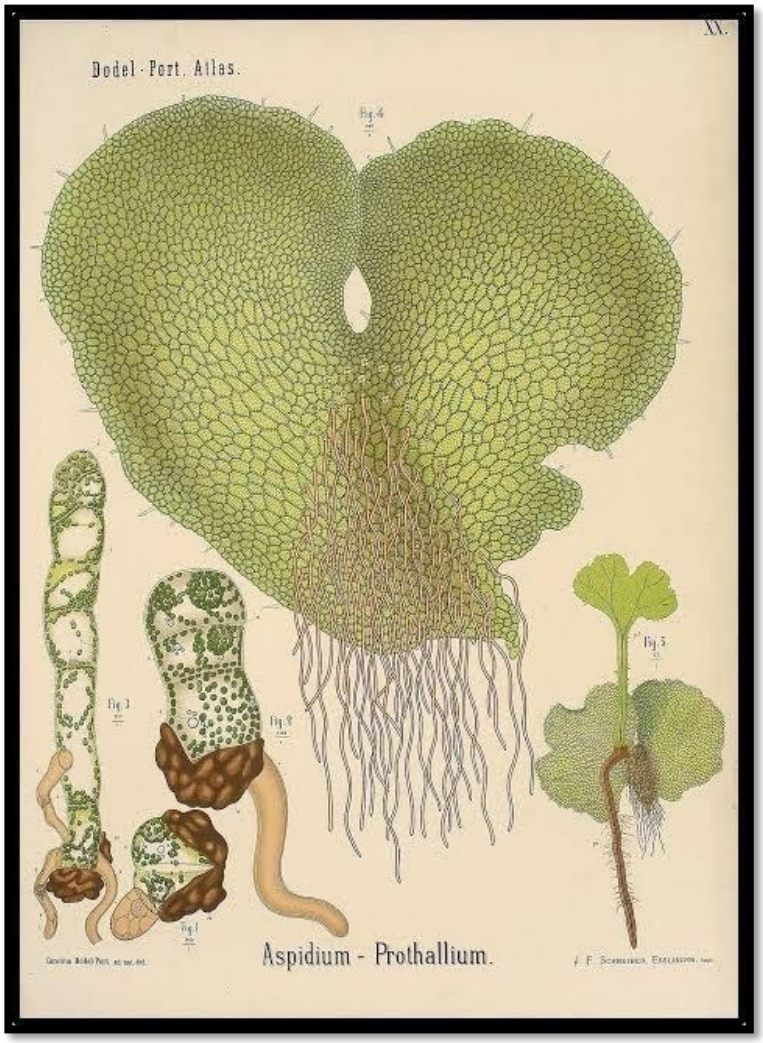


Figure 6. Fern gametophyte development from spore germination to sporophyte formation.

c. Look for the single, wedge-shaped apical initial cell sitting right in the deepest part of the notch. The gametophyte body is flat and very thin. Between notch and rhizoids is a thicker central region called the cushion and to either side of the cushion are thinner regions called the wings.

3. Gametophyte at lower power.

Next, using the compound scope at low to medium magnification, take a closer look at your gametophyte slide.

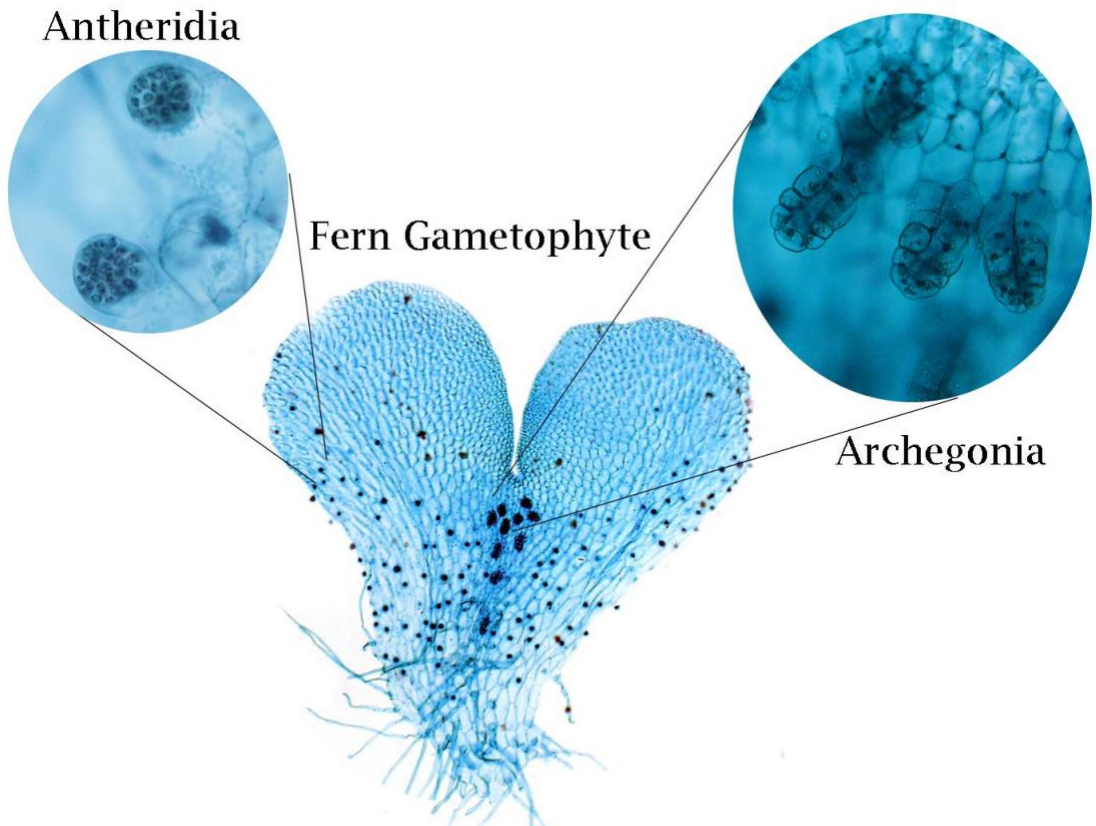
a. You may see little swimming things which are flagellated sperm cells, called **spermatozoans**. See if you can locate their source in a small, spherical **antheridium** near the base of the rhizoids.
b. If some of the sperm have not yet begun to swim, try to focus in on them under high power to see that they are **helical** and **multiflagellate**. If you don't see antheridia and sperm in your slide, look at someone else's.
c. Now search the cushion region near the notch meristem for **archegonia**. They look like tiny fingers sticking up from the cushion. The **neck canal cells** join to form a tube that the sperm cells swim down to fertilize a single egg cell.

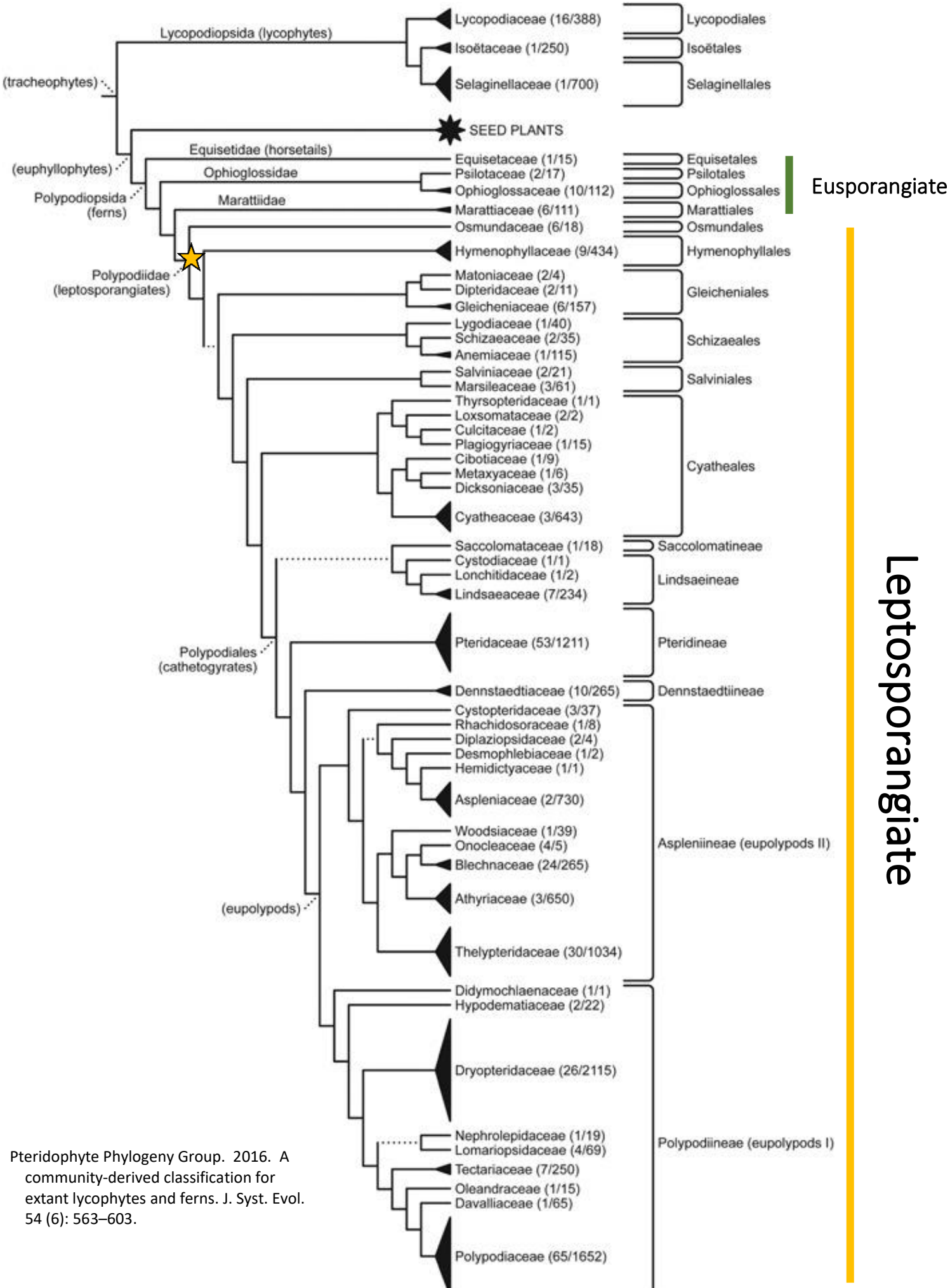
4. Prepared slides of *Adiantum* gametophytes.

In these slides, you can see the structure of the antheridia and archegonia in much more detail.

a. Young antheridia, composed of only a few cells, and **mature antheridia**, full of **spermatozoans**, should be visible. Notice that these antheridia are standing out from the surface of the gametophyte, not sunken in the cushion a characteristic of only the Polypodiids among the ferns.
b. The archegonia have a neck protruding below the surface of the gametophyte and a dark staining **egg cell** hidden beneath in the cells of the cushion. The younger archegonia have intact **neck cells**; in the **mature archegonia**, the neck cells have disintegrated to open a canal into the egg cell for the spermatozoans.

*S6. Sketch a gametophyte in detail using both your living gametophyte preparation and the pre-made slide of the *Adiantum* gametophytes. Label the entire gametophyte and all its features highlighted above. Sketch in detail one antheridium and one archegonium using the prepared slides of *Adiantum* gametophytes.





Pteridophyte Phylogeny Group. 2016. A community-derived classification for extant lycophytes and ferns. J. Syst. Evol. 54 (6): 563–603.