



Investigating the Capacity of Tree-Surface Mosses to Act as Bioindicators for Insect Infestation

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Abstract

Mosses are plants which can be used as bioindicators of environmental factors. However, it is not known if mosses can be used as bioindicators of hyper-specific environmental factors such as the infestation status of a singular tree. Using mosses as bioindicators of singular tree health could help rapidly identify extremely costly and damaging insect infestations. We investigated whether or not moss populations on NYC white ash (WA) trees could be used to determine if these trees were infested with the Emerald Ash Borer (EAB) pest. To answer this question, we sampled mosses from both EAB infested and non-infested WA trees and used DNA Barcoding technology to compare the moss populations from both types of trees. The observed ubiquity of *Orthotrichum consobrinum* across both populations suggests that using moss populations as a bioindicator for EAB infestation status in NYC WA trees is not viable. However, we anticipate that this research will produce attempts at analyzing whether organisms similar to Mosses (such as lichens) can be used as bioindicators of hyper-specific environmental factors as well.

Introduction

Previous research on the capacity of moss as a bioindicator focused on how it adapted to changes in air quality. Studies have demonstrated that mosses concentrate heavy metals within their system and as a result are able to act as reliable bioindicators for air pollution (Chen et al., 2019; Mahapatra et al., 2019), which could greatly help efforts to reduce pollution in major cities. The next step to this research is to find another environmental factor which is vital to moss survival and another condition which affects both this environmental factor and human quality of life to see if mosses can act as bioindicators for this condition. Mosses rely very heavily on humidity for their survival (Zanatta et al., 2018), and the very damaging EAB infestation in Ash trees can theoretically affect the humidity around these trees. We came to this theory by considering that EAB decimates Ash trees by interrupting the water transport within their circulatory system (Durham, 2017), and that this interruption of water transport will lead to impaired transpiration, which should in theory lead to decreased local humidity and affect mosses in some way due to their reliance on humidity. Based on this conjecture, we hypothesized that more drought-resistant mosses would grow on EAB-infested trees compared to EAB non-infested trees, which would effectively act as a population-based bioindicator for EAB infestation. EAB infestation often produces very broad-spectrum symptoms such as peeling bark in its early stages, and because these symptoms are difficult to trace back to EAB, such bioindicators could help discover the presence of EAB before it becomes irreversible. This in turn would have very positive large-scale impact, as infestations such as EAB cost the global economy \$70 Billion every year (Gula, 2023), and as such present a problem for which it could be incredibly advantageous to have bioindicators for.

Methods and Materials

We observed 10 WA trees located in the Upper West Side region of New York. They included 4 EAB infested trees (Figure 1a) and 6 non EAB-infested trees (Figure 1b). EAB infestation status was confirmed by observing D-shaped holes in the bark (Figure 2) which are markers of late-stage EAB infestation. We randomly collected approximately 10 moss samples per WA tree using alcohol-swabbed scissors. The moss samples were segregated by individual tree. We selected representative moss samples for both EAB-infested and non EAB infested WA trees by selecting 10 mosses from each category. The collected moss samples were extracted using the silica protocol of Cold Spring Harbor Laboratory DNA Learning Center (CSHL-DLC, 2023). DNA amplification was carried out using the PuReTaq Ready-To-Go (RTG) PCR Beads (Cytiva). PCR was carried out using plant cocktail primers consisting of primers rbcLaF and rbcLaRev according to the protocol of CSHL-DLC (2023). PCR succession was analyzed on 0.8 % agarose gel. The amplified products were sequenced by GENEWIZ using Sanger sequencing. The DNA sequence data was analyzed using DNA Subway software and compared using BLAST (Basic Local Alignment Search Tool) to identify the species of moss.



Figure 1a.
An EAB infested tree.



Figure 1b.
A non-EAB infested tree.



Figure 2.
An indicator of EAB infestation.

Results

Our main finding was that after sequence analysis, every single one returned *Orthotrichum consobrinum* as the most likely species match (Table 1). In addition, all but one sequence were absolutely identical after sequence trimming. The non-identical sequence, moss sample 12 from an EAB-infested tree, differs from the other 19 moss samples in three nucleotide positions. This difference is reflected in our Maximum Likelihood phylogenetic tree (Figure 3).

Category	Sample#	Species (from details)	Aln. Length	Bit Score	e-value	Mismatches
Gathered from EAB Non-Infested WA Trees	1	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	2	<i>Orthotrichum consobrinum</i>	599	1047	0.0	8
	3	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	4	<i>Orthotrichum consobrinum</i>	599	1054	0.0	6
	5	<i>Orthotrichum consobrinum</i>	599	1047	0.0	8
	6	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	7	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	8	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	9	<i>Orthotrichum consobrinum</i>	599	1049	0.0	7
	10	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
Gathered from EAB Infested WA Trees	11	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	12	<i>Orthotrichum consobrinum</i>	599	1063	0.0	4
	13	<i>Orthotrichum consobrinum</i>	599	1047	0.0	8
	14	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	15	<i>Orthotrichum consobrinum</i>	599	1049	0.0	7
	16	<i>Orthotrichum consobrinum</i>	599	1049	0.0	7
	17	<i>Orthotrichum consobrinum</i>	599	1054	0.0	6
	18	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	19	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7
	20	<i>Orthotrichum consobrinum</i>	599	1050	0.0	7

Table 1. Results from BLAST sequence analysis

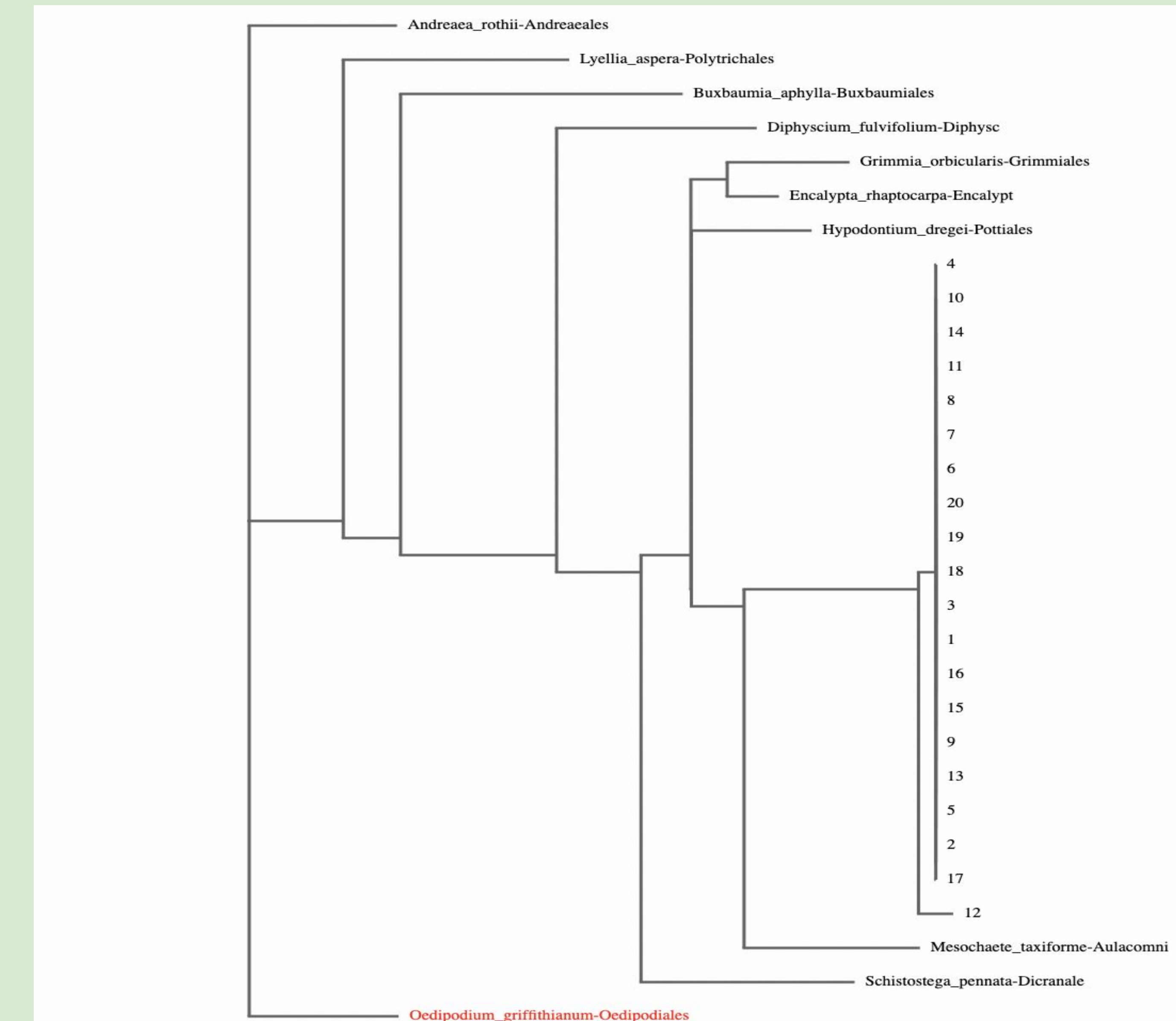


Figure 3. Maximum Likelihood Phylogenetic Tree

Discussion

As both non-EAB-infested and EAB-infested WA trees contain a similar amount of the same species of healthy *Orthotrichum consobrinum*, our results indicate that this particular moss is not an effective bioindicator of EAB infestation on WA trees. It is important to note that our experiment only consisted of a small sample size in a limited region, so our results may not be applicable to the effectiveness of all species of moss as bioindicators of pest infestations. Additionally, it is possible that we misidentified EAB-infested trees in the early stages of infection as non-EAB-infested trees or that there was cross-contamination between our moss samples, leading to the presence of the same moss in all samples. The presence of *Orthotrichum consobrinum* specifically is interesting due to the fact that it is a moss that had been considered restricted only to Eurasia until a morphologically identical species was found in North America in 2021 (Flagmier et al., 2021). Past research has indicated that *Orthotrichum consobrinum* is found only in less urbanized regions in Japan (Glime, 1993). Further research can be done into the resilience of this species of moss in its ability to be populous in an urban environment, as well as its ability to survive on an infested tree.

References

- Chen, Y.-E., Wu, N., Zhang, Z.-W., Yuan, M., & Yuan, S. (2019). Perspective of monitoring heavy metals by Moss visible chlorophyll fluorescence parameters. *Frontiers in Plant Science*, 10. <https://doi.org/10.3389/fpls.2019.00035>
- Durham, S. (2017). *Controlling Emerald Ash Borer*. Tellus. <https://tellus.ars.usda.gov/stories/articles/controlling-emerald-ash-borer>
- Flagmier, Maren & Draper, Isabel & Vigilando, Beatriz & Garilleti, Ricardo & Lara, Francisco. (2021). So close yet so far: The disjunct global range of *Orthotrichum consobrinum* (Orthotrichaceae) includes North America. *The Bryologist*, 124. 10.1639/0007-2745-124.3.403.
- Glime, Janice. (1993). Should mosses have common names? Part 9. The common names of Orthotrichales. *Evansia*, 10. 64-67. 10.5962/p.345994.
- Gula, L. T. (2023, February 6). *Researchers helping protect crops from pests*. National Institute of Food and Agriculture. <https://www.nifa.usda.gov/about-nifa/blogs/researchers-helping-protect-crops-pests>
- LIFE CYCLE OF THE EMERALD ASH BORER. (n.d.). Reforest Richmond. Retrieved from <https://www.reforestrichmond.org/emerald-ash-borer-eab>.

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