Laboratory 2 (cont.): Thalloid liverworts and their sporophyte

As discussed in class, liverworts can be divided based on their growth form into leafy and thalloid (ribbon like body) liverworts. Within the latter group, we can distinguish between complex (Marchantiales) and simple (Metzgeriales; Pallaviciniales) thalloid taxa, based on the anatomy of the thallus. Thalloid liverworts compose a polyphyletic group (meaning?________ ____).

Their high taxic diversity reflects a broad array morphologies or architectures. Their high diversity and their ecological success or significance may in part be explained by their associations with other organisms, primarily fungi and in some cases cyanobacteria.

The objectives of this lab are for you to visualize the organization of the gametophyte of complex thalloid liverworts (Marchantiopsida) and the simple thalloid liverworts (Jungermanniopsida), to describe various architectures and structures, such as asexual propagules (what does that word mean?) and "identify" some of the associates.

At the end of the lab you should be able to:

- distinguish complex from simple thalloid plants
- understand that not every plant with seemingly simple architectures are simple thalloids (concept of reverse evolution)
- be convinced that the embryo develops within a modified archegonium (i.e., the archegonium has two lives!)
- discuss other means of sporophyte protection
- describe the development and architecture of the sporophyte
- describe the associates and their distribution
- know examples of asexual diaspores of some liverwort
- have an understanding of how the liverwort sporophyte dehisces

As always, I encourage you to explore the plants freely (*for example, I won't ask you explicitly to find oil bodies, as a feature of liverworts they should be on your mind*) and document your observations as much as possible using the digital photographs and your sketches.

Note: liverworts are recognized here as a division of plants: Marchantiophyta

Major lineages of liverworts are treated as classes (ending –opsida), subclasses (ending –iidae) and orders (ending –ales).

I. Complex thalloid liverworts: Marchantiopsida

In the lab we have living material for the Blasiidae (*Blasia*), and Marchantiidae (*Conocephalum*, and *Marchantia*).

A. Conocephalum conicum is a species rather common in our area; it grows on stream banks, in shady habitats. It typically forms extensive colonies. The range of the species extends throughout much of the temperate zone of the Northern Hemisphere. Extensive studies on this species have offered the basis for discussion of **cryptic speciation** (more than the eye can see) in bryophytes. **Habit description**: thallus rather wide, typically with purple margins; dorsal surface with distinct areoles, and conspicuous single pores; ventral surface shows a midrib and on either side, a series of scales (ventral scales) and also rhizoids. **Make sure to sketch all portions of the gametophyte.**

Describe/illustrate **upper** surface. Characterize the upper surface. _________________________________

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Locate a pore and focus up and down. How is the pore defined? _____________________________________

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How do pores differ from stomata?

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Can you see the cells that form the rim of each pore? How many are there?________________

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Describe/illustrate **lower** surface. What structures do you distinguish and how are they distributed?

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Are rhizoids unicellular or multicellular?

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Are they all the same? Any filaments inside?

Let's understand the function of the pores on the upper surface.

What may be your prediction of their function?____

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Take a lobe of the thallus, clean the ventral surface, and remove a wing 9i.e., a portion of the thallus on one side of the median thickened portion; this will facilitate making transverse section. Proceed to make sections, by holding your dissecting needle at right angle to the axis and running your razor blade along the needle! Do not press hard on the tissue with your needle and use a sharp blade!

Describe/illustrate your transverse section.

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Are cells below the roof with pore, all the same? Sketch them.

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Make sure you focus your description on the overall architecture of the thallus (are “tissues” differentiated?, is the thickness uniform?,..) and the architecture of the upper layers! Can you now see why they are called complex thalloids? ____

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Based on your section you should now be able to explain the reticulate upper surface based on the section (i.e., what accounts for the lines you see on the surface, now that you see the internal structure?)

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Have you seen stomata so far?____

Symbionts of liverworts.
Most land plants establish associations with fungi, and some also with cyanobacteria.
What is your prediction of how each of these enhance fitness of the liverwort?

Advantage of associating with fungi?____
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Advantage of associating with cyanobacteria?____
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Mycorrhizal association:
Sample lobes of *Marchantia* with rhizoids, remove some rhizoids and observe under light microscopy. You should note that rhizoids come in two types, those with a smooth inner surface and one with a pegged inner surface. The former are alive while the latter are dead. They play distinct functions. The dead one may be involved in water transport and the live ones in nutrient transport and in hosting fungal hyphae.
Your notes: ___________________________________
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Perhaps we will not see endophytic fungi, as this sample is from our greenhouse and not a natural environment.
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Can you see fungal hyphae inside the rhizoids?
You may be able to confirm this by adding a drop of lactophenol cotton blue to the slide; LPCB will stain chitin, the main component of fungal cell wall. Ask the instructor: NOTE, LPCB is toxic.
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Cyanobacterial association:
Sample lobes of *Blasia.* Observe the lobe using transmitting light and document what you see.

The lobe may harbor endophytic cyanobacteria of the genus *Nostoc.* Isolate one such cluster, press the cover slip over it to spread/squash the cluster. Document your observation. *Nostoc* fixed N_2 but only in some of its cells, called _________________. Can you distinguish these?
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Asexual reproduction in liverworts.

Sexual reproduction has obvious genetic advantages, but has functional limitations.

Genetic advantages of sexual reproduction:

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Consequently: genetic disadvantages of asexual reproduction:

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Functional disadvantages of sexual reproduction:

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Consequently: functional advantages of asexual reproduction:

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Asexual reproduction provides an effective mean to propagate at least locally. Many liverworts develop specialized asexual propagules (i.e., gemmae).

Gemmae of *Lunularia* (Marchantiopsida). Locate gemmae on the thallus. Document their distribution and predict how they would be dispersed.

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Isolate some gemmae and observe in light microscopy.

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Since vegetative growth occurs through the activity of an apical cell, where would they be on the gemmae? How many new individuals would emerge from one gemma? Does this explain the effectiveness of asexual reproduction?

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Moving on to simple thalloids.
Note: simple thalloid liverwort do not compose a monophyletic group, one tlineage of them shares a unique ancestor with leafy liverworts, and all simple thalloids and leafies are accommodated in one class the Jungermanniopsida! Simple thalloids are distributed between two subclasses: Pelliidae and Metzgeriidae, for which we have one exemplar, *Pallavicinia* and *Aneura*, respectively.

Pallavicinia lyellii

Habit description: the lobes are elongated (long ribbons). It is common liverwort here in shaded mossy river banks.

Explore this body and document your observation. You should be able to contrast the traits of *Pallavicinia* to those you observed and documented for *Conocephalum* (surface of thallus, structures on lower surface, rhizoids, thallus anatomy, ...)

The sporophyte: architecture and maternal protection.

Like other land plants liverworts are embryophytes and matrotrophic: they develop a multicellular diploid generation with the embryo protected and nourished by the maternal plant!

We will examine more sporophytes next week, but today you have an opportunity to explore young stages in the maturation of the sporophyte.

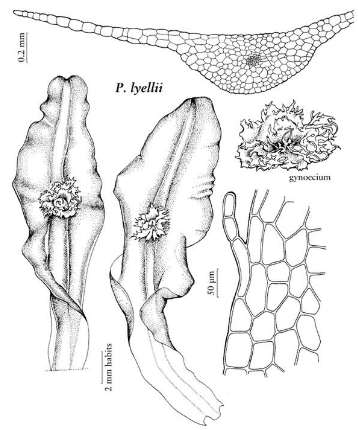
Individual plants are unisexual, meaning the species if __________. Most of the plants selected here are female plants, and they have undergone fertilization.

Archegonia are surrounded by an involucre of scales and following fertilization a pseudoperianth is developed. You should be able to see the young sporophyte by transparency.

Remove the entire structure and place on a slide.

Slice the entire structure (pseudoperianth and sporophyte) lenghtwise. Describe and document your observations.

You should see that the sporophyte is enclosed in a delicate sheath, and you should be able to convince yourself that this is derived from an archegonium.



PALLAVICINIA

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Since we may not have enough sporophytes of ***Pallavicinia***, work in pairs or also get some of the radula sporophytes (a leafy liverwort). The sporophyte architecture: can you distinguish the foot, seta and capsule?

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One distinguishing feature of liverwort sporophyte is the lack of a columella in the sporangium (no central axis)

Isolate the halves of the sporophyte and
1) empty the sporogenous mass
2) place the capsule walls upward, outer surface facing you.
Examine in light microscopy.

A. **Sporogenous mass**. Is it uniform? Are spores mature? What other cell type occurs in the spore mass? Such cells will always be lacking in mosses. Document your observations.

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Get a spore of another liverwort (***Fossombronia***) and compare.

B. **Capsule wall**. Are the cells all equal, do they all look the same? Do you see stomata? How does the thickness of the wall differ? Can you predict the lines along which the capsule would dehisce? How would the capsule dehisce?

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Now get a stem of ***Diplophyllum*** a leafy liverwort with immature sporophytes.
The capsules are immature, and still sunken among the perianth. But the capsule is enlarged, so you should be able to predict what comes next and how the sporophyte matures.
Isolate a sporophyte, and place in a drop of water. It should be enclosed or cover by a thin tissue. What is this tissue and why do you propose that?

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