**Project Title: Genomic and metagenomic analysis of *Polystichum* ferns**

**Investigators: (Name, affiliation, email)**
Dylan Mendenhall
Haven Ecology and Research, LLC
dylan@havenecology.com

Paul Shannon
Institute for Systems Biology
pshannon@systemsbiology.org

Ylva Lekberg
MPG Ranch
ylekberg@mpgranch.com

**Project Summary (500 words max):**

Over the past decade, dozens of mass die-off events affecting *Polystichum munitum* have been observed in western Washington and northwest Oregon, with a mean mortality rate of 61% across all sites (Goldstein-Miller et al., 2019). In 2013, an especially large mortality event was observed in Seward Park (Doughton, 2019). Since then, the Seward Park die-off site has continued to expand and now encompasses more than 20 acres (Shannon, 2019). The goal of this research project is to identify the cause of the sword fern die-off phenomenon observed at forested sites throughout the Pacific Northwest, with a principal focus on Seward Park.

Drought stress alone is unlikely to be the primary factor driving the decline of *Polystichum munitum* (Goldstein-Miller et al., 2019). As co-occurring plant species do not appear to be affected at sword fern die-off sites, a host-specific pathogen is likely responsible. Results from a recent greenhouse study suggest that a transmissible pathogen is indeed present in the foliage of symptomatic sword ferns, restricting their water uptake and reducing their foliar moisture content (Mendenhall et al., 2019). We intend to use microscopy and next generation sequencing to identify the purported pathogen which will support future investigations into its dispersal, life cycle and ecological function.

By exploring the ecological variables that may be driving the regional decline of *Polystichum munitum*, this research will help land managers develop strategies for mitigating the blight and restoring affected areas.

**Sampling Methods (500 words max):**

Sample collection

At each park, ferns will be sampled for foliar, rhizome and root tissue, including 12 healthy ferns and 3 symptomatic ferns from Schmitz Preserve Park, and 12 healthy and 12 symptomatic ferns from Seward Park. Diseased sword ferns will be identified based on their display of symptoms (hue and morphology of pinnae, low proportion of young fronds to old fronds) and their proximity to the primary die-off site. Healthy sword ferns will be identified by their lack of any apparent symptoms. For each fern, the rhizome, 10 pinnae and 10 root tips will be collected and placed in sterile sample bags. 10 mL of soil will be collected from under each plant. After sampling, excavated soil and organic matter will be put back into place. To reduce the chance of dispersing the purported pathogen, separate tools will be used to collect samples in Schmitz Preserve Park and Seward Park. All tools will be washed and sterilized with isopropyl

alcohol between samples. We will also mitigate the risk of contamination by sampling in the healthy areas prior to sampling the symptomatic ferns. Boots will be washed and the soles will be sterilized with bleach before moving between parks.

<u>Lab Analysis</u>

After surface-sterilizing the samples, total DNA/RNA will be extracted from plant tissues. PCR will be used to amplify the genetic barcode regions of bacteria, fungi and oomycetes. Universal primers will target the V4 region of 16S rRNA (bacteria) and the ITS region of rDNA (fungi, oomycetes). PCR products will be sequenced on the MiSeq platform. Plant DNA will be prepared PCR-free for analysis on the NovaSeq 6000 platform. Soil samples will be stored at -20°C for potential follow-up analysis.

## Data Analysis (300 words max):

The resulting MiSeq data will be processed through a bioinformatics pipeline (QIIME) using a 97% similarity threshold for determining operational taxonomic units (OTUs). Microbial taxa will be identified by comparing sequences to online repositories. Due to this being a pilot study with a limited sample size, the microbiome data will be analyzed primarily through descriptive statistics. After rarefying the sequence counts, community composition will be visualized with principal coordinates analysis (PCoA) and bar graphs displaying the relative abundance of taxa. Indicator species analysis and/or distance-based redundancy analysis (dbRDA) may be used to identify likely candidates for the purported pathogen.

## Data Management (100 words max):

During analysis, data will be stored a personal computer, a solid-state backup drive and in paper notebooks. All data will be made publicly available in an online repository such as GenBank and/or GitHub, or by request.

## Project Timeline (100 words max):
Sample collection: August and September, 2020
Lab work: September and October, 2020
Data analysis: November, 2020
Reporting: December, 2020

## Project Participants:
Dylan Mendenhall
Paul Shannon
Ylva Lekberg

Are any of the investigators volunteers?

Yes_____          No___X__

*If Yes, please contact [Lisa.Ciecko@seattle.gov](mailto:Lisa.Ciecko@seattle.gov) to complete a Volunteer registration form*

**Budget:**

| Expense | Details | Type | Cost |
|---|---|---|---|
| Sample bags | Sterilized sample bags (96) | | **55.08** |
| Isopropyl alcohol | 70% (1 L) | | **2.99** |
| Bleach | 6% sodium hypochlorite (128 oz) | | **11.90** |
| Distilled water | Lab grade (4 L) | | **23.72** |
| Detergent | Tween-20 (25 mL) | | **13.60** |
| Gloves | 1 box | | **38.85** |
| Test tubes | Centrifuge tubes, 15 mL | | **37.50** |
| Microcentrifuge tubes | Microcentrifuge tubes, 1.7 mL | | **35.29** |
| **Total:** | | | **$ 218.93** |

**Deliverables (100 words max):**

Results will be summarized in a written report in December 2020 and at a later meeting of the sword fern working group. Raw data will be available by request.

**Literature Cited (optional):**

Doughton, S., 2019. The Case of the Vanishing Ferns: Citizen sleuths can't figure out what's killing Seattle's sword ferns [WWW Document]. The Seattle Times. URL https://www.seattletimes.com/pacific-nw-magazine/whats-killing-our-sword-ferns/ (accessed 9.9.19).

Goldstein-Miller, C., Ramirez, A.R., Kildisheva, O., Cieko, L., 2019. Polystichum munitum (Western Sword Fern) Decline in the Pacific Northwest. Report.

Mendenhall, D., Elliott, M., Shannon, P., 2019. Sword ferns from the Seward Park die-off site can induce wilting and obstruct water uptake in healthy ferns. Report.

Shannon, P., 2019. Seward Park Sword Fern Die-off: Is there a pattern to the 10% sword fern survival at Ground Zero? Seward Park Sword Fern Die-off. URL https://sewardparkswordferndieoff.blogspot.com/2019/09/is-there-pattern-to-10-survival-at.html (accessed 1.27.20).