VII. Salviniales (Polypodiidae) and Ophioglossidae

C. Salviniales, the Heterosporous Leptosporangiate Ferns

Within the Polypodiidae is the order Salviniales, comprising two families of aquatic, heterosporous ferns - the Salviniaceae and Marsileaceae. 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 elsewhere in the Polypodiidae. What's more, the features are fun to watch in operation!

1. Salviniaceae: Salvinia

a. Node Design and components in Salvinia

i. 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.

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

- 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.

- 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.

Make a carefully labeled DIAGRAM of the shoot design of Salvinia.

2. Azollaceae: Azolla

a. Shoot Layout of Azolla

i. Take a bit of stem 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 leaf. Look for the alternating leaves in two rows and the branchpoints in the axis.

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

iii. Now look for the attachment location of the roots. 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.

iv. 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 cyanobacterium 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".

3. Marsileaceae

a. Living Plants

i. Living plants of Marsilea from the greenhouse are in the lab this week. They look a lot like four-leaf clover, but they are ferns. Look closely at the plants - and look at the sample of plant pulled form the pot to see the basic life form. A simple rhizome creeps around in the

pot, giving rise to leaves and roots.

The leaves of Marsilea are made up of a petiole and four pinnae clustered at the leaf tip. Notice that the leaf venation is free, not net-like, as you'd expect to see in a flowering plant such as clover. Another clue that you are in fact looking at a fern is that vernation is circinate, just as in the rest of the Polypodiidae.

ii. Remove a bit of stem from the covered dish of rhizomes. Make your own freehand transverse section of the stem. Stain with toluidene blue. 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.

iii. Check your interpretation of your hand section by comparing it with the photomicrograph at the bottom of page 56.

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. 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, out of the sporocarp wall (Fig. 17).

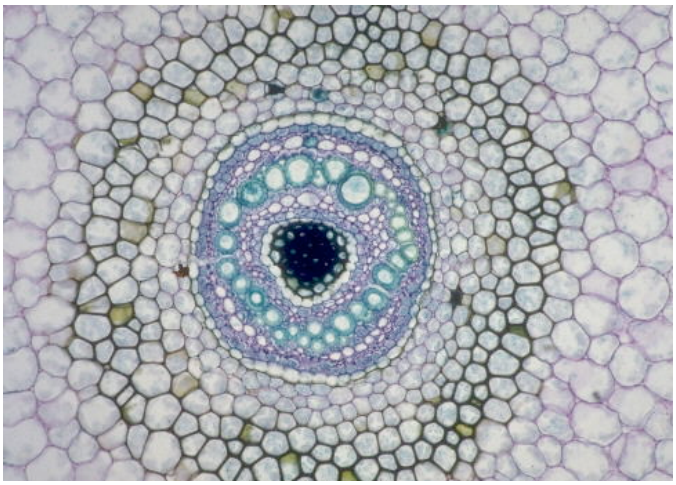
i. Note the dark, hardened sporocarp wall and the veins protruding from within, especially later in opening.

ii. 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.

DIAGRAM the sorus-bearing axis and a single sorus.

Fig. 17: <u>Marsilea</u> sorophore, expanded in water

c. When you come to lab next week, look at the mature female gametophyte and embryonic sporophyte that have developed. Mount one of the gametophytes on a microscope slide and stain it with a bit of toluidine blue stain to see the sperm lake - the gelatinous funnel through which the sperm swim to the archegonium - and the helical, multiflagellate sperm trapped in the gelatin.



<u>Marsilea</u> rhizome, cross section

Ophioglossidae: Psilotales and Ophioglossales

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.

A. General Features of Psilotum Sporophytes: Living Plants

Fig. 18

Fig. 19

1. Note the pattern of branching (Fig. 18). The green, ridged, aerial axes fork repeatedly to form a shrubby life form. Look for occasional *trichotomies*.

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

3. *Synangia* (singular synangium) - sets of sporangia evolutionarily fused together - areapparently attached laterally, in the axils of a pair of projections, toward the apices of the aerial axes (Fig. 19). 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.

4. 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.

5. 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.

B. 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, and include a TABLE that reports the major characters of each genus and a summary of numbers of shared and different character states in your lab notebook.

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

2. Notice that the synangia, though shaped differently, are morphologically equivalent to Psilotum synangia. The synangia are located on a short lateral branch just above a pair of microphylls. Only the number of synangium lobes are different. Make a DIAGRAM of the fertile axis and synangia of the two genera, to compare them.

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

Look at the interior of the creeping axis as you make a DIAGRAM of it in cross section.

1. The surface of the creeping axis has old, broken-off rhizoids. Just to the interior, in the cortex, there are cells containing mycorrhizal fungi.

2. In the interior look for an endodermis with a casparian strip.

3. Note that there are one or two small xylem masses surrounded by a single phloem cylinder (why are there sometimes two?) These steles are simple protosteles: at their core is a solid mass of xylem.

4. Now look at sections of the aerial axes. These differ in having 1) a lignified epidermis with sunken stomates, 2) a spongy cortex layer that functions in gas exchange, 3) a zone of sclerenchyma in the cortex, 4) a siphonostele with a pith of sclerenchyma (not parenchyma, as is common in siphonosteles in other groups), and 5) an absence of rhizoids. Look for exarch protoxylem in these aerial axes.

Make a TABLE comparing the creeping and the aerial axes of Psilotum.

D. Longitudinal Sections of the Stem With Synangium

Make a fully labeled DRAWING of the stem and synangium in longitudinal section. 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.

1. At the very outside is an epidermis; stomates should still be visible in the surface layer.

2. 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.

3. 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.

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.

1. 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).

2. Two of the three chambers of the synangium are visible. The wall

resembles that of the lycophyte sporangium, an outer layer of large cells and two inner layers of smaller cells. Remember that this sort of sporangium with a several-layered wall, which is very common in vascular plants, is called a *eusporangium.*

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

E. Gametophyte of Psilotum

Finally, look at the gametophyte of Psilotum on display. The important visible features are:

1. axial life form and forking

2. rhizoids

3. superficial antheridia

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.

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.

A. General Features of Botrychium Stem: Prepared slide of stem cross section

1. Look at the prepared slide of the stem cross-section as you make a DIAGRAM. 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. 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.

2. Compare the *Botrychium* stem to the preserved *Tmesipteris* stem. Do you notice any differences?

B. General Features of Botrychium Gametophyte: Prepared Slides

1. Look at the prepared slide of the gametophyte as you make a DIAGRAM. 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.

Remember Botrychium and Psilotum share the character of subterranean gametophytes.

C. General Features of Ophioglossum Fertile Spike: Prepared Slides

1. Look at the prepared slide of the fertile spike as you make a DIAGRAM. Look for the two rows of embedded sporangia. Note the main axis of the vascular system, do you see it branch between the sporangia?

2. How many cell walls do you count around each sporangium? Remember the Ophioglossaceae is eusporangiate.