

Laboratory 2: hornworts

Objectives of this lab

At the end of this lab, you should be able to distinguish a hornwort and describe their general features. You should also be able to distinguish and describe the general features of thalloid liverworts and contrast the sporophyte of liverworts to that of hornworts.

To start the lab, we will briefly lay out the concept forming the basis for the paper to be discussed next week.

Villarreal, J. C., & Renner, S. S. (2012). Hornwort pyrenoids, carbon-concentrating structures, evolved and were lost at least five times during the last 100 million years. *Proceedings of the National Academy of Sciences* 109(46), 18873-18878.

What you need to know?

What is a phylogeny?

How is it constructed?

What can it be used for?

If evolution reflects the transformation of traits and transformed traits are retained if selected for than what is the selection "force"?

Can traits transformation be linked to environmental change?

Questions?

Anthocerotophyta (the scientific name for hornworts)

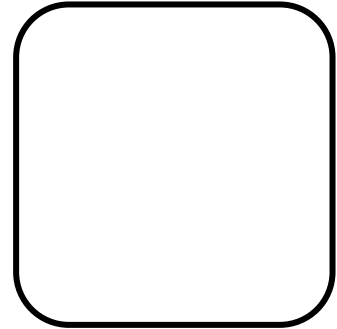
The species we'll be examining today is *Phaeoceros laevis* L. subsp. *carolinianus* (L.) Schust. This is cosmopolitan taxon, growing on inorganic soil. Here are some features: Thallus growing in rosettes, with the margins more or less entire, lacking central cavities (schizogenous cavities), but with chambers developing below the mucilage clefts, these chambers becoming invaded by *Nostoc*.

We are using a live, fertile specimen of this species to examine the various features characteristic of hornworts. The species grows in the fall, and we try to keep it alive for this class. You will be looking at gametophytes and sporophytes separately.

A. Macroscopic features:

The hornwort gametophyte is thalloid, meaning bilaterally symmetric, that is flat and sheet like. The defining feature of hornworts is of course the shape of their sporophyte (we look at those latter).

Under the dissecting scope you should be able to see the endophytic colonies of *Nostoc*. How are they distributed? Sketch out the plant.



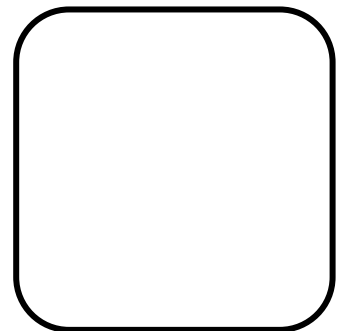
B. Microscopic features:

B1. The gametophyte: each individual thallus develops from a spore. Take one thallus and examine the under the dissecting scope. Distinguish the dorsal (upper) and ventral surface (lower). What feature do you use to identify the two surfaces?

a. Place the thallus or one or more lobes on the slide **dorsal surface** up (always use a cover slip when examining tissues with the compound scope). Focus on and describe the following:

- **epidermal cells:** are they all the same, describe the number and shape of chloroplasts,...

- the organization of cells near the tip of a lobe: look for the **apical cell**, the **mucilage**, and further from the apex, the **mucilage clefts**. Can you locate any of these?



- do cells of the dorsal epidermis define **pores** or develop **rhizoids**?

b. Flip the thallus over and examine the **ventral surface**.

- the thallus of hornworts, like other bryophytes is attached to the substrate by **rhizoids**. Describe the shape, architecture and location of the rhizoids. Do you see any filaments **inside** the rhizoids? What may these be? Are the rhizoids multicellular? How can you confidently tell?

- do cells of the ventral epidermis define **pores** (mucilage clefts)? _____. Since the underlying chamber will be invaded by *Nostoc* you should look for these near the apex (tip) of the thallus.

The *Nostoc* colonies are visible under the dissecting scope but should not be mistaken for the male gametangia. You can squeeze out the *Nostoc* colonies (cut the thallus close to the colony and gently press the colony out), and they'll appear as small chains of beads (unless the cover slip was pressed too hard on the colony, then the cells separate). Can you see any heterocysts (nitrogen fixing cells)?

c. Cross sections of the thallus. Follow the instruction on how to section a thallus.

Examine your section and look for the following:

- Is the thallus solid or with cavities? _____

- Are the cells composing the thallus all the same or do they differ and if so randomly or in a structured way? Do they hold chloroplasts,...? Describe.



Although *Phaeoceros* is bisexual many populations including this one, are protandrous. Do you know what this means and what the significance of this feature is?

B2. The sporophyte

The defining feature of hornworts is of course the shape of their sporophyte. You can see that the sporophyte is an **unbranched axis** that dehisces along **two longitudinal lines**, starting from the apex. This exposes the mass of yellow **spores**. The most mature spores are found toward the apex, and the level of maturation decreases as you go down the sporophyte.

Get a thallus fragment with a sporophyte and place the sporophyte on a slide. Is this thallus fragment a female or a male gametophyte?

What is the function of sporophyte? _____

Given this function, what needs to happen, for this function to be enabled? _____

Can you see where and how the sporophyte would dehisce?

Remove the sporophyte and separate the two halves. If your sporophyte began to dehisce you should simply further separate the two halves of the sporophyte downward.

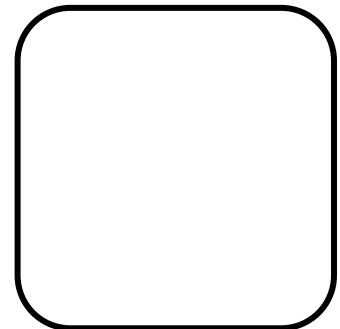
Observe the spore mass; you should notice that the color of the mass changes from the base of the sporophyte to its apex. Is your spore mass yellow or black? Does this matter? Well, we actually have two local species and sometimes they grow intermixed: *Phaeoceros laevis* has yellow spores, whereas *Anthoceros punctatus* has black spores!

Can you see the central axis (thread-like), which is the columella. Describe the cells composing it.

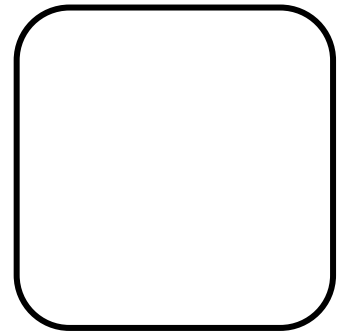
What may be its function?

Take one sporophyte half (with its spore mass) and move to another slide. Remove the spore mass from one half (hold the half at one end and gently scrape the spore mass off and place this half with the epidermis upward. Cover the sporophyte half and spore mass and examine under the compound scope.

Describe the cells of the epidermis. Are all the cells similar or can you distinguish cells that are differentiated in their shape and that may play critical functions in sporophyte development?

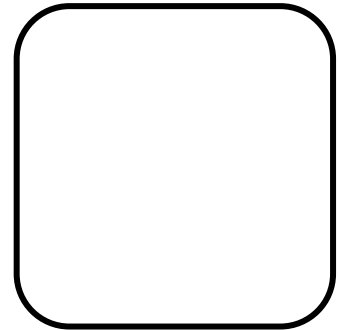


Examine the spores (note the ornamentation and trilete mark) and the (pseudo)elaters (how are they shaped and do they have thickenings?).



Go back to the other half of the sporophyte. Keep it with the spore mass, and place it with the spore mass up (facing you). Cover with a slip and examine under the compound scope.

You should be able to see distinct stages in spore maturation, considering that meiosis is _____ in hornworts. The spores from portion higher up are mature (well ornamented surface), whereas the ones from further down are rather smooth, still in tetrads, or not even formed yet.



Can you distinguish sterile cells ([pseudo]elaters) among the spores?
How do you distinguish them?

