Measuring Biodiversity of Moss Species in Several Microenvironments of the Pine Barrens Region of Long Island

Brianna Donnelly and Torre Ann Parrinello
Shoreham-Wading River High School

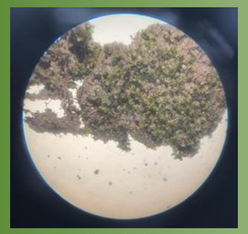
SEPA SCIENCE EDUCATION PARTNERSHIP AWARD
Supported by the National Institutes of Health
Cold Spring Harbor Laboratory DNA LEARNING CENTER

Abstract

Samples of moss were collected from various areas on the campus of the Shoreham-Wading River High School to measure the health of the soil in this community. Samples were identified by taxonomic criteria, then DNA was extracted to confirm genetic identity using the Barcode Long Island methods. By visual inspection, species were identified from seven different moss genuses. Upon completion of the DNA analysis, none of our samples were confirmed to be the same as the visual identification.

Introduction

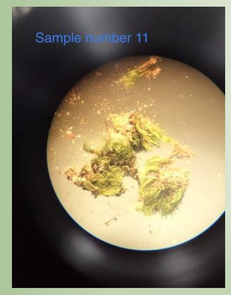
Mosses are important to a forest ecosystem because they filter out the water from rain, and other runoff sources. Mosses also produce nutrients such as phosphates and nitrates that the pond needs to survive. The role of mosses in an ecosystem is also to serve as a food source for animals such as rabbits, groundhogs, deer and other small rodents. This study will measure the biodiversity of moss species on our high school campus, located within the Long Island Pine Barrens region, and use this information to evaluate the health of the campus pond. In this study, we will be comparing moss species found around the campus pond, located adjacent to a local roadway, to moss species found in the surrounding forested area of campus. We hypothesize that there will be a greater biodiversity of moss species in the forest compared to mosses located near the roadways.



Sample 2

Sample 8

Collecting moss specimens



Sample #	Common Name	Scientific name
1	Anomodon	*Anomodon rostratus*
2		*Diphyscium foliosum*
3	Greater whipwort	*Bazzania trilobata*
8	Broom moss	*Dicranum scoparium*
9		*Tetraphis pellucida*
10		*Leucobryam glaucum*
11	Haircap	*Polytrichium commune*
		\

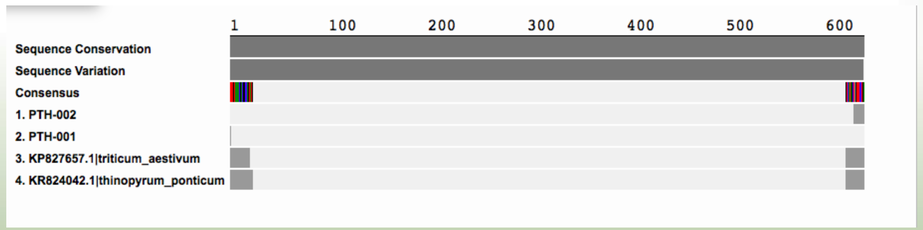
Preliminary identification of moss samples

Materials and Methods

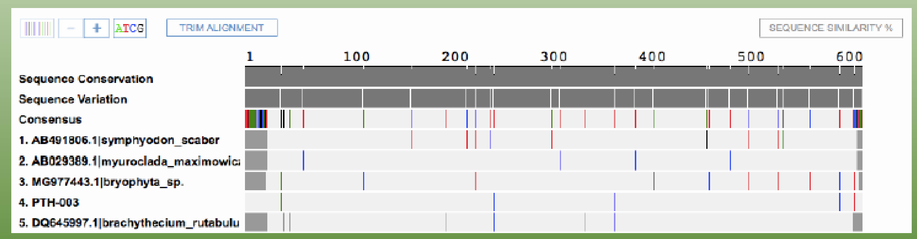
Mosses were removed carefully from the surface in which they were connected to with the help of a clean dissecting scalpel, rinsed with ethanol and left overnight to dry to remove all traces of water. Specimens were then stored at -20C until extraction. A field guide was used to taxonomically identify mosses prior to DNA extraction.
Following the Barcode Long Island protocol, DNA was first extracted, cells were broken open using lysis buffer and manually crushing using micropestles. Supernatant was removed to a fresh tube and silica bead solution was added to bind the DNA. Samples were then washed twice with buffer to remove impurities, and finally released from the silica beads with distilled water. DNA in the gene of interest was amplified by PCR, using the *rbcL* primer. Amplification was then confirmed by gel electrophoresis. Samples 1-3, 8-11 were sent for sequencing.

Results

Gel electrophoresis to confirm presence of DNA after PCR



Samples PTH-001 and PTH-002 appear to be the same species



Sample PTH-003

Discussion

None of the mosses we identified using field guides are matching up to the species names we assigned them from visual inspection. Samples 1, 2, 8 and 11 are matching up with sequences of various types of wheat. Sample 3 does not match the name we assigned it, but the appearance of the identified species is very similar to the appearance of sample 3, so we are confident in that result.

We have discovered that it is very difficult to obtain clean DNA samples from our moss species through extraction. The sediment that the mosses are grown in is difficult to completely clear away, and the samples themselves are very difficult to crush to lyse cells. As a result of these difficulties, the PCR was not overly successful, as evidenced by our gel photo.

We hypothesize that the appearance of the wheat sequence is an environmental contaminant, perhaps from a seed that blew into the area, or perhaps something that came from the windows of the adjacent building, where a cafeteria is located in close proximity to the collection site.

Samples PTH-001 and PTH-002 were incorrectly identified as *Triticum aestivum*, Bread wheat

Sample PTH-003 was identified as *Brachythecium rutabulum*, Rough stalked Feather moss

References

Adirondack Mosses, wildadirondacks.org/adirondack-mosses.html.
"Forest Moss." *Penn State Extension*, extension.psu.edu/forest-moss.
Fleury, Bruce E. "Lab 8 - Primitive Plants - Bryophytes, Ferns and Fern Allies ." *Bryophytes, Ferns and Fern Allies*, 2016, www.tulane.edu/~bfleury/diversity/labguide/mossfern.html.
Frank, T. "The Temperate Deciduous Forest Food Web." *Study.com*, Study.com, 2018, study.com/academy/lesson/the-temperate-deciduous-forest-food-web.html.
Glime, J. M. "Chapter 7-3 Gardening: Private Moss Gardens" *Bryophyte Ecology* November 2017
Liu, Yan. "A Case Study of DNA Barcoding in Chinese Grimmiaceae and a Moss Recorded in China for the First Time." *Taxon*, 1 Feb. 2011, www.jstor.org/stable/41059832?seq=1#page_scan_tab_contents.
Monthey, Roger, Kenneth R. Dudzik "Learning About Mosses in Our Northeastern Woodlots: An Introduction" *U.S. Forest Service* June 2010
Monthey, Roger, Marilyn Mollicone "Common Mosses and Liverworts of New England Woodlots" *U.S. Forest Service* November 2000
S, Carpenter. "Nutrients in Lakes and Streams." *Water Encyclopedia*, 1998, www.waterencyclopedia.com/Mi-Oc/Nutrients-in-Lakes-and-Streams.html.

Acknowledgements

We would like to thank Dr. Sharon Pepenella and Megan Capobianco from Barcode Long Island for all of their assistance and patience on helping to prepare this project.