Insights into the current distribution and population genetics of the old growth specklebelly lichen, *Pseudocyphellaria rainierensis*

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INTRODUCTION

Pseudocyphellaria rainierensis **(Imshaug) is a rare, epiphytic cyanolichen endemic to old growth forests in western North America from Alaska to Oregon (Glavich 2013) (Figure 1).**

This species is considered vulnerable in Oregon, imperiled in Washington, and is currently under assessment by the International Union of the Conservation of Nature (IUCN) to determine its global status as part of the IUCN Red List of Threatened Species (Glavich 2013, Allen et al 2021).

Populations of *Pseudocyphellaria rainierensis* have experienced a >30% decline in the last 90 years due to timber extraction (Allen et al 2021). Climate-driven fire has become a threat to the species, as well. The Beachie Creek Fire, which burned 193,572 acres in the Willamette National Forest in fall 2020, destroyed one of the largest known populations of *Pseudocyphellaria rainierensis* in Oregon (Allen et al 2021). These threats reflect the need to re-evaluate current conservation and management practices of critical habitat for the species.

RESEARCH OBJECTIVES

The primary objectives of our research are :

1) **to delimit the current distribution of *Pseudocyphellaria rainierensis* in Washington state and examine the role forest continuity and stand age in the distribution of the species, and**
2) **to characterize the population genetic structure of *Pseudocyphellaria rainierensis* in Washington state**

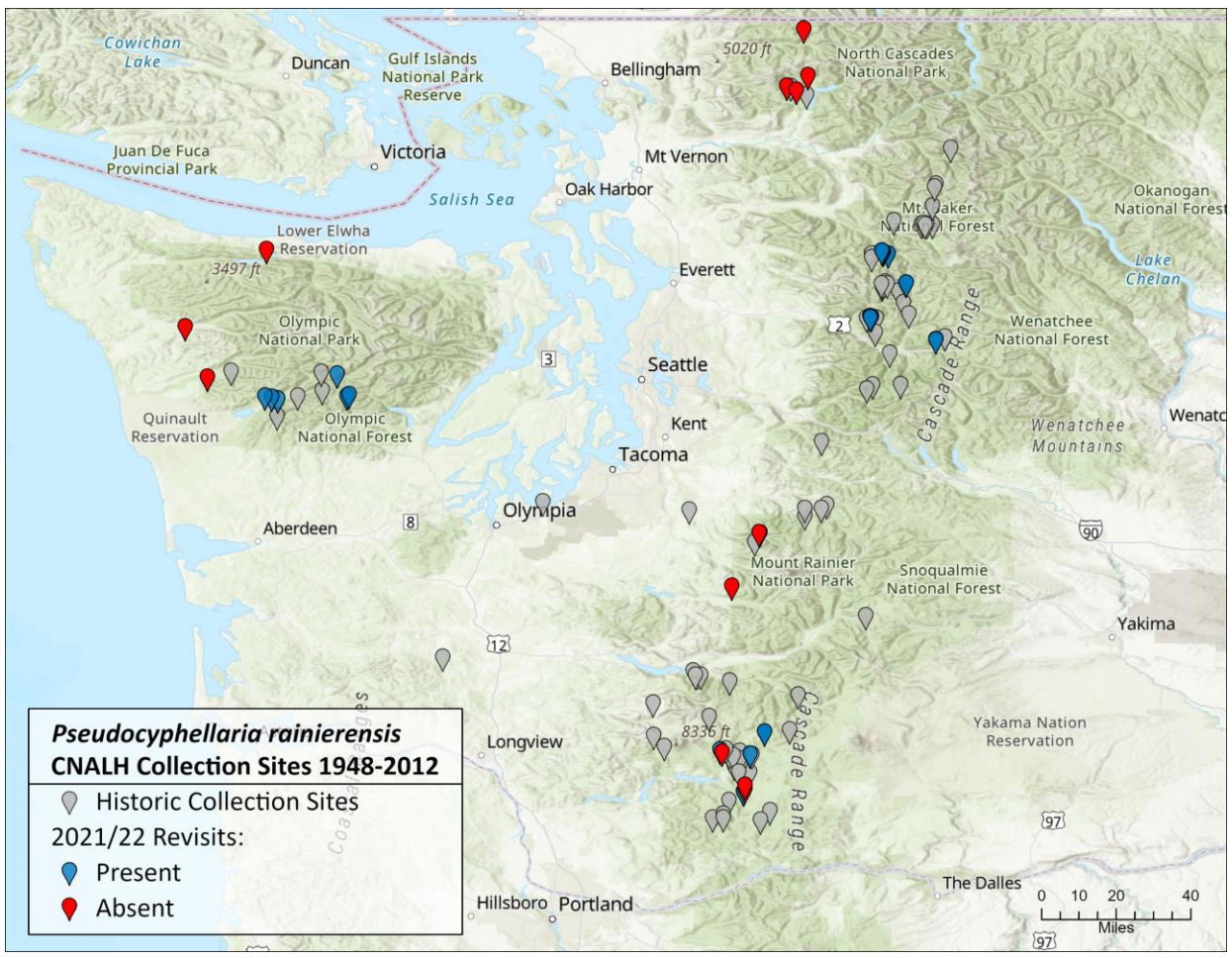

Figure 2. **Map displaying *Pseudocyphellaria rainierensis* collection sites cataloged in the Consortium of North American Lichen Herbaria (CNALH) database from 1948-2012. Presence/absence data was obtained during revisits to 42 historic collection sites from June 2021 to February 2022. *P. rainierensis* was present at 28 of 42 sites revisited.** (Map by B. Glenn)

PRELIMINARY FINDINGS AND NEXT STEPS

Pseudocyphellaria rainierensis **was present at 28 of the 42 sites revisited suggesting there may be significant declines of the species within some parts of its historic range in Washington state (Figure 2).**

We plan to visit an additional 50 historic collection sites in Washington state during the summer of 2022. With this information, we will update current patterns of occurrence for *Pseudocyphellaria rainierensis* and delimit current threats to the species and the old growth forests in which it occurs. This research will ultimately help to inform forest management practices and conservation strategies.

DNA samples will soon undergo Restriction-site Associated DNA sequencing at the Tripp Lab at University Colorado at Boulder. Whole genome sequencing will be performed at the Allen Lab at Eastern Washington University. The results from Restriction-site Associated DNA sequencing in tandem with the whole genome sequence will allow us to characterize the genetic structure within and among populations of *Pseudocyphellaria rainierensis* in Washington state.

Figure 1. Pseudocyphellaria rainierensis **in the Olympic National Forest near Quinault, WA**. (Photograph by S. Sharrett)

METHODS

To assess the historic and current distribution of *Pseudocyphellaria rainierensis* in Washington state we first reviewed collection data from herbarium specimens via the Consortium of North American Lichen Herbaria (CNALH) (Figure 2). Beginning in early summer 2021 we revisited 42 historic collection sites of *Pseudocyphellaria rainierensis* and recorded the presence/absence, abundance and element occurrence data for each site (Figure 2).

To examine the population genetic structure of *Pseudocyphellaria rainierensis* in Washington state, we collected 91 tissue samples from 12 populations in the Gifford Pinchot, Olympic, Mount Baker-Snoqualmie National Forests. Populations were defined as spatially discrete groups of individuals occupying a single forest stand (Devkota et al 2019). Tissue collections were performed according to protocols described in Devkota et al (2019) with slight modifications. One tissue sample (2 cm^2) per carrier tree was randomly collected with a minimum spacing of 50 ft (15.24 m). The total number of tissue samples collected was dependent on the local abundance of the species. Tissue samples were collected using nitrile gloves to reduce contamination then kept in acid-free paper packets, air dried, and stored at 32°F (0°C).

DNA extractions were performed according to the protocols outlined in McKenzie et al (2020) at the Calabria Lab at The Evergreen State College.

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References:

Allen, J., Calabria, L., Glew, K., Villella, J., & Dillman, K. (2021). Pseudocyphellaria rainierensis. Retrieved February 21, 2021, from http://iucn.ekoo.se/iucn/species_view/369711/

Devkota, S., Chaudhary, R. P., Werth, S., & Scheidegger, C. (2019). Genetic diversity and structure of the epiphytic foliose lichen Lobaria pindarensis in the Himalayas depends on elevation. Fungal Ecology, 41, 245-255.

Glavich, D. (2013). Conservation Assessment for Lichens. U.S.D.A. Forest Service Region 6 and U.S.D.I Bureau of Land Management Interagency Special Status and Sensitive Species Program. https://www.blm.gov/or/plans/surveyandmanage/files/ca-li-5-lichens-2013-04.pdf.

McKenzie, S. K., Walston, R. F., & Allen, J. L. (2020). Complete, high-quality genomes from long-read metagenomic sequencing of two wolf lichen thalli reveals enigmatic genome architecture. Genomics, 112(5), 3150-3156.

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