Moss sporophytes with a higher proportion of leptoids have higher water transport rates

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Abstract: **The sporophytes of moss plants are dependent on the gametophytes for both photosynthesis and water, which makes conducting cells (hydroids and leptoids) an important part of the sporophyte anatomy. A previous study found that *Physcomitrium pyriforme,* which has shorter sporophytes, had higher rates of water transport than *Funaria hygrometrica,* which has taller sporophytes. The aim of this study is to test for differences in the conducting cell anatomy between these two moss species, which could be responsible for differences in water transport rates. We used histology methods to fix, embed, and section sporophyte seta and then quantified the numbers and sizes of the conducting cells. The results revealed that leptoids comprise a higher proportion of the conducting cell area in *P. pyriforme,* while hydroids comprise more of the conducting cell area in *F. hygrometrica.* These results point toward the leptoids playing a role in water transport in the moss sporophyte.**

***Keywords*:** anatomy, bryophytes, conducting cells, hydroids, seta

INTRODUCTION

Mosses are small, photosynthetic green plants that include over 13,000 morphologically diverse species (Patel et al. 2021). Mosses have sporophytes that are physically attached to the leafy gametophyte throughout their life and are dependent on the gametophyte for both photosynthates and water. Despite being classified as non-vascular, moss plants contain cells that are specialized for internal conduction and transport (i.e., hydroids and leptoids; Ligrone et al. 2000). Hydroids are water conducting cells that are dead at maturity and elongated, whereas leptoids are food conducting cells that are elongated and alive at maturity (Hébant 1977). Together these cells transport water and nutrients from the gametophyte to the sporophyte, which facilitates spore development (Scheirer 1980). This makes conducting cells an important part of the functional anatomy of the sporophyte and spore production.

Funariaceae is a family of mosses that have diverse sporophyte morphologies and can be easily grown in the laboratory. Across the approximately 255 species in the family, sporophytes have diverse capsule shapes and sizes as well as a variety of seta lengths, whereas the gametophyte morphology is quite uniform (Medina et al. 2018). The geographical distributions of Funariaceae are also diverse with many species occurring on more than one continent (Fife 1985). Sporophytes across the family range from only a few millimeters tall in *Physcomitrium patens* (Hedw.) Mitt. to over several centimeters tall in *Funaria hygrometrica* Hedw. This diversity makes species in the Funariaceae useful for comparative studies (Budke & Goffinet 2016).

In a previous study using two Funariaceae species (Whitaker & Budke 2021), we found that *Physcomitrium pyriforme* (Hedw.) Brid. sporophytes had higher rates of water transport (2.78 mm per min) than the taller sporophytes of *Funaria hygrometrica* (2.30 mm per min). This contradicted our hypothesis that taller sporophytes, extending beyond the still air of the boundary layer, would have higher rates of water transport compared to shorter sporophytes. Based on these findings, in this study we are testing for differences in the conducting cell anatomy between *P. pyriforme* and *F. hygrometrica*, which could be responsible for differences in the water transport rates. We predict that *P. pyriforme* sporophytes will have a larger number of and larger transverse area devoted to conducting cells compared to *F. hygrometrica.* Examining these cells might give us insights into the structure-function relationship for water transport rates in moss sporophytes.

MATERIALS AND METHODS

Study Taxa

Specimens of *Funaria hygrometrica* (Budke 145, CONN) and *Physcomitrium pyriforme* (Goffinet 9276, CONN) were used to generate laboratory cultures of gametophytes and sporophytes as outlined in Budke & Goffinet (2016). Both species were grown in the laboratory under the same temperature, light, water, and soil conditions. These species have diverse sporophyte morphologies with *F. hygrometrica* having an average height of 35 mm (Fig. 1A), while *P. pyriforme* is smaller with an average height of 15 mm (Fig. 1B; Whitaker & Budke 2021). *Funaria hygrometrica* also has larger capsules that have an average length of 2-3.5 mm (Budke et al. 2011), whereas *P. pyriforme* has smaller capsules with an average length of 1-3 mm (McIntosh 2007).

Histology

Laboratory grown sporophytes of *F. hygrometrica* and *P. pyriforme* with fully expanded capsules were collected and the middle 50% of the sporophyte stalk was fixed in formalin-aceto-alcohol (FAA). The tissues were fixed at room temperature for 4-8 hrs, and then overnight under a vacuum for a total of 24 hrs of fixation. Post-fixation specimens were placed into 70% ethanol for initial dehydration and then processed through a graded series of cold ethanol: 95% for 30 min and then 100% for 15 minutes, which was repeated twice. After dehydration, tissues were embedded in Spurrs resin (5g ERL 4221, 4g DER 736, 12.50g NSA, 0.14g DMAE) using a graded series. They were first placed in a 1:1 solution of resin to ethanol (100%) for 1.5 hrs, then 3:1 resin to ethanol for 3 hrs, and finally 100% freshly made resin overnight. These steps were carried out on a rolling mixer with perforated lids. Some of the 100% Spurrs resin was used to make shims that served as a platform to center the specimens in the resin and anchor the specimen labels. Specimens were placed in silicone molds on top of the shims and fresh 100% resin was added. The resin was polymerized overnight at 60-65°C.

Sectioning and Mounting

Once polymerized, the blocks containing the tissues were sectioned 700 to 1000 nm thick using a microtome (EM UC7; Leica Microsystems, Wetzlar, Germany) and glass knives. Sections were placed onto a drop of water on a glass slide coated with 2% formvar (Ruzin 1999), which helped the sections stick. The slides were then placed on a slide warmer at 45°C overnight. Next, the sections were stained with 1% toluidine blue and coverslips were mounted on the slides using permount and xylene. The slides were placed onto a slide warmer at 50°C overnight with one small fishing weight (3.013 g) placed on top of the coverslips to prevent bubbles from forming as the mounting media dried. After 12-24 hrs the slide warmer was turned off, but the weights were left in place for another 12-24 hrs.

Microscopy

A compound light microscope (BX60; Olympus, Tokyo, Japan) with an attached digital camera (EOS Rebel T6i; Canon, Tokyo, Japan) was used to take images of the stained tissue on the slides at 40X magnification.

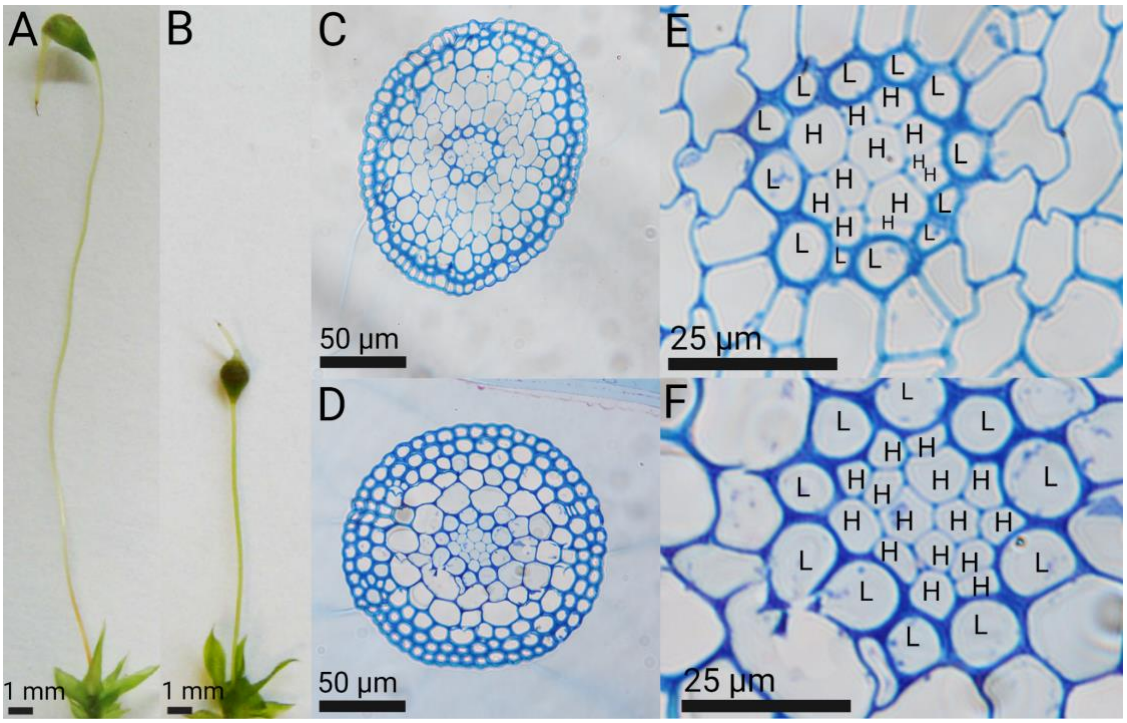

Figure 1. Sporophyte morphology and anatomy of Funariaceae species. A,C,E. *Funaria hygrometrica.* B,D,F. *Physcomitrium pyriforme.* A,B. External sporophyte morphology with expanded capsules topped by calyptra and attached leafy gametophytes at the base. Images used with permission under CC-BY, version 4.0 from Figure 1J,L in Budke & Goffinet (2016). C-F. Transverse sections through the middle of the sporophyte seta. E,F. Close-up of the conducting cells from C and D. L = leptoid cells; H = hydroid cells. This figure was created with BioRender.com.

Data Collection & Analysis

ImageJ was used to count cells and measure areas from the digital images (Schneider et al. 2012). Specifically, measurements and counts were made from the transverse sections of the seta, including the total seta area; the area devoted to conducting cells; hydroid cell area; leptoid cell area; as well as the number of conducting cells, hydroids, leptoids, and non-conducting

cells. Ratios between these area measurements and cell counts were also calculated, such as conducting cell area divided by seta area and conducting cell area divided by number of conducting cells. These data were recorded in an Excel spreadsheet (Microsoft, Washington, USA). RStudio (RStudio Team 2020) was used to perform statistical analysis and generate figures.

T-tests were used to test for significant differences between the two species. Prior to these tests we carried out F-tests to compare variances between the data collected for the two species. If there was not a significant difference between the variances ($p > 0.05$), then the variance was set as equal in the following t-test. However, if there was a significant difference ($p < 0.05$), then the variance was set as unequal.

RESULTS

Based on our anatomical observations we tested for significant differences in conducting cell anatomy between *Physcomitrium pyriforme* (N = 7) and *Funaria hygrometrica* (N = 6; Fig. 1C-F). We did not find a significant difference in the number of conducting cells (leptoids + hydroids) and number of hydroids between the two species. In contrast, we found that *F. hygrometrica* had a larger number of leptoids compared to *P. pyriforme* (t = 2.2893; df = 11; $p = 0.04283$; Fig. 2A). In order to account for variation in seta area among individual sporophytes, the conducting cell areas were divided by the total seta area for each individual. The conducting cell area per seta was greater in *P. pyriforme* than in *F. hygrometrica* (t = -5.6457; df = 7.5267; $p = 0.0006019$; Fig. 2B).

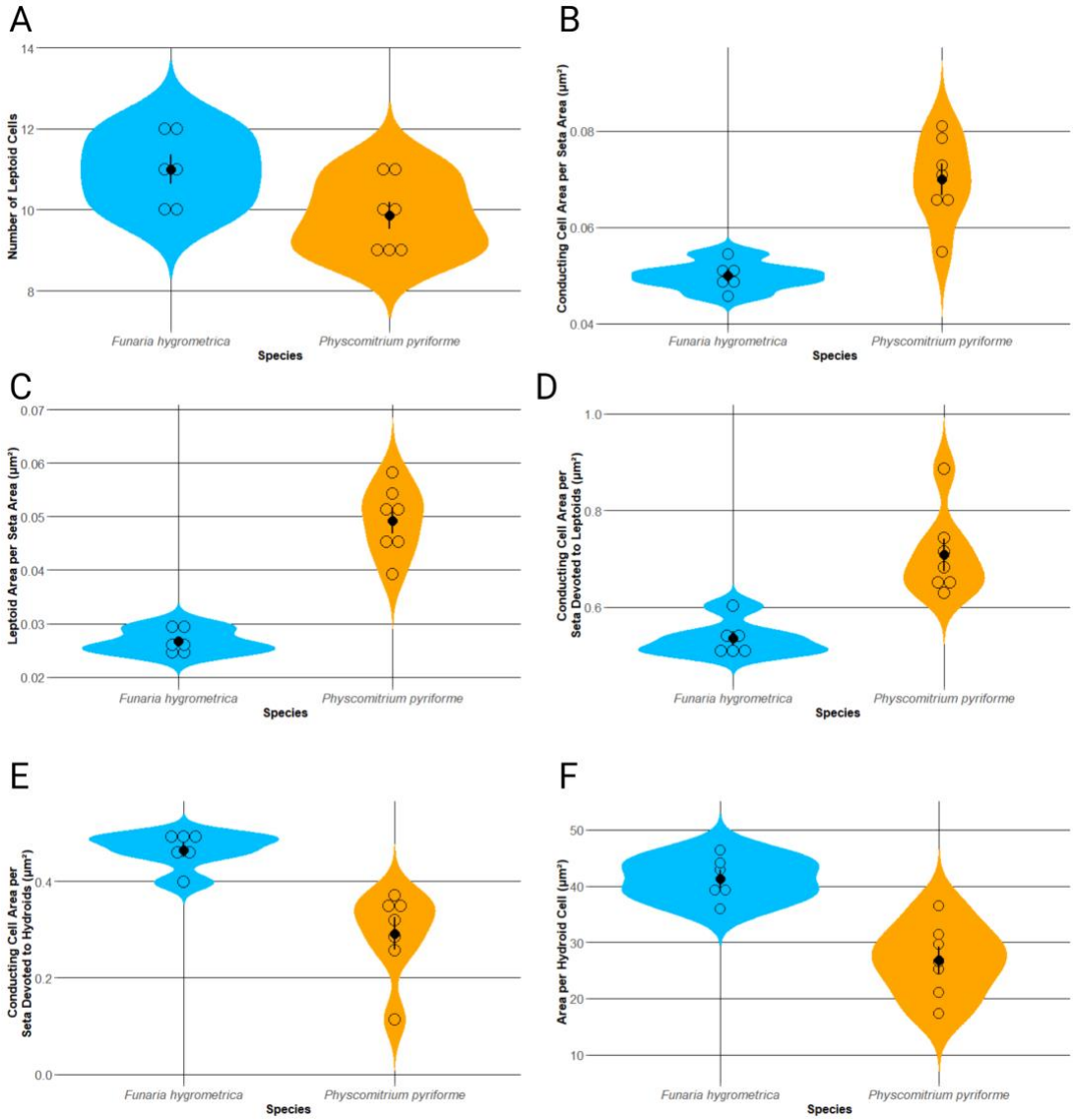

Figure 2. Violin plots with the data from each sample displayed as open circles, the mean as a solid circle, and standard error of the mean as bars. Data from *Funaria hygrometrica* (N = 6) is on the left of each panel in blue and *Physcomitrium pyriforme* (N = 7) is on the right in orange. (A) Number of leptoid cells. (B) Conducting cell area per seta area in µm^2. (C) Leptoid area per seta area in µm^2. (D) Conducting cell area per seta devoted to leptoids in µm^2. (E) Conducting cell area per seta devoted to hydroids in µm^2. (F) Area per hydroid cell in µm^2. This figure was created with RStudio and BioRender.com.

Examining the leptoids separately, *P. pyriforme* was found to have a larger leptoid area per seta than *F. hygrometrica* (t = -8.7787; df = 7.5939; p = 3.071e-05; Fig. 2C) and more of the conducting cell area per seta is devoted to leptoids (t = -4.4791; df = 11; p = 0.0009328; Fig. 2D). However, there was no significant difference in the size of the leptoid cells between the species. Examining the hydroids separately, *F. hygrometrica* had a more of the conducting cell area per seta devoted to hydroids (t = 4.4791; df = 11; p = 0.0009328; Fig. 2E) than *P. pyriforme*. Also, *F. hygrometrica* had a greater hydroid cell size (t = 4.8163; df = 11; p = 0.0005391; Fig. 2F).

DISCUSSION

We predicted that *Physcomitrium pyriforme* sporophytes would have a larger number of and larger transverse area devoted to conducting cells compared to *Funaria hygrometrica*. Our results support this prediction, showing not only that *P. pyriforme* sporophytes have a proportionally larger conducting cell area than *F. hygrometrica* sporophytes, but also that more of this area is dedicated to leptoids, rather than hydroids. In our previous study, *P. pyriforme* sporophytes were found to have higher rates of water transport than *F. hygrometrica* (Whitaker & Budke 2021). This points towards the leptoids playing an important and potentially larger role in water movement through the sporophyte in this moss species.

Hydroids and leptoids in bryophytes have anatomical similarities to xylem and phloem in tracheophytes. In both lineages, water conducting cells (hydroids and xylem) are located centrally with the food conducting cells (leptoids and phloem) surrounding them. Both leptoids and phloem sieve elements are elongated with thick walls and alive at maturity (Scheirer 1980). Leptoids retain a microtubular cytoskeleton in addition to mitochondria, which differs from phloem sieve elements that typically lack these organelles and are supported by companion cells (Woudenberg et al. 2022). Hydroids and xylem tracheids are also elongated cells that are both dead at maturity, lacking all cell contents, including organelles (Ligrone et al. 2000). In terms of their cell walls, the hydroids lack the secondary wall thickenings that are present in xylem (Scheirer 1980).

A significant difference between the hydroids of bryophytes and xylem of tracheophytes is the presence of lignin in the cell wall. Lignin are complex hydrophobic heteropolymers that are crosslinked to carbohydrate polymers (Ligrone et al. 2007). Hydroids lack lignin, but they do have lignin-like compounds in their cell walls. Both of these are aromatic compounds that contain cinnamyl, but lignin-like compounds lack methoxyl groups, which are present in lignin (Edelmann et al. 1998). While the lignin and lignin-like compounds both decrease lateral permeation of water through the cell walls, lignin is significantly more effective, resulting in xylem being better at retaining water compared to hydroids (Scheirer 1980). Additionally, hydroids have very thin cell walls, which not only makes them poor at mechanical support (Woudenberg et al. 2022), but also provides less volume for lignin-like compounds to be deposited into the cell walls. On the other hand, the thin, and potentially more flexible, cell walls of the hydroids may enable them to recover from cavitation events more easily, in comparison to xylem.

Since leptoids and phloem cells are anatomically similar, they may also have similar capacities for internal water conduction. Sugars are loaded into phloem sieve elements at the source (photosynthetic tissue) and are then unloaded at the sink (fruits, seeds, roots; Gould et al. 2005). The higher solute concentration at the source causes water to flow in the sieve elements via osmosis (De Schepper et al. 2013). This generates a pressure gradient resulting in the movement of both sugars and water from source to sink (Knoblauch et al. 2016). Moss gametophytes have lower rates of photosynthesis compared to vascular plants (Martin & Adamson 2001). Thus, mosses may have a higher proportion of water per unit sugar transported through the leptoids in comparison to the phloem of vascular plants, which could explain their role in water movement in moss sporophytes. Another way that moss leptoids could play a role is by facilitating water movement laterally into the hydroids, which have more permeable cell walls due to their lack of lignin. Additional research could help to determine the role leptoids play in water transport and whether it is facilitated in similar ways to phloem.

CONCLUSION

This study found that *P. pyriforme* has a higher proportion of conductive tissue, larger transverse area of the seta devoted to leptoids and that a larger proportion of the conducting cell area is composed of leptoids in comparison to *F. hygrometrica*. These results have the potential to explain why, despite having shorter sporophytes, *P. pyriforme* has higher rates of water transport compared to *F. hygrometrica* (Whitaker & Budke 2021). In combination, these findings point toward leptoids, when present, playing a role in sporophyte water movement, in combination with hydroids.

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