VII. Polypodiidae: Salviniales / Ophioglossidae: Psilotales and Ophioglossales

A. Polypodiidae: *Salvinia*, *Azolla*, and *Marsilea*, the Heterosporous Leptosporangiate Ferns

Within the Polypodiidae is an order of aquatic, heterosporous ferns - the Salviniales. Included within the Salviniales are two families, the Salviniaceae and the Marsiliaceae. These families together form a distinctive clade within the ferns (see again the PPG phylogeny, Lab 6, last page). Though these families include very few species altogether, they are excellent material to study in vascular plant morphology, because they present a series of highly derived characteristics that we assume to be homologous with features of leaf and stem in the Polypodiids. What's more, the features are fun to watch in operation!

1. Salviniaceae: *Salvinia*

Node design and components in *Salvinia*

a. Look at the living *Salvinia* plants in the lab. The sun-gathering leaves are unusually good at repelling water, which you can prove by dribbling water onto the leaves in the dish.

b. Once you're done with the dribble experiment, take a few nodes back to your workplace and investigate the features of *Salvinia's* node.

   i. First, note the two simple sun-gathering leaves at each node. Remove a leaf and look at it under the dissecting scope (or put the whole shoot under the scope) and look at the distinctive hairs. These hairs look like eggbeaters: if you put some water on the leaves under the 'scope, the role of the hairs in water repelling becomes clear.

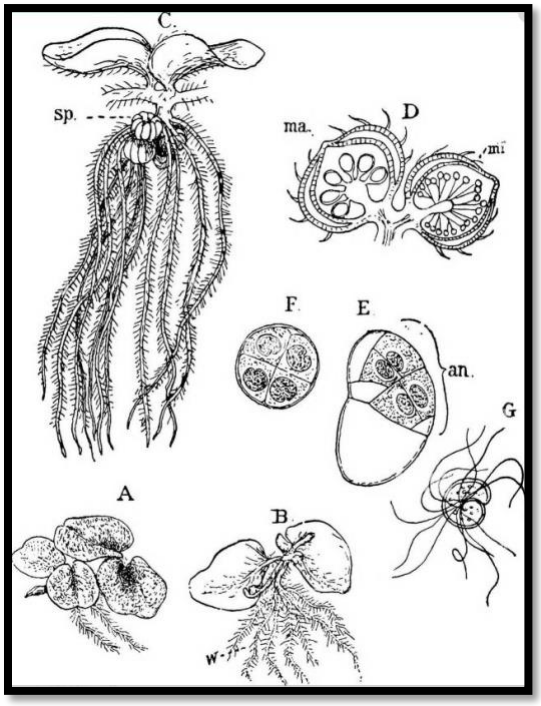

Figure 1. *Salvinia natans*. A, Small plant, X2, seen from above; B, a similar one from below; w, root-like submerged leaf; C, fragment of a fruiting plant, X2; sp, sporocarps; D, a megasporangial {ma) and microsporangial (mt) sporocarp in longitudinal section (slightly magnified); E, male prothallium with the single antheridium {an) from the side; F, a similar one seen from above; G. spermatozoids

   ii. Second, note that there is a third structure at each node. You should be able to guess that it is involved in absorption, but the favored interpretation is that it's an absorptive leaf, not a root. Underwater leaves are often highly dissected like this one, and this structure shares bilateral symmetry and attachment at the node with *Salvinia's* more traditional leaves. There are no true roots in *Salvinia*.

*S1. Sketch a whole plant of *Salvinia*. Pay special attention to the shoot design and organization of its parts at each node. Draw a detailed close up of a single leaf hair as well.

2. Salviniaceae: *Azolla*

Shoot Layout of *Azolla*

a. Take a bit of <u>stem</u> axes from the supply of material and make a simple dissection to understand branching, leaf layout, and features of the node. First, figure out what is branch and what is <u>leaf</u>. Look for the alternating leaves in two rows and the branch points in the axis.

b. Now look at the layout and design of the leaves. Probe until you demonstrate to yourself that each leaf does have <u>two lobes</u>, the upper one of which is larger.

c. Now look for the attachment location of the <u>roots</u>. They should be arising from the same place as a leaf, which is at a node. Branches also arise from nodes: check to see if you can identify the leaf associated with a branch somewhere on your axis.

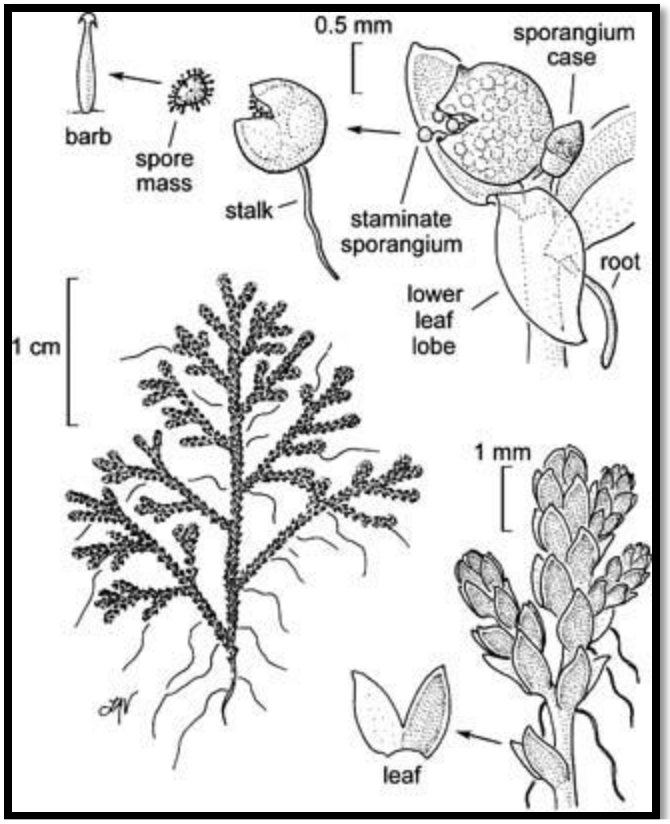

Figure 2. *Azolla filiculoides* demonstrating the tiny scale and characteristic features the Azollaceae.

d. Mount a bit of your shoot system on a microscope slide and chop at it a bit with a razor so that the leaves are split open into small shreds. Now add a drop of water or two and a coverslip, and look at your preparation under the microscope. You are looking for small colonies of the <u>cyanobacterium</u> *Anabaena* - they look like greyish-green strings of beads. Remember, these are the prokaryotes that provide fixed nitrogen to *Azolla*. In parts of Asia, *Azolla* is cultivated in rice paddies for green manure.

*S2. Sketch a whole plant of *Azolla* and its symbiont cyanobacterium *Anabaena*. Label the terms highlighted above.

3. Marsileaceae: *Marsilea*

Are those four leaf clovers? Look more closely… what vegetative features can you observe that will help you to determine if they are ferns or flowering plants? These living plants of *Marsilea* from the greenhouse, are heterosporous water ferns. They may resemble four leaf clovers, but they are in fact ferns. A simple rhizome creeps around in the pot, giving rise to roots and leaves with fiddleheads!

a. Whole Plants

i. The leaves of *Marsilea* are made up of a **petiole** and <u>four pinnae</u> clustered at the leaf tip. Notice that the leaf venation is <u>free</u>, not net-like, as you would expect to see in a flowering plant such as clover. Another clue that you are in fact looking

at a fern is that it has **circinate vernation**, just as in the Polypodiales.

ii. Remove a bit of **stem** from the covered dish of **rhizomes**. Make your own freehand transverse section of the stem. Stain with phloroglucinol. Under the 'scope, look for a ring of **cortical cavities**, an inner cortical **sclerenchyma ring**, and a **central vascular cylinder**. The vascular cylinder is a **siphonostele** with sclerenchyma instead of parenchyma in the **pith**.

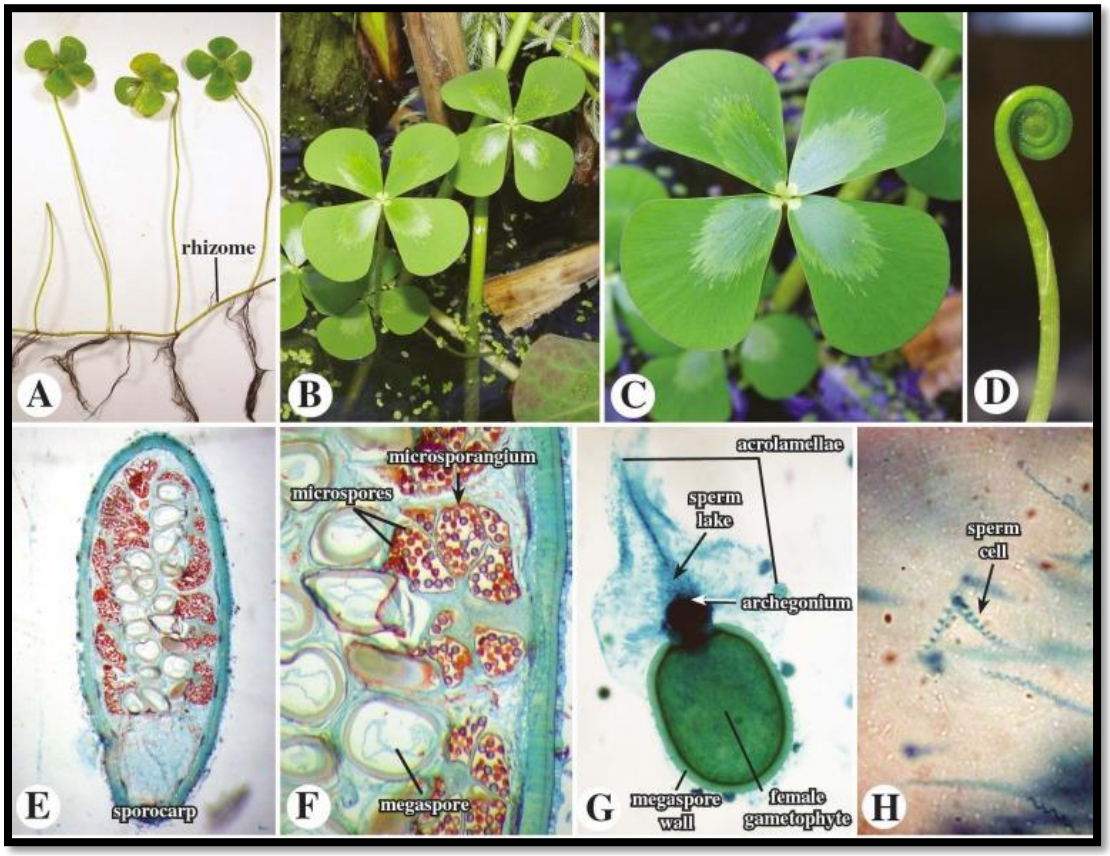

Figure 3. Vegetative and reproductive features of *Marsilea*. A. long-creeping rhizome, B. aquatic to emergent habitat, C. four pinnae, D. circinate vernation of growing leaf, E. a sporocarp in transverse section, F. detail of the mega- and microsporangia and mega- and microspores, G. aquatic gametophytes with their gametangia and gametes, H. single sperm cell.

b. Sporocarps

In the lab this week, your teaching fellow has set up a dish with a sporocarp of *Marsilea* for you to see dehisce and develop.

i. These **sporocarps**, traditionally interpreted as fused pinnae, are highly drought-resistant. In fact, we have to file a notch in the sporocarp wall in order to get them to germinate. The actual process you're seeing is the extrusion of the group of **sori**, usually about 10 to 15 minutes, out of the sporocarp wall

ii. Note the dark, hardened sporocarp wall and the veins protruding from within, especially later in opening.

iii. Long, thin ivory-colored sori are evident, first tightly clustered and later stretched out on a gelatinous axis called a **sorophore**. Look closely at these sori - along one side are large **megasporangia**, each with a single small bump protruding from the sporangium. The entire rest of the sorus is much smaller **microsporangia**. The whole group of sporangia in the sorus is surrounded by a transparent **indusium**.

*S3. Sketch a whole plant of *Marsilea* and one sporocarp in detail. Label the terms highlighted above.

*When you come to lab next week, look at the mature female gametophyte and embryonic sporophyte that have developed.

i. Mount one of the gametophytes on a microscope slide and stain it with a bit of Toluidine Blue stain to see the sperm lake.

ii. the gelatinous funnel through which the sperm swim to the archegonium - and the helical, multiflagellate sperm trapped in the gelatin.

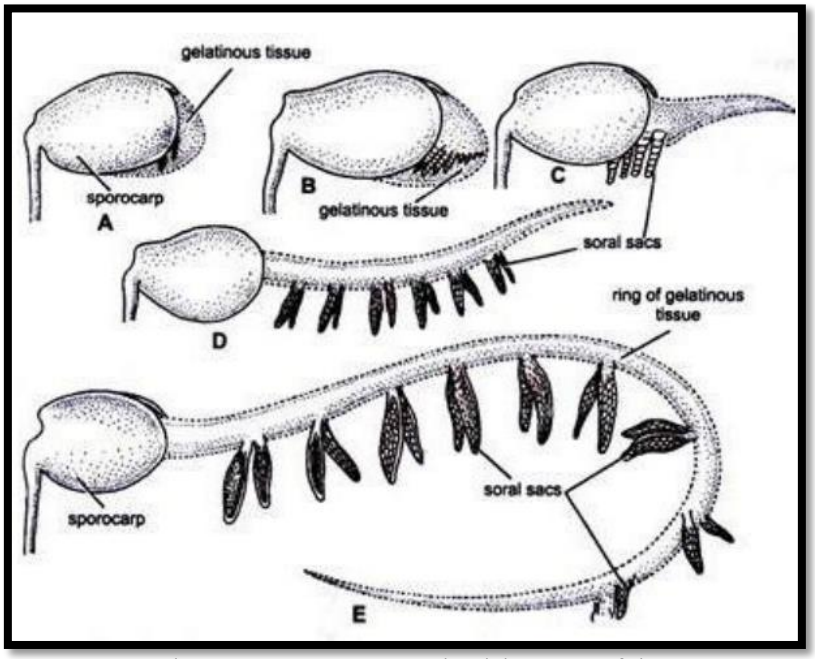

Figure 4. *Marsilea.* Successive stages in the dehiscence of the sporocarp.

B. Ophioglossidae: Psilotales: Psilotaceae

Psilotum and *Tmesipteris*, which together comprise the Psilotaceae, have been the subject of endless arguments among botanists. The problem is that they present a peculiar combination of primitive and derived features - and the derived features do not argue convincingly for their inclusion in any one division or order. The usual solution is to avoid the problem by putting them in their own division and order, however recent molecular evidence suggests that they are part of the monilophyte clade, together with the ferns and the horsetails. See if you think they belong there. The question is, are they simple plants reduced from more complex plants like ferns, or are they primitively simple plants? We consider them at the very end of our study of the ferns, because you need to know about both the ferns and the primitive plants to understand the problem that the Psilotaceae presents.

1. General Features of *Psilotum* Sporophytes: Living Plants

a. Note the pattern of branching and the green, ridged, **aerial axes** fork repeatedly to form a shrubby life form. Look for occasional **trichotomies**.

b. Minute **microphylls** (technically just projections because they are not fully vascularized) areattached in a few places along the aerial axes.

c. Synangia (singular **synangium**) - sets of sporangia evolutionarily fused together – are apparently attached laterally, in the axils of a pair of **projections**, toward the apices of the

aerial axes. Notice that these synangia are three lobed, and that they dehisce along three lines radiating from the center of the synangium. The popular idea is that this synangium is the evolutionary product of the fusion of three sporangia along their sides.

d. Remove a single synangium and dissect it on a slide. Decide how many chambers there are inside the synangium. Remove a few spores and look at them under the microscope to determine whether they are monolete or trilete.

e. In an herbarium specimen of *Psilotum*, note the difference between the aerial axes and the underground axes - the underground axes have no microphylls, no roots, and they are not green even when alive.

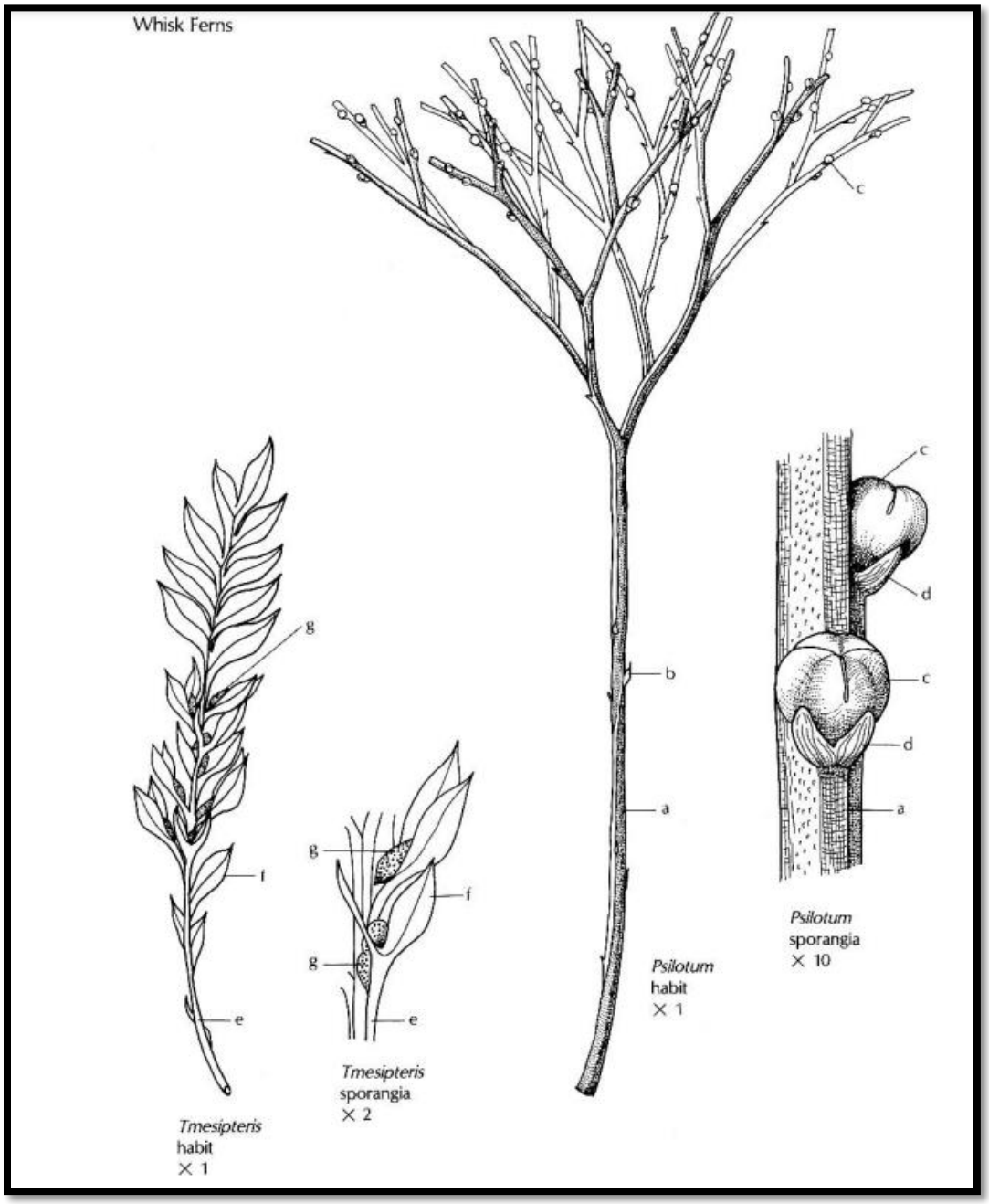

Figure 5. *Psilotum* and *Tmesipteris* morphology comparison. *Psilotum*: a. areal axis, b. stem microphyll, c. synagium, d. paired synangia microphylls;, *Tmesipteris*: e. stem, f. large flat microphyll, g. bilocular synangium.

2. Transverse Sections of Creeping and Aerial Axes of *Psilotum*: Prepared Slides

a. The surface of the creeping axis has old, broken-off rhizoids. Just to the interior, in the cortex, there are cells containing **mycorrhizal fungi**.
   i. In the interior look for an endodermis with a casparian strip.
   ii. Note that there are one or two small xylem masses surrounded by a single phloem cylinder (why are there sometimes two?)
   iii. These steles are simple **protosteles**: at their core is a solid mass of xylem.

b. Now look at sections of the aerial axes. These differ in having:
   i. a lignified epidermis with **sunken stomates**,
   ii. a spongy cortex layer that functions in gas exchange,
   iii. a zone of sclerenchyma in the cortex,
   iv. a **siphonostele** with a pith of sclerenchyma (not parenchyma, as is common in siphonosteles in other groups). Look for exarch protoxylem in these aerial axes.

3. Longitudinal Sections of the Synangium

Now that you've looked at the axes in transverse section, you can look at a longitudinal section of an aerial stem and attempt to conceptualize the stem in three dimensions. This slide also introduces you to sporangia and spore production. Look at the stem first: identify the parts from outside in as usual.

a. Review stem anatomy from your first lab:
   At the very outside is an epidermis; stomates should still be visible in the surface layer. Next, you should be able to recognize the spongy layer of the cortex from what you saw of it in the transverse section. There is a sort of internal atmosphere inside the stems of these plants, equivalent to the space in the spongy mesophyll of leaves in angiosperms and ferns. The xylem, which appears central and solid in many of these sections, may be made up of annular, helical, or scalariform tracheids. Small elongate phloem cells lie outside the xylem.

b. Now look at the **synangium**, which is the big, apparently two-parted organ on a short lateral branch. In some slides you can see that the synangium is attached to a short stem, and the unvascularized **microphylls** are attached to the stem as well.
   i. The synangium is supplied with vascular tissue that is connected to the vasculature of the stem. Look for **tracheids** right in the synangium base itself (visible only in median sections).

   ii. Two of the three chambers of the synangium are visible. The wall resembles that of the lycophyte sporangium, an outer layer of large **sporangial wall cells** and two inner layers of smaller **jacket cells**. Remember that this sort of sporangium with a several-layered wall, which is very common in vascular plants, is called a **eusporangium**.

   iii. Look at the **spores** themselves. They are monolete, just as you saw before in your dissection.

4. Gametophyte of *Psilotum*

Finally, look at the gametophyte of *Psilotum* on display. The gametophyte is especially interesting in that it is most like a sporophyte axis of any gametophyte in this course. People fond of the idea that primitive vascular plants had sporophytes and gametophytes that look similar think of *Psilotum* as an example of a primitive life cycle. The important visible features are:

1. axial life form and forking
2. rhizoids
3. superficial antheridia

*S4. Sketch a whole *Psilotum* plant. Label the features highlighted above. In your sketch include the following: 1) the whole plant from living specimen or herbarium specimens, 2) areal axes transverse section, 3) longitudinal section of the axis and synangium.

5. General Features of *Tmesipteris* Sporophytes: Pickled Plants

Vegetatively, *Tmesipteris* is quite different from *Psilotum*, but reproductively they are very similar. Keep comparing and contrasting the two in your mind as you work.

a. Note the strikingly different **microphylls**. In *Tmesipteris* they are large and flat - and they are fully vascularized by a **single leaf trace**.

b. Notice that the **bilocular synangia**, the shape is different but is morphologically equivalent to *Psilotum* synangia. The synangia are located on a **short lateral branch** just above a pair of **microphylls**.

*S5. Sketch a whole *Tmesipteris* plant. Label the features highlighted above .

C. Ophioglossidae: Ophioglossales: Ophioglossaceae

The order Ophioglossales has one family and 3-10 genera. In lab today we will look at two genera found in Vermont, *Ophioglossum* and *Botrychium*. The third genus, *Helminthostachys*, is only found in the Old World tropics. Start by looking at the herbarium specimens provided to get a general sense their morphology and then the prepared slides provided for each genus.

1. General Features of *Botrychium*

a. Herbarium specimens
   i. Take a look at the herbarium specimens to identify the single leaf or frond, which is divided into two parts: a **vegetative segment**, called a “trophophore”, and a non-green **reproductive segment**, sometimes called a “sporophore” that carries numerous **small, globular, sporangia**.

b. Prepared slide of stem cross section
   i. You are seeing an **ectophloic siphonostele,** a type of stele where phloem is located only on the outside of the xylem. The phloem cells will be conspicuously thickened.

ii. Note the lines of radial cells - you are seeing wood. The **unifacial vascular cambium** produces this **secondary xylem**. *Botrychium* is the only living fern to produce secondary xylem.

c. General Features of Botrychium Gametophyte: Prepared Slides
   i. You should be able to see **antheridia** embedded in an outgrowth of tissue called the **dorsal ridge**. Look closely along each side of the dorsal ridge to spot the **archegonia**. In addition, you should be able to recognize **rhizoids** on the gametophyte.
   ii. Remember *Botrychium* and *Psilotum* share the character of subterranean gametophytes.

2. General Features of *Ophioglossum*

a. Herbarium specimens
   i. Take a look at the herbarium specimens of this new genus and identify the same vegetative and reproductive morphology present in the *Botrychium*.

b. Prepared slide of stem cross section
   i. Look for the two rows of **embedded sporangia**. Note the **main axis** and vascular system, how is it different from in *Botrychium*?
   ii. How many cell walls do you count around each sporangium? Remember the Ophioglossaceae is eusporangiate. Identify the each layer: a single **sporangial wall** cells, and two layers of **jacket cells**.

*S6. Compare *Botrychium* or *Ophioglossum* in a sketch of whole plant. Label the features highlighted above for each and include a detailed sketch of one the prepared slides.

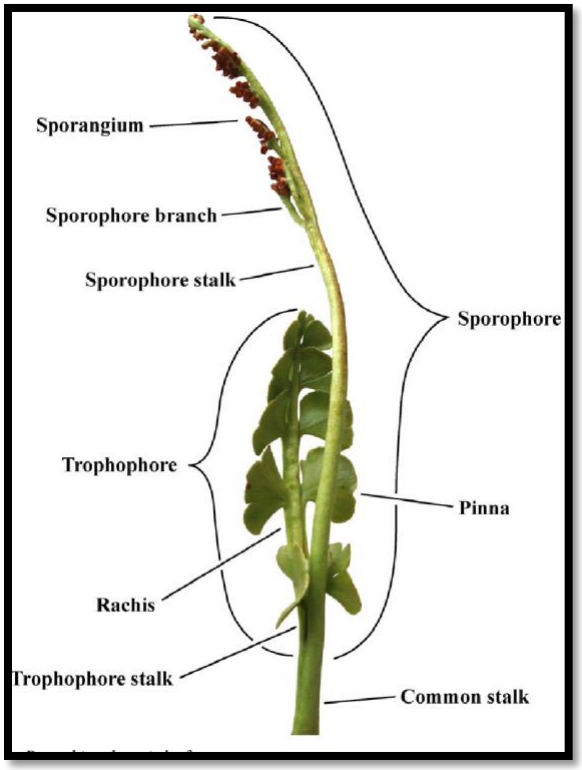

Figure 6. Morphological terms used to describe the members of the Ophioglossales. Note: there is still an ongoing debate about the nomenclature used to describe these plant parts. Some experts prefer to use the more specific terms outlined here sand others prefer to call them all "vegetative and reproductive segments" on the account that we still do not understand there homology. Some hypotheses even state that the photosynthetic portion of the plant is a flattened stem and not a leaf at all. More work is need until we can say so with confidence.