FACTORS AFFECTING THE DISTRIBUTION AND ABUNDANCE OF ALPINE AND SUBALPINE POPULATIONS OF PUMICE MOONWORT (*BOTRYCHIUM PUMICOLA*): ECOLOGY OF A RARE CASCADE ENDEMIC

By

SUSAN MARIE ROE-ANDERSEN

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THESIS APPROVAL PAGE

Approved:

10 May 2010
Date

Dr. Stewart Janes, Committee Chair

10 May 10
Date

Dr. Darlene Southworth, Committee Member

10 May 2010
Date

Dr. Steven Jessup, Committee Member

May 17, 2010
Date

Dr. Alissa Arp, Dean,
College of Arts and Sciences

To my parents, Keith and Eunice, who inspired and
nurtured my early love of plants.

and

To my husband, Eric Andersen who helped
me accomplish this life-long dream.

me ke aloha pau'ole.
~

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….. I especially thank the moonworts who quietly endure, despite our poking and prodding, continually holding secrets of life on lonely, windswept mountain peaks…..

FRONTISPIECE

Botrychium pumicola trophophore and sporophore bearing ripe sporangia.

Photo courtesy of Janelle Cossey. Hawaiian phrase from Pukui (1997).

ABSTRACT OF THESIS

FACTORS AFFECTING THE DISTRIBUTION AND ABUNDANCE OF ALPINE AND SUBALPINE POPULATIONS OF PUMICE MOONWORT (*BOTRYCHIUM PUMICOLA*): ECOLOGY OF A RARE CASCADE ENDEMIC

by Susan Marie Roe-Andersen

Subalpine and alpine populations of *Botrychium pumicola* (Ophioglossaceae), pumice moonwort, are restricted to volcanic habitats in the Cascade Range of central and southern Oregon and extreme northern California. The disjunct distribution of this threatened species and its absence from habitat that appears suitable is of conservation and land management concern. The focus of this study was to determine if adjacent areas with and without *B. pumicola* differ in abiotic and biotic factors and to better understand how these factors affect the distribution of the species. *Botrychium pumicola* occurs on exposed, windswept volcanic domes, ridgelines and summits. However, the species is not restricted to pumice substrates as previously reported. Populations occur on flat to steep slopes up to 27°, and aspects of N, E, SE, S, SW, and W. South, southwest, and west aspects were preferred. Soil potassium levels and soil density were higher, and soil temperatures lower in plots with *B. pumicola*. *Botrychium pumicola* populations occur with a diverse suite of sparse herbaceous perennial plants. A nearest neighbor analysis for *B. pumicola* and its plant associate showed a clumped dispersion. Species richness of

plant associates was similar in areas with and without *B. pumicola*. An investigation of the spore bank revealed a low spore density for all populations including the largest with 1300 *B. pumicola* individuals. The disjunct distribution of *B. pumicola* may be influenced by a variety of factors creating “habitat islands” suitable for growth and development of *B. pumicola* spores, gametophytes and sporophytes and their obligate AM fungal symbionts.

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INTRODUCTION

Botrychium pumicola Coville (Ophioglossaceae), the pumice moonwort, is a rare eusporangiate fern, endemic to the Cascade Range of central and southern Oregon, and the Mazama, Newberry and South Sister pumice plume areas of central and southern Oregon. It is also reported from northern California (CNPS, 2010). Populations occur in volcanic substrates of basalts, dacites, rhyolites, andesites, and other pyroclastics. Oregon populations of *B. pumicola* occur in fragmented distributions at Crater Lake National Park, Deschutes and Winema National Forests, and the Prineville District of the Bureau of Land Management (Hopkins et al., 2006).

A former Federal Category 1 Candidate for listing as Threatened, and a former Federal Species of Concern (USFWS, 1996), *B. pumicola* is currently listed as Threatened by the State of Oregon (ODA, 2008; ONHIC, 2007). It is also on the U.S. Forest Service Region 6 Sensitive Species List and the Bureau of Land Management Special Status Plant List (ISSSP, 2010).

Botrychium pumicola occurs on pumice scree substrates (FNAEC, 1993), with the exception of Mt. Bachelor in the Deschutes National Forest where the substrate is volcanic rubble and boulder fields (Hopkins et al., 2006). Subalpine and alpine populations occur on exposed, high elevation ridges and montane populations occur in *Pinus monticola* (lodgepole pine) frost-pocket basins at lower elevations (Hopkins et al., 2006; FNAEC, 1993; Hickman, 1993). Estimated total population size ranges from 24,000 - 27,560 individuals (Raven, 2008; Hopkins et al., 2006).

Frederick V. Colville first described *B. pumicola* from his discovery of the plant on Llao Rock in what would become Crater Lake National Park (Underwood, 1900). *Botrychium pumicola* has been observed growing in pumice substrates in association with *B. lanceolatum* subsp. *lanceolatum* in subalpine sites and with *B. simplex* in montane sites (Roe-Andersen, personal observation; FNAEC, 1993). The genus *Botrychium* consists of 50-60 species worldwide, 24 of which occur in Western North America (FNAEC, 1993).

The distinguishing characteristic of the Ophioglossaceae is the division of the frond into fertile (sporophore) and sterile (trophophore) blades (Wagner, 1990). *Botrychium pumicola* consists of an above-ground structure with a photosynthetic fern-like blade (trophophore), a reproductive sporophore, and a below-ground stipe and stem with a persistent root which extends down 7.5-10 cm into the substrate.

Plants emerge soon after spring snow-melt, as early as mid-April in montane habitats and by mid-July in subalpine/alpine habitats with peak emergence from mid-July to mid-August (Hopkins et al., 2006). Plants reach a height of 2-8 cm above the surface, with 7.5-10 cm of the plant below ground. Primordia for the next year's trophophore and sporophore over-winter underground.

Reproduction occurs through spores and vegetative gemmae (Camacho, 1996). The use of gemmae as a reproductive strategy may be an adaptive response to dry habitat (Farrar and Johnson-Groh, 1990). In this family, spores require a period of darkness for germination (Johnson-Groh , 2002). Photoinhibition of spore germination ensures below ground germination where the likelihood of sufficient moisture and close proximity of a mycorrhizal symbiont improve chances for colonization (Whittier, 2006).

Botrychium species form obligate symbioses with arbuscular mycorrhizal (AM) fungi, predominately with *Glomus* spp. (Kovacs et al., 2007; Winther and Friedman, 2007). Ascomycete hyphae also have been documented in *B. pumicola* (Camacho, 1999). The mycoheterotrophic stages of *Botrychium*, including subterranean gametophyte and sporophyte stages, can last as long as 10 years (Winther and Friedman, 2007). Individual photosynthetic sporophytes often remain underground for 1-3 years at which time they rely on their mycorrhizal partners for photosynthate (Johnson-Groh, 1998). During this secondary subterranean stage the sporophyte depends on fixed carbon sources from neighboring autotrophic plants, likely through a shared network of glomalean fungal networks (Winther and Friedman, 2007).

The distribution of *B. pumicola* is disjunct along the southern and central Cascade Range and in Mazama, Newberry and South Sister pumice plume areas of central and southern Oregon. However, it is absent from similar habitat that appears suitable. Disjunct populations range from a reported population on Shastina cone on the western flank of Mt. Shasta in northern California, to Broken Top in central Oregon, a distance of 350 km. A distance of 160 km separates the southernmost population from its nearest neighbor. Other populations are separated from each other by 1.6 to 104 km.

The goals of this study were to characterize the habitats of alpine/subalpine populations of *B. pumicola*, to provide baseline data for future study, and to determine if areas inhabited by *B. pumicola* differ in environmental variables from adjacent areas without *B. pumicola* with regard to (1) site characteristics (slope, aspect, elevation), (2) plant communities (associated plant species richness, *B. pumicola* and associated plant species distribution) (3) soil textural properties, soil nutrients and (4) the presence

of *B. pumicola* and AM fungi spore bank. This information will be useful for informed management and conservation decisions in regards to this threatened species. To address these research goals, I asked following questions:

1. Do abiotic factors, specifically soil nutrients, soil texture and site characteristics differ in plots with and without *B. pumicola*?
2. Do biotic factors, specifically associated plant species and *B. pumicola* individuals, presence of AM fungi and *B. pumicola* spore bank differ in plots with and without *B. pumicola*?

METHODS

Study Sites

Nine subalpine/alpine sites were chosen for study: eight in central and southern Oregon and one in northern California (Figure 1). Subalpine/alpine populations receive heavy snow accumulation with minimum temperatures below freezing for half the year (WRCDC, 2010; Hopkins et al., 2006). Summer precipitation is low and there are wide daily temperature fluctuations. Plants have very little shelter and are subsequently exposed to intense heat, cold, UV radiation and desiccating wind. Sites were chosen from the twelve documented high elevation (2096 m – 2761 m) subalpine/alpine populations. *Botrychium pumicola* populations located in montane sites were not considered in this comparative study focusing on subalpine/alpine populations. Montane sites are located in lower elevation *Pinus monticola* frost pocket basins (Hopkins et al., 2006). These sites differ from subalpine/alpine sites in their biotic factors (different plant community, earlier *B. pumicola* emergence and senescence) and abiotic factors (gentler slope, lower elevation, higher average summer temperatures).

Crater Lake National Park

Study sites at Crater Lake National Park were located in subalpine pumice fields surrounding the caldera of Crater Lake. Substrates consisted predominately of volcanic "popcorn" pumice and mixed volcanic gravel of various sizes. All known populations within the Park were studied, including Llao Rock (two populations), Cloud Cap (two populations), Skell Head, Grotto Cove and Dutton Ridge (Figure 2). All the sites were at

high elevation (2096-2448 m) in exposed, wind-swept, pumice fields from the Mt. Mazama pumice outfall c. 7700 years ago (Klimasauskas et al., 2002). From 1919 - 2009 Crater Lake National Park received an average of 12.8 m of snow per year, with average low temperatures above freezing for the months of June – September (WRCDC, 2010).

Deschutes National Forest

The Dome

This site was located in Newberry National Volcanic Monument at an elevation of 2193 m. The Dome is a horseshoe shaped volcanic cinder cone approximately 11,000 years old, covered with a 0.5 - 1m layer of Newberry "popcorn" pumice from the Big Obsidian Flow eruption c. 1,300 years ago (Sherrod et al., 1997).

Broken Top

This site was located on the SE flank of Broken Top at an elevation of 2316-2333 m. The substrate consists of eroded andesite, dacite and rhyodacite from the100,000 year old Broken Top eruptions mixed with pumice and volcanic gravel from the South Sister eruption c. 2000 years ago (Scott et al., 2003).

Mount Bachelor

This alpine site was located on the summit of Mt. Bachelor at an elevation of 2761 m in open, windswept volcanic rubble from the Mt. Bachelor summit eruptions c. 12,000 years ago, and small amounts of pumice from the Mt. Mazama tephra plume c. 7700 years ago (Scott, 1990).

Shasta-Trinity National Forest

Shastina Cone

A single population on Shastina Cone was re-located on 13 July 2009 by staff from Shasta-Trinity National Forest (Eric White, USFS, personal communication). This

population was located on an exposed, windy southwest flank of Shastina Cone near the upper end of Diller Canyon at an elevation of 2755 m. Shastina Cone was formed 9700-9400 years ago as a subsidiary andesite cone that rises 3758 m on the west flank of Mt. Shasta (Christiansen, 1990).

Figure 1. Central and southern Cascade Range showing *Botrychium pumicola* study sites (2009) in Oregon (Broken Top, Mt. Bachelor, The Dome, Crater Lake National Park) and northern California (Shastina Cone).

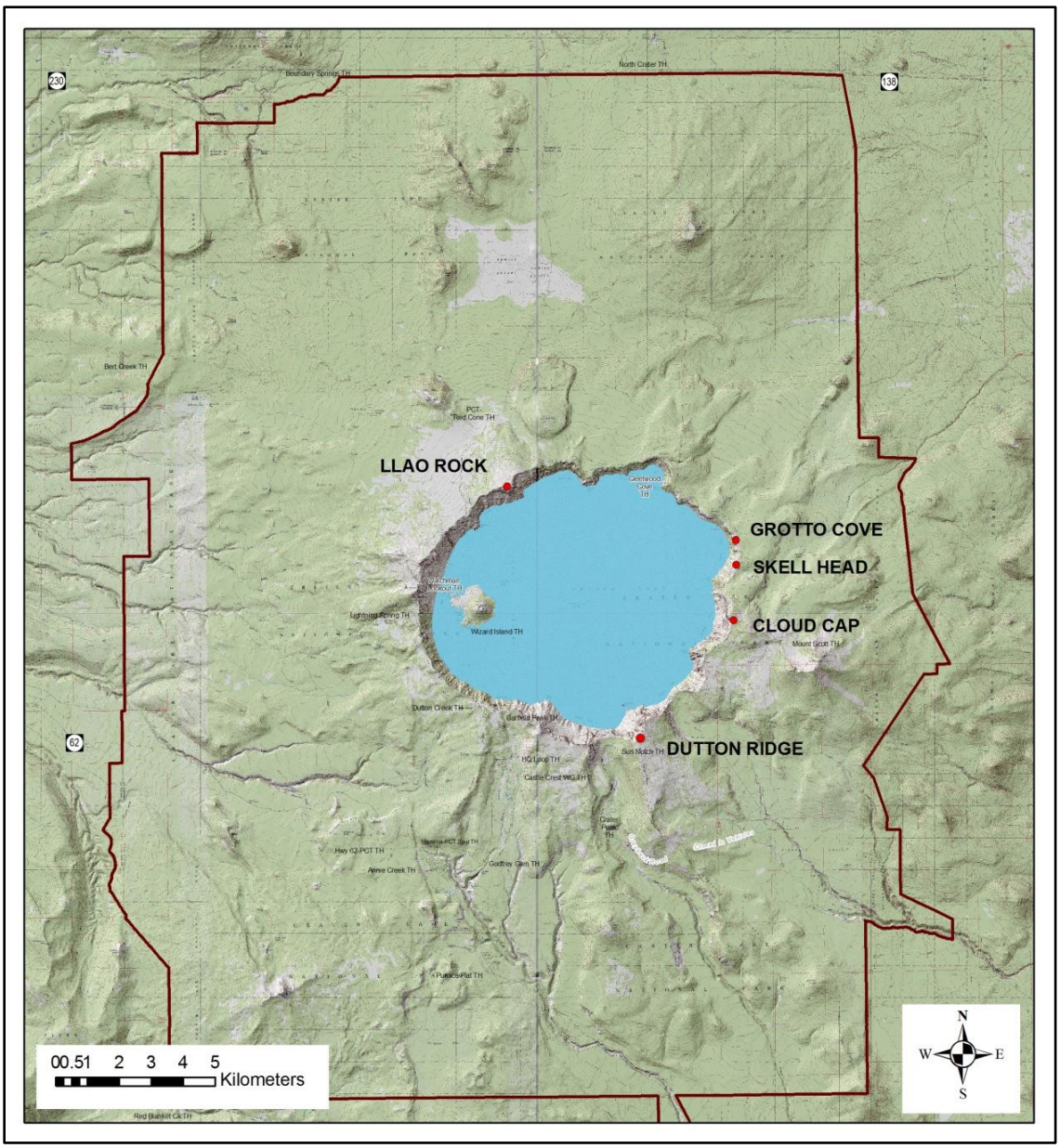

Figure 2. *Botrychium pumicola* study sites at Crater Lake National Park, Oregon (2009).

Sampling Design

Plots

Data collection began on 22 June 2009 and ended on 28 August 2009 at which time most *B. pumicola* plants had senesced. To minimize researcher impact on fragile pumice substrates in the sampled areas, light weight, soft soled, foam resin "pumice shoes" were worn during data collection.

Each population was located and its boundaries delineated by temporary pin flags. Individual *B. pumicola* plants were marked with pin flags located 5 cm from the plant. Pin flags were counted to determine population size. Five 1-m^2 plots were selected within each *B. pumicola* population from a random numbers table for sampling of environmental variables (Elzinga et al., 1998). Ten randomly selected plots were sampled at the Llao Rock site due to the large population size.

Environmental variables were divided into two categories for sampling: abiotic factors which included soil nutrients, soil textural properties and site characteristics (slope, aspect and elevation), and biotic factors which included associated plant community, nearest neighbors (*B. pumicola* and closest other plant species) and distribution of *B. pumicola* and other plant species. A 1-m^2 sampling frame was used to delineate each plot for sampling.

Adjacent areas that were similar in slope, aspect, elevation and associated plants but lacked *B. pumicola* were paired with a corresponding population at each site and sampled similarly at the same time. Karst et al. (2005) found that environmental variables at fine spatial scales may influence the distribution and abundance of ferns. Therefore, for this study, paired sites were chosen within 5-10 m of the *B. pumicola* population. This

proximity was far enough away from the population to avoid sampling where there were subterranean sporophytes but close enough to capture micro-site factors that might be influencing *B. pumicola* distribution.

Populations were located and documented using a Garmin GPSmap 60CSx handheld GPS unit. All maps were created using ArcGIS v. 9.3 (ESRI, 2008). Elevation was determined with the Garmin GPS unit and field checked with USGS 7 minute topographic maps (Crater Lake East, Crater Lake West, Broken Top, Mt. Bachelor, East Lake, and Mt. Shasta topographic quadrangles). Slope was determined with a Suunto clinometer and aspect with a Silva Ranger compass. In each 1-m^2 plot all plants species were identified and stems counted.

Within each plot with *B. pumicola*, community structure, nearest neighbors and potential competition or mycorrhizal association with nearest autotroph was investigated. For each *B. pumicola* plant, measurements were taken of number and lengths (measured to nearest 0.5 cm) of trophophores and sporophores, presence or absence of spores, presence or absence of any damage or desiccation of the trophophore or sporophore, nearest *B. pumicola* and nearest plant associate.

Sampling occurred at nine of the twelve known subalpine/alpine populations. This included the five subalpine populations within Crater Lake National Park (Figure 2), two subalpine populations (Dome and Broken Top) and one alpine population (Mt. Bachelor) in the Deschutes National Forest. The Shastina Cone (Shasta-Trinity National Forest) subalpine site was sampled for some, but not all, environmental variables.

In order to ensure that the timing of measurements was equivalent across sites, sampling was carried out in relation to *B. pumicola* phenology. Sampling began at the

lowest elevation site (Grotto Cove) and moved along an elevational gradient from low to high. Each population was examined during the "separation stage" which included elongation of the sporophore prior to spore release and the "spore-release stage" which included full size plants releasing spores (Johnson-Groh and Lee, 2002).

Signs of herbivory on *B. pumicola* individuals and signs of *Thomomys mazama* (pocket gopher) activity was noted for each site.

Isolation of AM fungal and *Botrychium pumicola* spores

Ramirez et al. (2004) found the density of fern spores was significantly higher in the first 10 cm of soil. Therefore, for this study a soil sample containing surface rock and soil was taken to a depth of 10 cm and placed in 1-L re-sealable plastic bags. Samples were taken from all populations except for the Dome and Shastina Cone. Soil samples were stored at room temperature (15-21°C) for 90 - 120 days until processing. Soils were tested for the presence of AM fungal spores and *B. pumicola* spores October-December 2009. Spores from a *B. pumicola* sporophore at Grotto Cove were examined under the microscope to confirm identity in subsequent tests.

Spores were isolated from 17 soil samples via centrifugation (Brundrett et al., 1996; Mason and Farrar, 1989). For each soil sample, 250 ml of dry soil was mixed with 250 ml of distilled water and soaked for 30 min, stirring every ten min to ensure even saturation. Wetted soil was decanted through a series of soil sieves (Tyler Standard Screen numbers 9, 16, 28, 100, 200, 325) using tap water to flush soil through each sieve. *Botrychium pumicola* spores average 35 µm, however they tend to remain in tetrad (Farrar, 2006; Wagner, 1998). AM fungal spores range from 20-1000 µm (Brundrett et al., 1996). Number 200 (75 µm opening) and 325 (43 µm opening) sieves plus the catch

basin, were chosen to capture both types of spores. Sievings were removed with a plastic spoon from number 200 and 325 sieves and from the catch basin and each placed into 50 ml plastic centrifuge tubes. Tubes were spun in a Sorvall RC 5C Plus Centrifuge for 5 min at 2000 RPM. Supernatant was discarded and the pellet re-suspended in 50% sucrose solution, stirred vigorously and centrifuged for 3 min at 2000 RPM. Four samples from the top of each tube were transferred to a microscope slide. Slides were examined using a Leica DMLB compound microscope. Spores were photographed using a SPOT Insight digital camera. Spore density was obtained by counting the number of *B. pumicola* and fungal spores and placing them into the following groupings: None=0, few=1-5, some=6-20, many >20.

Soils

Soil temperature readings were taken for each 1-m² plot at root depth (10 -12 cm below soil surface) using a Taylor Commercial digital probe thermometer model 9842 (accuracy range ± 0.6° C). Readings were taken at a distance of 2 - 3 cm from one *B. pumicola* plant in plots with *B. pumicola* and from one associated plant species in plots without *B. pumicola*.

Soil samples were taken from one randomly selected plot with *B. pumicola* and one plot without *B. pumicola* for each population except for Shastina Cone which was not sampled. The soil sample at Dutton Ridge was taken 10 cm from the *B. pumicola* individual to avoid removing gemmae, gametophytes or juvenile sporophores. Two *B. pumicola* plots at Broken Top were sampled for comparison due to its highly different visual characteristics including lack of nearby plant associates and clay soil. Soil samples were taken at *B. pumicola* root depth of 10 - 12 cm, placed in 1-L re-sealable plastic bags

and transported to a freezer for storage. All samples were stored at a temperature of 0 - 4° C. Soils were transported in a cooler to the Soil Analytical Laboratory at Oregon State University, Corvallis, OR for testing of soil moisture, organic matter, pH, P, K, Mg, Na, Ca, NO_3, NH_4, soil density and particle size using the methods of Horneck et al. (1989).

Data Analysis

Environmental variables were divided into abiotic factors which included soil nutrients, soil textural properties and site characteristics (slope, aspect and elevation), and biotic factors which included *B. pumicola* measurements and phenology, other plant species, and nearest neighbors (*B. pumicola* and closest associated plant species). The 95% confidence interval was used as the level of significance for all analyses. Given the limited number of subalpine/alpine populations, values between P = 0.05-0.1 were considered for biological relevance and potential management recommendations for Federal land managers.

Data were analyzed with a Wilcoxon Matched Pairs Test using Statistica v.7 (StatSoft Inc., 2007). This is a non-parametric test that compares two matched groups. Wilcoxon Matched Paired Tests were performed to determine if there were significant differences between plots with and without *B. pumicola*. Due to the number of comparisons, a Bonferroni correction was applied to decrease Type I error (Rice, 1989). Because the Bonferroni correction has been considered by some researchers to be overly conservative or likely to introduce type II errors, results were considered under both conditions (Bland and Altman, 1995; Perneger, 1998; Batzer et al., 2004; Narum, 2006).

Descriptive statistics were generated for each population and for each environmental variable (abiotic and biotic factor) for plots with and without *B. pumicola*

using Statistica v. 7. Differences in abiotic and biotic factors between plots with and without *B. pumicola* were evaluated using *t*-tests (Minitab, 2009).

In addition to evaluating environmental variables separately, a multivariate analysis was conducted using PC-ORD (McCune and Mefford, 2006). A non-metric multidimensional scaling (NMS) ordination was created for each group of environmental variables to visually represent any separation between plots with and without *B. pumicola*. NMS is an ordination technique that creates a spatial representation of the measurements of each environmental variable, based on the rank order of distance between these points in a matrix. All NMS ordinations used the Sorensen (Bray-Curtis) distance measure. NMS was used to visually compare differences and similarities between plots with and without *B. pumicola* at each site in regards to each environmental variable. McCune and Mefford (2006) recommend removing outliers due to their potential to skew data so an Outlier Analysis was conducted for all environmental variables, outliers removed and the NMS test was re-run.

Differences revealed in NMS were subsequently analyzed using Multi-Response Permutation Procedures (MRPP) in PC-ORD. MRPP is a non-parametric procedure that tested the hypothesis of no difference between two or more groups of environmental variables. As with NMS, outliers were removed and the test was re-run.

Associated plant species data was modified by removing species occurring in fewer than 5% of the plots. Removal of species with relative rarity in the data set reduced “noise” in the data (McCune and Grace, 2002). In order to minimize the effect of disproportionately large numbers in the plant associate data set, a relativization was

applied. Any plant associate that occurred in only one population (singleton) was also removed from the data set.

Nearest Neighbor Analysis was conducted for each *B. pumicola* plot to determine the degree of spatial dispersion in the distribution between each *B. pumicola* individual and nearest neighboring *B. pumicola* individual and between the nearest other plant species. A Nearest Neighbor Index (NNI), where NNI = average distance/expected distance was determined following the procedure of Clark and Evans (1955) with each *B. pumicola* individual and associated plant species NNI value ranging between 0 - 2.1491. A value approaching zero indicates a clumped distribution, a value near one indicates a random distribution and a value nearing 2.1491 indicates a uniformly spaced distribution.

Aspect measurements for each population were binned into one of eight cardinal directions to match the following: North (N), 336-25°; North East (NE), 26-70°; East (E), 71-110°; South East (SE), 111-160°; South (S), 161-200°; South West (SW), 201-250°; West (W), 251-290°; North West (NW), 291-335°. A Chi-square Goodness of Fit Test (Minitab, 2009) was performed to determine how close the observed binned aspects were to expected binned aspects.

Mean and standard deviation were determined for all *B. pumicola* trophophore and sporophore measurements. Trophophore lengths were binned into the following categories: small (0.05-1.5 cm); medium (2.0-3.5 cm); large (4.0-5.5 cm); extra-large (>6.0 cm). Presence of trophophore necrotic damage was calculated as a percentage of each population.

RESULTS

Study Site Descriptions

Crater Lake National Park

Grotto Cove

Grotto Cove was the easternmost and lowest elevation subalpine *B. pumicola* population in Crater Lake National Park (Figure 2; Table 1). The substrate consisted of light colored "popcorn" Mazama pumice 1-5 cm in diameter, mixed with gravel and silt (Figure 3A, B). Associated plant cover was sparse with *Carex breweri*, *Phlox hoodii*, and *Lupinus lepidus* the most common associated plant species. The site was surrounded on three sides by an open stand of wind-dwarfed *Pinus contorta* with the lake precipice on the west side. The *B. pumicola* occurred in a scattered distribution on the east (upper site) and west (lower site) side of a paved walkway that bisected the population (Figure 3D). Pocket gopher mounds were present.

This site has a history of disturbance beginning with construction of the original roadway, followed by grading and planting of *P. contorta* and *Arctostaphylos patula* when the road was rerouted c.1940. The path was re-developed c. 1968 into the "Grotto Cove Nature Trail" as an all-accessible interpretive trail. Interpretive signs placed on both sides of the path explained the flora at the site (Figure 3C). This nature trail was abandoned by c.1978 (Mark and Watson, 2009).

There was evidence of heavy disturbance from foot traffic in the lower site where visitors leave the paved walkway to get closer to the lake precipice. *Botrychium pumicola* populations were not recorded at Grotto Cove until Zika (1995) documented the upper site. One can speculate that *B. pumicola* was in a prolonged subterranean stage, overlooked or ignored, or that this is a relatively recent colonization.

Skell Head

The Skell Head site (Figure 2; Table 1) consisted of light colored "popcorn" Mazama pumice 1-5 cm in diameter, mixed with gravel and silt (Figure 3E). Associated plant cover was sparse with the dominant species consisting of *Erigeron elegantalus, C. breweri* and *Achnatherum occidentale*. The distribution of *B. pumicola* was scattered, with clumps of three to five *B. pumicola* (Figure 3F). This site was open and windswept with a stand of *Pinus albicaulis* to the east, the lake precipice to the west and little sign of human disturbance. Deer scat was observed but no signs of herbivory. Pocket gopher mounds were present.

Cloud Cap

The Cloud Cap site consisted of two populations, Cloud Cap North and Cloud Cap South (Figures 2 and 3G; Table1). Slope at Cloud Cap South was 27°, steepest of all study sites. A stand of *P. albicaulis* bordered Cloud Cap North to the east and south with the lake precipice to the west. The substrate consisted of Mazama pumice 1-3 cm in diameter, mixed with gravel and silt. There was less pumice and more dark gravel than at Grotto Cove, Skell Head or Llao Rock (Figure 3H). Some areas of the populations had little or no pumice. Associated plant cover was moderate with *Antennaria rosea, E. elegantalus,* and *C. breweri* the most common associated plant species. A scattered

distribution of *B. pumicola* occurred at the site. The populations occurred along a southwest to northeast axis in line with the prevailing winds (WRCDC, 2010). Cloud Cap South was 15 m to the south of Cloud Cap North, below the overlook rock wall. Both populations were subject to visitor disturbance due to their proximity to the overlook. Visitors were observed leaving the parking area and overlook to walk on the fragile pumice fields below the rock wall. A popular social trail was located directly east of Cloud Cap North, and while most visitors stayed on the path, there was evidence of foot traffic into the *B. pumicola* population. No signs of herbivory were observed at this site. Pocket gopher mounds were present.

Llao Rock

This site was located in rolling terrain at the summit of Llao Rock (Figures 2 and 4A, C). This site had the largest number of *B. pumicola* in four populations (Table 1). Population 1 (Llao West) was located on the western flank of the summit (Figure 4A,B); population 2 (Llao East) 20 meters east of Llao West (Figure 4C-E); population 3 (not sampled) 10 m from Llao West on the northwest flank of Llao Rock; population 4 (not sampled) 50 m from population 3 down the northwest flank. *Botrychium lanceolatum* subsp. *lanceolatum* also occurred near Llao West and population 3 (Figure 4F).

The substrate consisted of white “popcorn” Mazama pumice 1-5cm in diameter, mixed with gravel and silt. Soil excavation revealed buildup of organic matter; soil tests confirm this site has the highest organic matter (2.75%) of all the Crater Lake sites (Table 2).

Associated plant cover was dense with *Raillardella argentea, C. breweri, Elymus elymoides* and *Agoseris sp*. the most common plant associates. The distribution of *B.*

pumicola at this site was dense with several clumps of three to five *B. pumicola* (Figure 4B). The site was open and windswept with clumps of *P. albicaulis* scattered along the southern and western edge of the population. To the south was the caldera precipice.

Human foot traffic was minimal although occasional hikers reach the site via the west and north. Elk and deer scat were observed below the site but no signs of herbivory. Pocket gopher mounds were present at this site.

Dutton Ridge

This site was located 10 m below the summit of Dutton Cliff in open, windswept "popcorn" pumice substrate similar to Llao Rock (Figure 4G, H; Table 1). Although two *B. pumicola* individuals were observed during separate site visits, only one individual was re-located for sampling. The site was bordered on the south by a stand of *P. albicaulis* with other *P. albicaulis* scattered around the site. Associated plant species were sparse with *A. occidentale, R. argentea* and *C. breweri* the most common. This was the second Crater Lake population where *R. argentea* occurs, but in much lower density than at Llao Rock. The density of *B. pumicola* was very low, with smallest number of individuals of all populations (Table 1). Pocket gopher mounds were present.

Deschutes National Forest

The Dome

This site was located in Newberry Volcanic National Monument, administered by the USDA Forest Service, Deschutes National Forest (Figure 1; Table 1). The Dome is a horseshoe shaped volcanic cinder cone with a layer of Newberry "popcorn" pumice outfall from the Big Obsidian Flow eruption (Figure 5A, B). The *B. pumicola* population

was scattered along the southwest bend in the "horseshoe". The site was open and windswept with scattered *P. albicaulis*.

The substrate resembles Grotto Cove but the pumice source was from Newberry volcano, not from Mt. Mazama. There was a mix of Newberry pumice with gravel and silt and a different suite of plant associates than at Crater Lake. The most common associated plants were *P. hoodii, Castilleja applegatei,* and *Achillea millefolium.*

Broken Top

One of two populations on Broken Top, this site was located on the SE flank (Figures 1 and 5C; Table 1). A hiking trail was located 5 m east of the site, however there was little sign of disturbance within the *B. pumicola* population. This site has an unusual mix of pumice, gravel and hard packed clay soil substrate. The latter had little associated plant cover but the most robust *B. pumicola* of all sites visited (Figure 5D). Soil tests revealed very high calcium levels (2300 mg/kg), the highest of all sites (Table 2).

There were scattered *P. albicaulis* throughout the site; the most common plant associates were *A. millefolium* and *Penstemon davidsonii*. No *B. pumicola* occurred on the slope above or directly below the population, only in the reddish/brown substrate.

Mount Bachelor

This alpine site was located on the summit of Mt. Bachelor in open, windswept volcanic rubble from the Mt. Bachelor eruptions (Figures 1 and 5E-H; Table 1). The upper site was located on Mt. Bachelor summit (Figure 5G) and the lower site on the eastern flank of the summit (Figure 5H). *Botrychium pumicola* were found between rocks in pockets of Mazama and Sisters pumice, gravel and silt (Figure 5F). Associated plant cover was sparse with *P. davidsonii* and *C. breweri* the most common. *Botrychium*

pumicola were found nestled in micro-sites with other plants where some soil development had occurred. This site resembles the Shastina Cone site, but with more soil development and plant cover and *B. pumicola* scattered throughout the site, unlike Shastina Cone where they only occurred nestled in rock shelters. A hiking trail bisects the upper and lower sites and leads directly to the summit. The upper site was fenced to protect the *B. pumicola* population (Charmane Powers, USFS, personal communication; Figure 5E).

Shasta -Trinity National Forest

Shastina Cone

A single population of *B. pumicola* occurred in the Shasta-Trinity National Forest of northern California (Figure 1; Table 1). This population was located on an exposed, windy southwest flank of Shastina Cone near the upper end of Diller Canyon (Figure 6A, E). The substrate was andesitic volcanic rubble from the Shastina Cone eruptions mixed with gravel and silt (Figure 6B, F); no pumice was observed at this site. Associated plants were sparse with *P. davidsonii* and *C. breweri* the most common. The site was scattered with clumps of *P. albicaulis* krummholz (Figure 6E). *Botrychium pumicola* were located in rock caves and crevices with small amounts of soil and shelter from the wind (Figure 6C, D, G, H).

This population was documented by Cooke (1941) and had been discounted as representing *B. pumicola* for many years (Hickman et al.1993). It had been suggested that the Shastina Cone plants may have been a mistaken identification of *B. pinnatum* (Hopkins et al., 2006). Staff from Shasta-Trinity National Forest rediscovered three *B. pumicola* individuals on 13 July 2009 (Eric White, USFS, personal communication). The

author accompanied Dr. Donald R. Farrar and staff from Crater Lake National Park and Shasta-Trinity National Forest to the site on 4 August 2009 to confirm the plants as *B. pumicola*. Three more *B. pumicola* plants were located in rock crevices near the first sightings.

Due to the small population size, soil samples were not taken here. Soil development only occurred around *B. pumicola* individuals and associated plants, and thus the risk of disturbance and removal of *B. pumicola* gemmae, gametophytes, and juvenile sporophytes was too great to justify soil removal.

Table 1. *Botrychium pumicola* study sites arranged by lowest to highest elevation in the Cascade Range of central and southern Oregon and northern California. CLNP, Crater Lake National Park, Oregon; NVNM, Newberry Volcanic National Monument; DSNF, Deschutes National Forest, Oregon; STNF, Shasta-Trinity National Forest, California.

Population	Location	Size (ha)	Elev. (m)	Aspect + bin	Slope	Total *B. pumicola*
Grotto Cove	CLNP	0.025	2096	12° N	3-10°	13
Skell Head	CLNP	0.025	2180	250° SW	7.5°	37
The Dome	NVNM	0.051	2193	18° N	12°	75
Broken Top	DSNF	0.101	2333	82° E - 140° SE	15°	636
Cloud Cap South	CLNP	0.025	2400	250° SW	27°	22
Cloud Cap North	CLNP	0.404	2402	185° S -210° SW	19-25°	115
Llao Rock West	CLNP	0.05	2444	110° E, 290° W	9°	≥1000
Llao Rock East	CLNP	0.404	2444	250° SW	0-19°	257
Dutton Ridge	CLNP	0.025	2468	190° S	9°	2
Shastina Cone	STNF	0.025	2755	220° SW	15-17°	6
Mt. Bachelor	DSNF	0.101	2761	129° SE - 172° S	15°	372

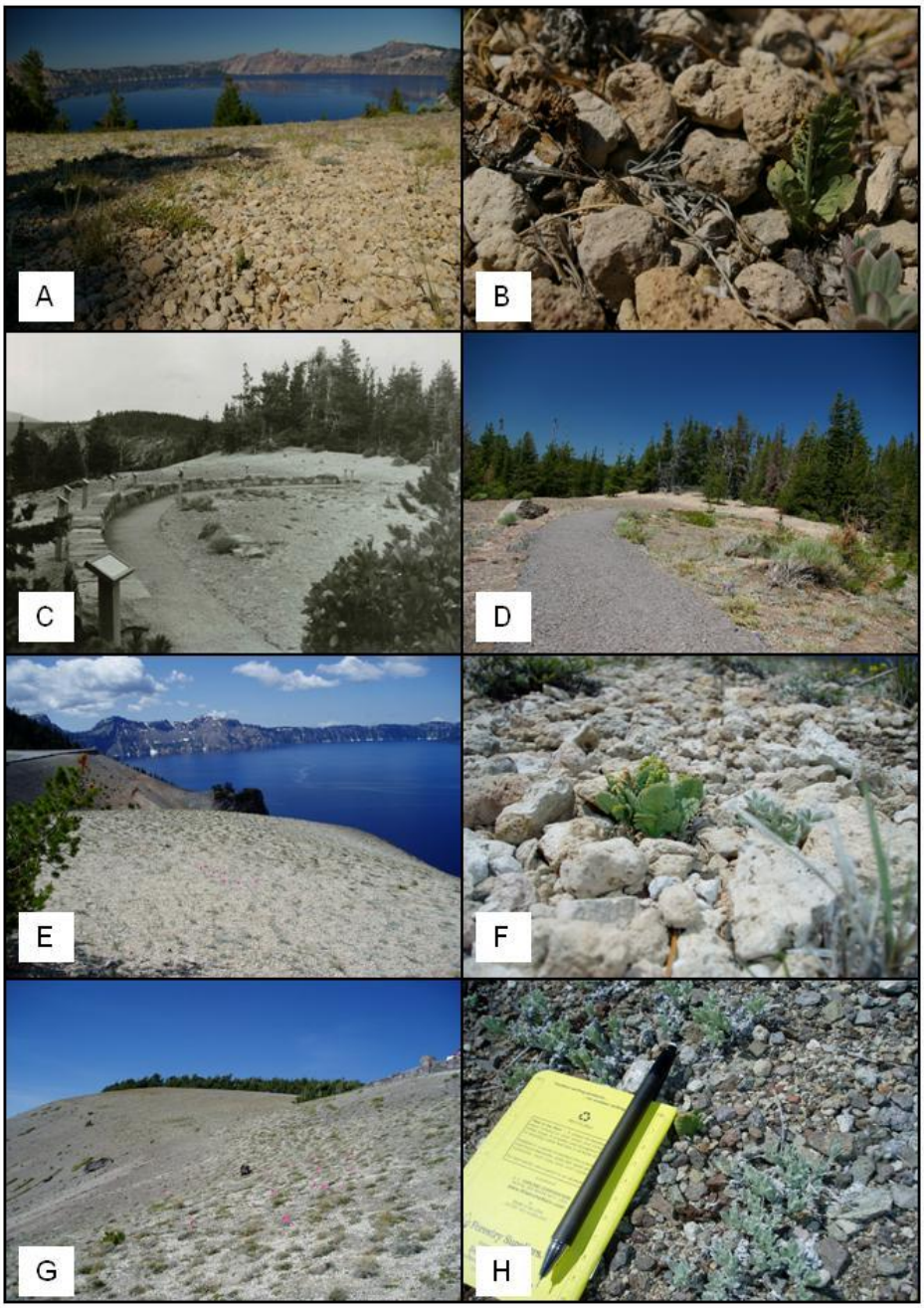

Figure 3. *Botrychium pumicola* study sites at Crater Lake National Park, Oregon: (A) Grotto Cove; (B) Grotto Cove *B. pumicola*; (C) Grotto Cove Nature Trail c. 1968 (Courtesy Crater Lake National Park); (D) Remains of Grotto Cove Nature Trail 2009; (E) Skell Head; (F) Skell Head *B. pumicola*; (G) Cloud Cap North and South; (H) Cloud Cap South *B. pumicola* in dark gravel substrate.

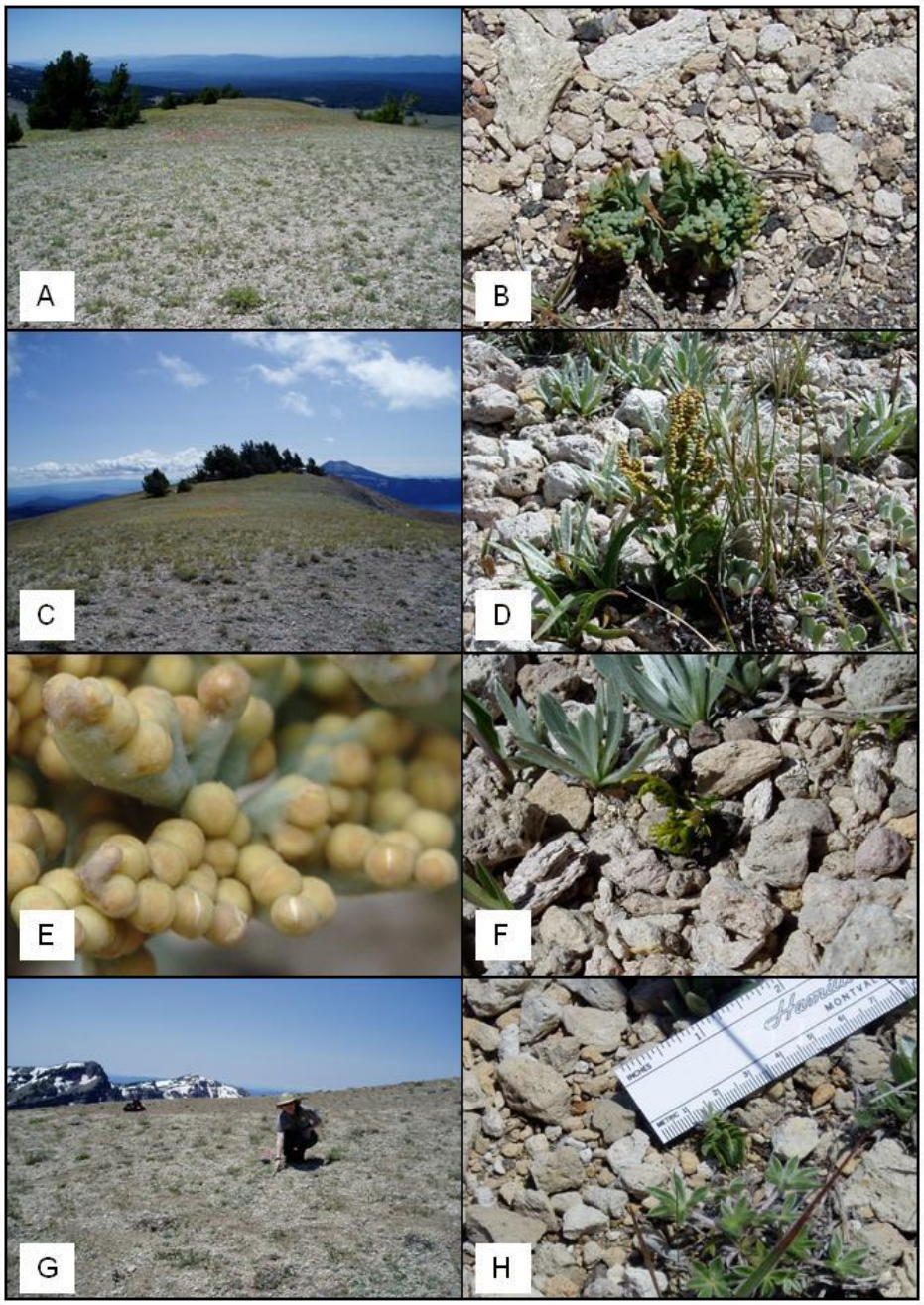

Figure 4. *Botrychium pumicola* study sites at Crater Lake National Park, Oregon: (A) Llao Rock West; (B) Llao Rock West *B. pumicola*; (C) Llao Rock East; (D) Llao Rock East *B. pumicola*; (E) Llao Rock East *B. pumicola* sporangia opening to reveal spores; (F) Llao Rock *B. lanceolatum* subsp. *lanceolatum* between East and West *B. pumicola* populations; (G) Dutton Ridge; (H) Dutton Ridge *B. pumicola*.

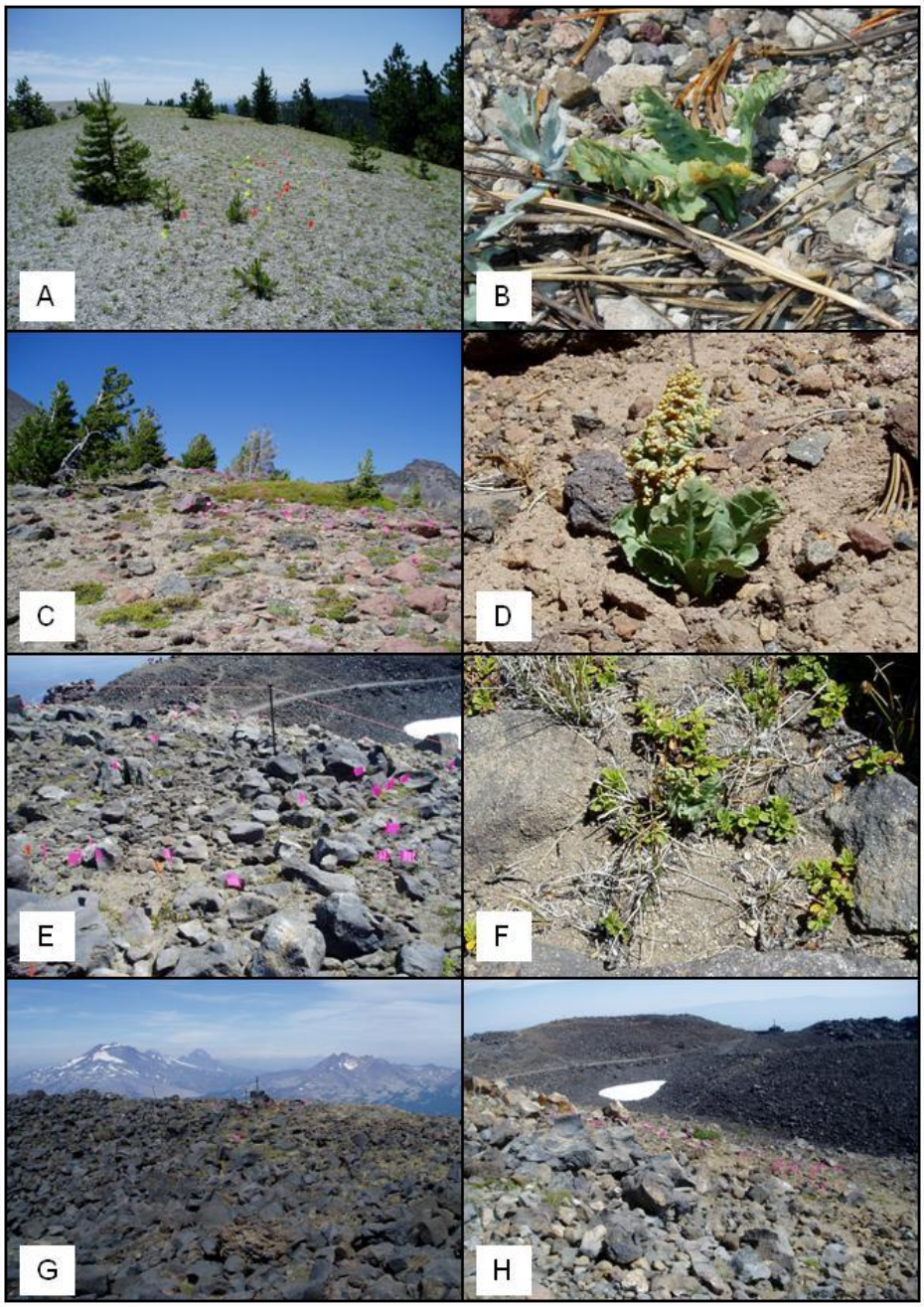

Figure 5. Deschutes National Forest, Oregon *Botrychium pumicola* populations: (A) The Dome; (B) The Dome *B. pumicola*; (C) Broken Top; (D) Broken Top *B. pumicola*; (E) Mt. Bachelor upper site; (F) Mt. Bachelor *B. pumicola*; (G) Mt. Bachelor site looking north toward Broken Top, North and Middle Sister; (H) Mt. Bachelor lower site.

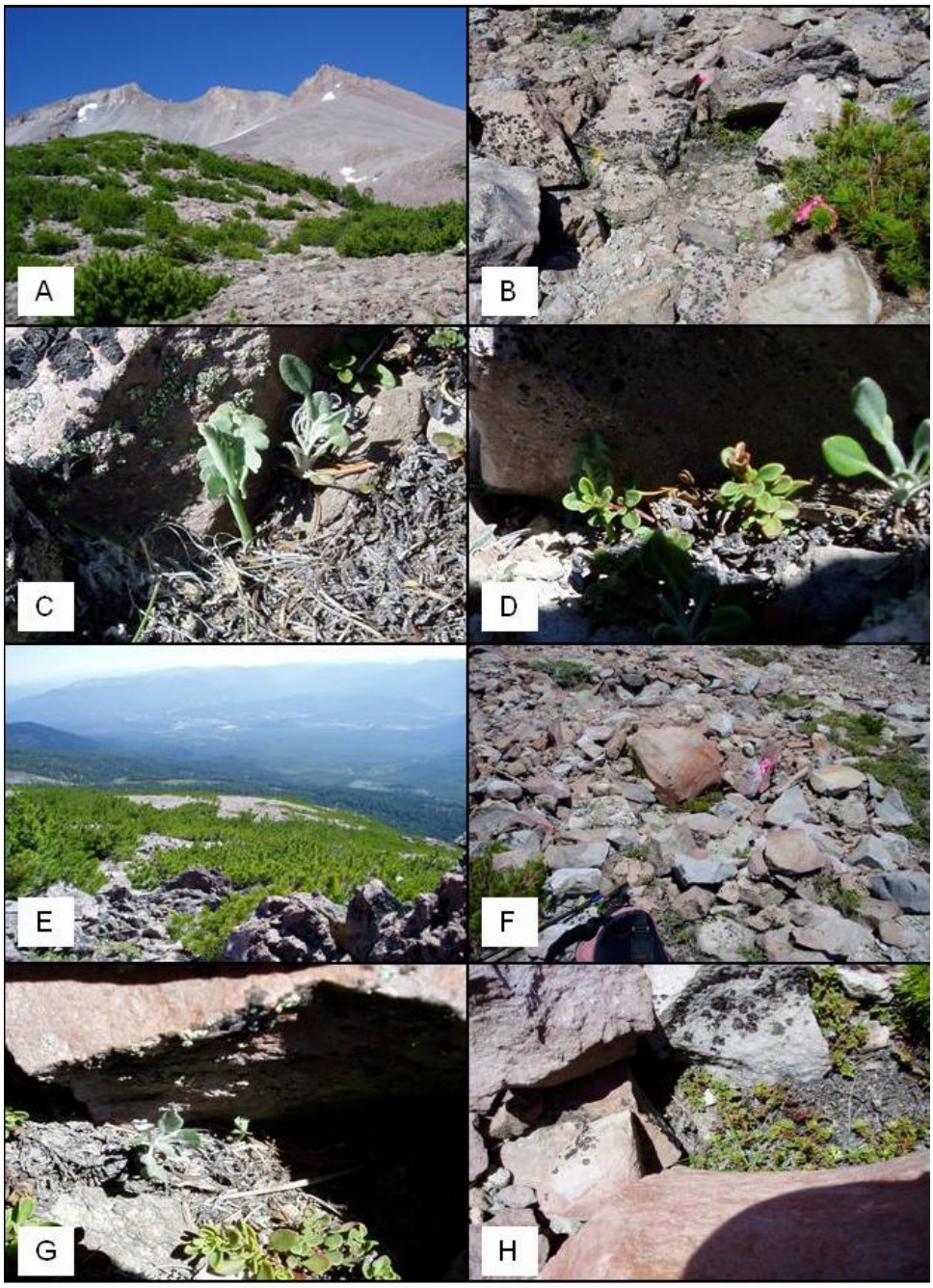

Figure 6. Shastina Cone *Botrychium pumicola* site, Shasta-Trinity National Forest, California: (A) Upper site view west to Shastina Cone summit; (B) Upper site rocks sheltering *B. pumicola*; (C) Upper site largest *B. pumicola*; (D) Upper site *B. pumicola* with *P. davidsonii*; (E) View east to Lower site and *P. albicaulis* krummholz; (F) Lower site rocks sheltering *B. pumicola*; (G,H) Lower site *B. pumicola* in crevices.

Abiotic Factors

Soil nutrient composition did not differ between paired plots with and without *Botrychium pumicola* in regards to pH, P, K, Mg, Na, Ca, NO_3, NH_4, and percent organic matter (% OM) (Wilcoxon Matched Pairs Test and *t*-test; Table 2). Without the Bonferroni correction, K was significantly higher in plots with *B. pumicola* (Wilcoxon Matched Paired Test $P = 0.024$ and *t*-test $P = 0.027$; Table 2). Percent organic matter was higher in plots with *B. pumicola* (Wilcoxon Matched Paired Test $P = 0.066$; Table 3).

Soil textural properties did not differ between plots with and without *B. pumicola* in soil density, percent gravel, sand, silt, or clay (Wilcoxon Paired Test and *t*-test). Without the Bonferroni correction, soil density was higher in plots without *B. pumicola* (Wilcoxon Paired Test $P = 0.032$; Table 3). Percent gravel was higher in plots without *B. pumicola* (Wilcoxon Matched Paired Test $P = 0.051$; Table 3).

Site characteristics did not differ between plots with and without *Botrychium pumicola* in soil temperature (Wilcoxon Matched Pairs Test or *t*-test). Without the Bonferroni correction, soil temperature was higher in plots without *B. pumicola* (Wilcoxon Matched Pairs Test $P = 0.006$; Table 4). Most *B. pumicola* plots occurred on S, W, and SW aspects (Chi-square Goodness of Fit Test $P = .045$; Figure 7).

NMS ordination of all abiotic factors showed a high degree of overlap between plots with and without *B. pumicola* (Figure 8). No significant differences in abiotic factors were observed using MRPP for all populations ($P = 0.180$). When outliers (Broken Top plots with *B. pumicola* and Mt. Bachelor plots without *B. pumicola*) were removed, significant differences were observed (MRPP, $P = 0.049$).

NMS ordination of all abiotic factors and plant associates with singletons removed (any plant associate that occurred in only one population), showed no significant difference between plots with or without *B. pumicola* (MRPP, $P = 0.875$).

An NMS joint/bi-plot of all plant associates and soils data showed correlations between soil nutrients and soil characteristics on distribution of *B. pumicola* populations (Figure 9). The Dome correlated with higher soil temperatures, percent gravel and levels of NO_3; Skell Head and Llao Rock correlated with higher percentage of sand; Broken Top correlated with higher percentage of clay; and Mt. Bachelor and Broken Top correlated with higher levels of Ca.

Table 2. Soil nutrients in plots with and without *Botrychium pumicola* by *t*-test and Wilcoxon Matched Pairs test for alpine/subalpine populations in the Cascade Range of central and southern Oregon. Significant differences without applying Bonferroni correction indicated with (*). No significant differences were observed when Bonferroni correction was applied.

Population	pH	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Ca (mg/kg)	NO_3 (mg/kg)	NH_4 (mg/kg)
B. pumicola present								
Llao West	6.3	30	88	47	21	405	1.7	2.0
Llao East	6.5	20	88	59	19	513	1.3	1.6
Broken Top	6.3	40	82	129	20	461	1.3	4.9
Broken Top2	6.6	29	152	1190	18	2300	1.1	2.1
Dome	6.3	10	119	136	27	597	3.1	2.3
Grotto Cove	6.2	48	52	27	29	95	0.3	0.9
Dutton Ridge	6.3	45	71	31	13	167	0.09	1.2
Cloud Cap N	6.8	10	115	154	17	519	0.5	1.0
Cloud Cap S	6.6	12	91	111	14	329	0.7	1.1
Skell Head	6.5	30	63	43	28	176	0.4	1.0
Mt. Bachelor	6.5	19	107	194	24	1230	0.6	1.5
Mean (sd)	6.45 (0.181)	26.64 (13.68)	93.45 (28.42)	192.82 (335.44)	20.91 (5.49)	617.46 (636.46)	1.01 (0.852)	1.78 (0.143)
B. pumicola absent								
Llao West	6.5	27	63	54	15	217	0.8	1.4
Llao East	6.5	35	47	31	15	117	0.9	1.0
Broken Top	5.7	50	39	23	13	45	1.3	1.2
Dome	6.3	12	98	145	22	589	3.2	1.7
Grotto Cove	6.5	43	47	26	19	112	0.8	1.0
Dutton Ridge	6.4	39	76	38	18	171	1.3	1.4
Cloud Cap S	6.5	14	90	122	19	372	1.0	1.2
Skell Head	6.4	31	59	38	19	157	0.7	1.0
Mt. Bachelor	6.4	15	82	488	20	2010	0.9	1.4
Mean (sd)	6.36 (0.255)	29.6 (13.6)	66.8 (20.8)	107 (149)	17.78 (2.86)	421 (618)	1.21 (0.775)	1.26 (0.240)
P (*t*-test)	0.389	0.640	0.027*	0.460	0.122	0.495	0.585	0.165
P (Wilcoxon)	0.866	0.953	0.024*	0.594	0.086	0.594	0.401	0.123

Table 3. Soil textural properties in plots with and without *Botrychium pumicola* by *t*-test and Wilcoxon Matched Pairs test for alpine/subalpine populations in the Cascade Range of central and southern Oregon. Soil properties include soil density and arcsine transformed percent organic matter (OM), water (H_2O), gravel, sand, silt and clay. Significant differences without applying Bonferroni correction indicated with (*). No significant differences were observed when Bonferroni correction was applied.

Population	Soil density (g/L)	% OM	% H_2O	% Gravel	% Sand	% Silt	% Clay
B. pumicola present							
Llao West	371.25	2.75	4.26	23.67	91.30	7.50	1.30
Llao East	371.73	2.46	11.86	20.01	91.30	5.00	3.80
Broken Top	405.62	2.77	2.51	34.66	83.80	12.50	3.80
Broken Top 2	454.11	2.51	4.66	22.19	63.80	15.00	21.30
Dome	342.51	2.32	5.54	56.00	85.00	12.50	2.50
Grotto Cove	532.36	1.02	0.97	25.14	92.50	3.80	3.80
Dutton Ridge	499.67	1.81	4.26	13.65	92.50	5.00	2.50
Cloud Cap N	591.70	1.64	1.15	57.60	90.00	6.30	3.80
Cloud Cap S	465.71	1.78	1.94	36.92	90.00	7.50	2.50
Skell Head	357.00	1.02	5.10	21.73	96.30	2.50	1.30
Mt. Bachelor	494.68	1.89	8.97	20.44	86.30	10.00	3.80
Mean (sd)	444.21 (81.069)	1.99 (0.623)	4.66 (3.312)	30.18 (14.692)	87.53 (8.671)	7.96 (4.034)	4.58 (5.631)
B. pumicola absent							
Llao West	505.05	1.52	0.99	17.93	93.80	3.80	2.50
Llao East	559.59	0.85	8.05	35.37	97.50	1.30	1.30
Broken Top	508.36	2.74	1.85	49.11	82.50	13.80	3.80
Dome	404.47	2.84	9.15	67.00	81.30	15.00	3.80
Grotto Cove	460.35	0.87	1.06	25.33	97.50	1.30	1.30
Dutton Ridge	542.09	1.49	7.53	31.30	92.50	6.30	1.30
Cloud Cap S	558.77	1.65	1.41	37.41	92.50	3.80	3.80
Skell Head	483.64	0.99	9.41	22.97	90.00	6.30	3.80
Mt. Bachelor	493.68	1.86	11.32	20.32	82.50	10.00	3.80
Mean (sd)	501.8 (49.811)	1.65 (0.739)	5.64 (4.228)	34.08 (15.733)	90.01 (6.399)	6.84 (5.068)	2.82 (1.216)
P (*t*-test)	0.069	0.249	0.718	0.558	0.427	0.478	0.343
P (Wilcoxon)	0.032*	0.066	0.678	0.051	0.889	0.484	0.800

Table 4. Soil temperature of plots with and without *Botrychium pumicola* by *t*-test and Wilcoxon Matched Pairs test for alpine/subalpine populations in the Cascade Range of central and southern Oregon. Significant differences without applying Bonferroni correction indicated with (*). No significant differences were observed when Bonferroni correction was applied.

Population	Soil Temp °C	
	B. pumicola present	*B. pumicola absent*
Llao West	17.87	20.81
Llao East	10.25	14.35
Broken Top	20.58	20.32
Dome	22.32	23.92
Grotto Cove	24.30	20.68
Dutton Ridge	15.60	15.70
Skell Head	19.10	18.68
Mt. Bachelor	13.74	15.08
Mean (sd)	18.80 (4.48)	18.69 (3.37)

P (*t*-test)	0.955
P (Wilcoxon)	0.006*

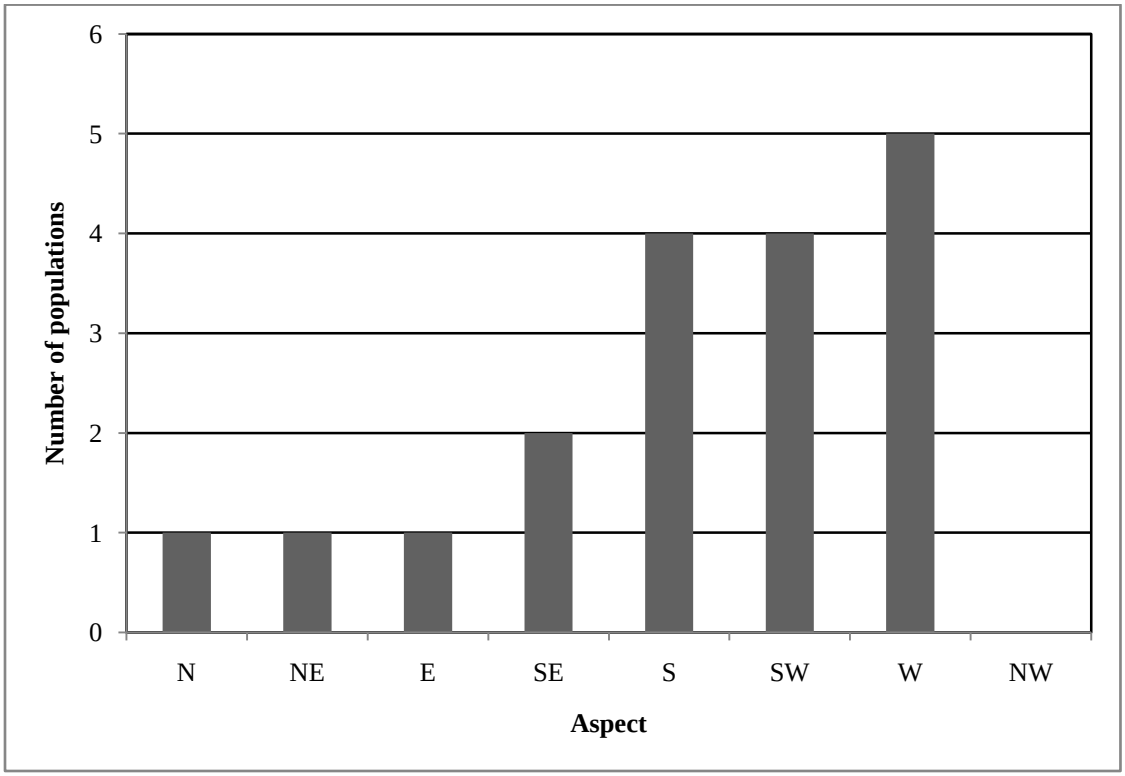

Figure 7. Aspect of *Botrychium pumicola* populations in the Cascade Range of central and southern Oregon and northern California. Aspect measurements taken for each population were binned into one of eight cardinal directions: North (N), 336-25°; North East (NE), 26-70°; East (E), 71-110°; South East (SE), 111-160°; South (S), 161-200°; South West (SW), 201-250°; West (W), 251-290°; North West (NW), 291-335°.

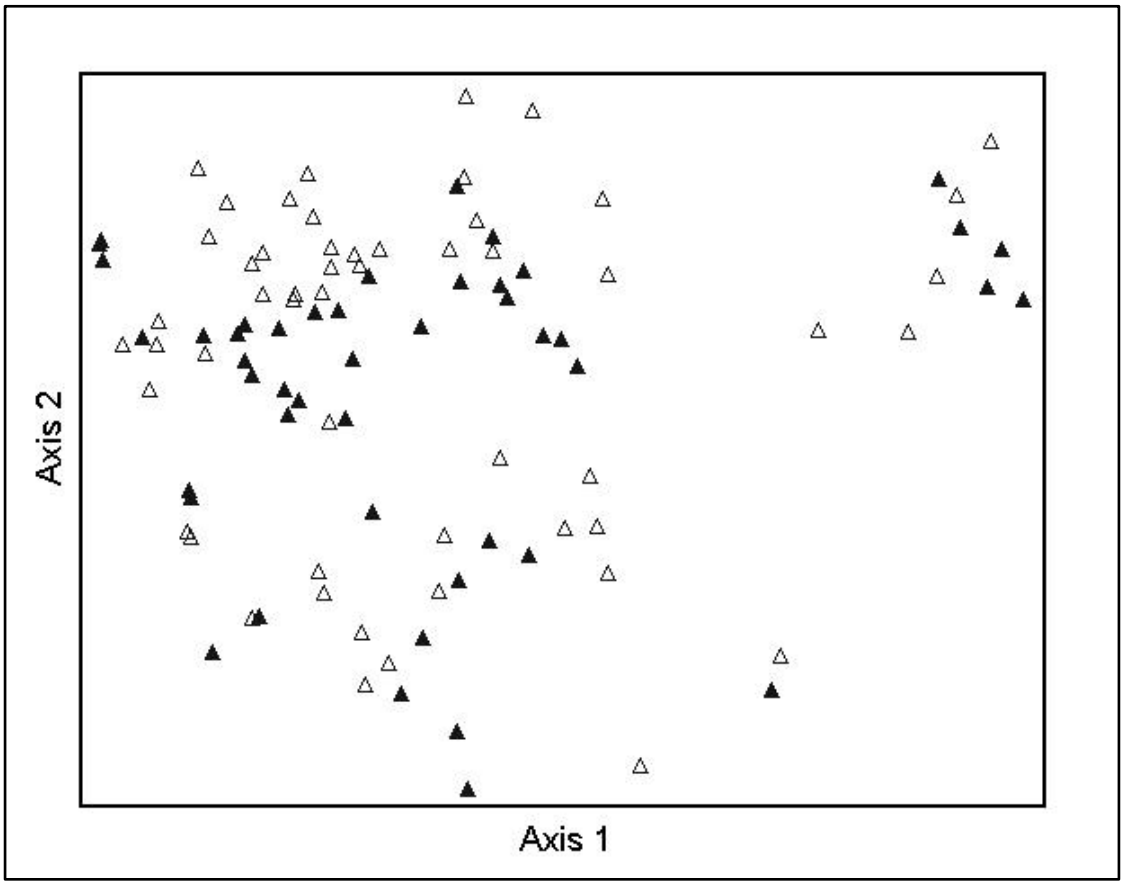

Figure 8. NMS ordination of all abiotic factors for *Botrychium pumicola* populations in the Cascade Range of central and southern Oregon. Δ = with *B. pumicola* ;▲ = without *B. pumicola.*

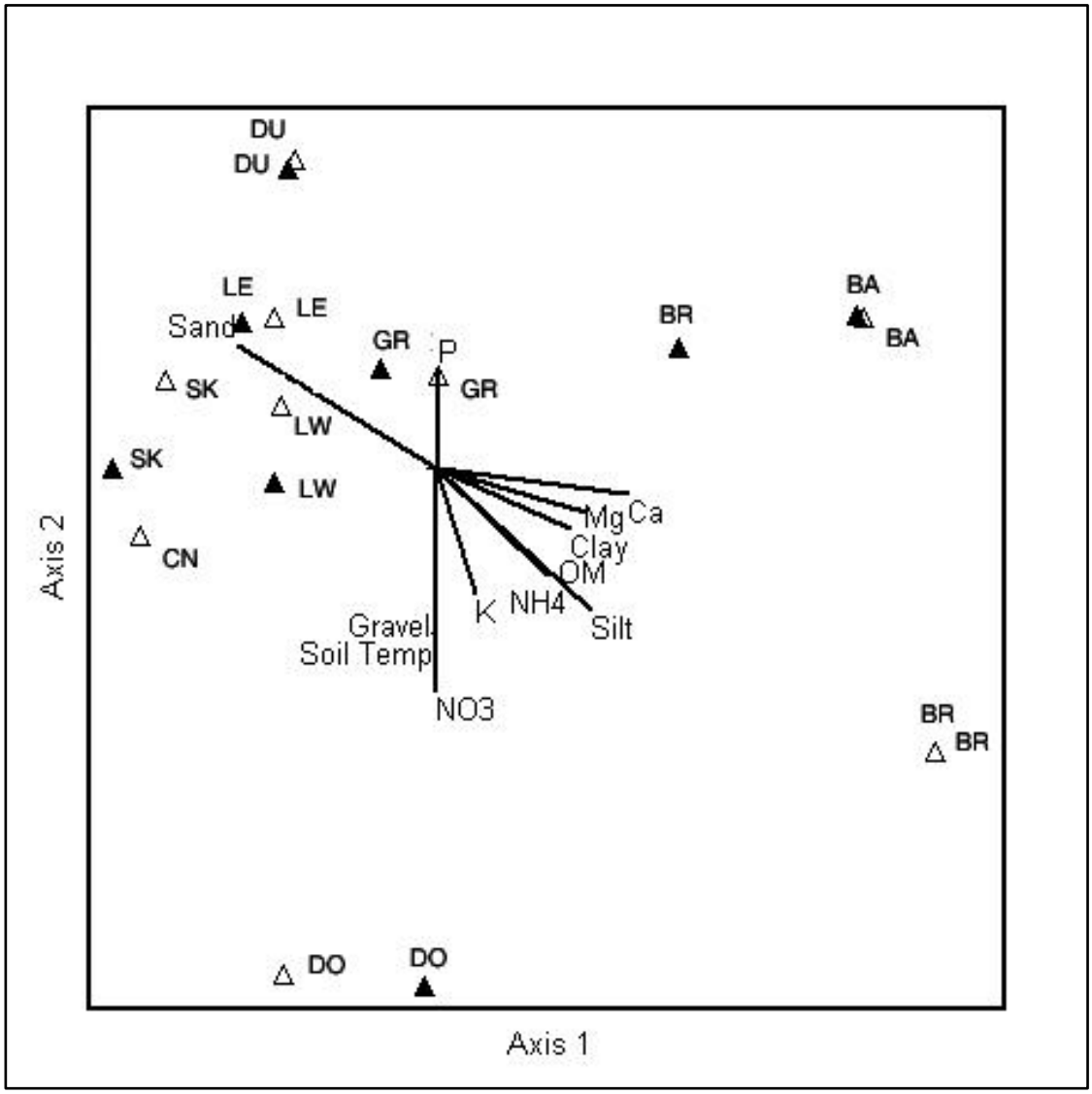

Figure 9. NMS joint/bi-plot of all plant associates and soil factors for *Botrychium pumicola* populations in the Cascade Range of central and southern Oregon. Δ = with *B. pumicola* . ▲= without *B. pumicola*. BA, Mt. Bachelor; BR, Broken Top, CN, Cloud Cap North; DO, The Dome; DU, Dutton Ridge; GR, Grotto Cove; SK, Skell Head.

Biotic Factors

Thirty-five associated plant species occurred in plots with *B. pumicola* and 32 in plots without *B. pumicola* (Appendix A and B). NMS ordination of all plant associates showed some separation of plots with and without *B. pumicola* (Figure 10) Differences in plant associates were observed using MRPP (P = 0.015).

When outliers (*Raillardella argentea* and *Carex breweri*) were removed, no difference between groups was observed (MRPP, P = 0.140). In order to minimize the effect of plots with disproportionately large numbers, a relativization was applied, then plots with and without *B. pumicola* differed (MRPP, P = 0.007; Figure 11).

When associated plant species occurring in fewer than 5% of plots were removed, plots with and without *B. pumicola* differed (MRPP P = 0.018; Figure 12). When outlier *R. argentea* was removed from the data set, plots with and without *B. pumicola* did not differ (MRPP, P = 0.268; Figure 13).

The density of associated plants (number per m^2) showed a high range of variability in plots with and without *B. pumicola* (Appendix C and D). Neither NMS ordination of all plant associates per m^2 with singletons removed, or MRPP differed between sites with and without *B. pumicola* (P = 0.875). Species area curves (Figure 14A, B) indicated that plots with *B. pumicola* were not richer in plant associate species (first order jackknife estimate = 44.9) than plots without *B. pumicola* (first order jackknife estimate = 42.5). At eight plots, species area curves with and without *B. pumicola* had 32 species of associated plants.

Nearest neighbor indices (NNI) of *B. pumicola* showed a clumped to random dispersion (Table 5). Four populations (Broken Top, The Dome, Mt. Bachelor and

Shastina Cone) were below 1.00 which indicated some clumping. The remaining populations, Llao East, Llao West, Cloud Cap North, Grotto Cove and Skell Head, had NNIs of 1.101- 1.328 indicating a random distribution. No population approached the maximum 2.1491 value; at this scale the NNIs did not indicate a uniformly spaced dispersion.

Nearest neighbor indices between each *B. pumicola* individual and its nearest associated plant species showed a clumped dispersion except at Broken Top (Table 5). Eight populations, Llao Rock, Cloud Cap, the Dome, Dutton, Grotto Cove, Mt. Bachelor, Skell Head and Shastina Cone, had NNIs less than 1.00 indicating a clumped dispersion. Broken Top had an NNI average of 2.041 indicating a uniform dispersion.

Mean trophophore and sporophore measurements ranged from 1.5 to 2.625 cm and 1.5 to 3.5 cm respectively (Figures 15 and 16; Table 6). Mean trophophore length was highest at Mt. Bachelor, followed by Broken Top and Grotto Cove. Lowest mean trophophore length was found at Dutton Ridge. Mean sporophore length was highest at Broken Top and lowest at Dutton Ridge.

Dutton Ridge, Cloud Cap North, Skell Head, Shastina Cone and the Dome had the highest percentage of trophophores in the small (0.5 - 1.5 cm) size class (Figure 17). Llao East and West and Grotto Cove had the lowest percentage of small size class trophophores, with most of these populations in the medium (2-3.5 cm) size class. Only Mt. Bachelor had trophophores in the largest (≥6 cm) size class.

Trophophore brown necrotic tissue was observed at every population with the exception of Shastina Cone (Figure 18; Table 7). All *B. pumicola* sampled at Dutton

Ridge, Dome, Grotto Cove and Skell Head had brown necrotic tissue. Of the populations with necrotic tissue, Mt. Bachelor and Llao West had the lowest percentage.

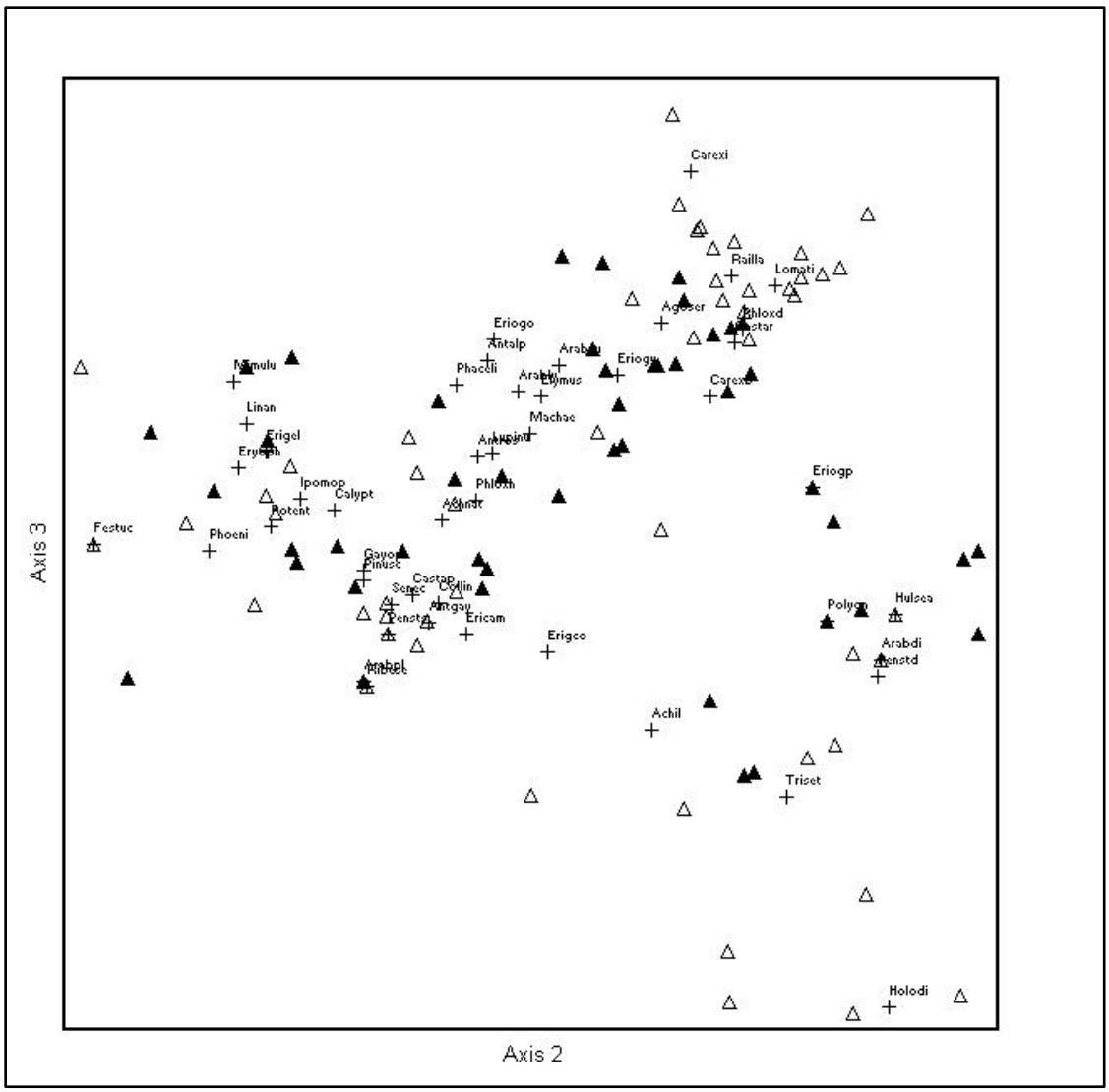

Figure 10. NMS ordination for all plant associates for all populations of *Botrychium pumicola* in the Cascade Range of central and southern Oregon and northern California. Δ = with *B. pumicola*, ▲ = without *B. pumicola*.

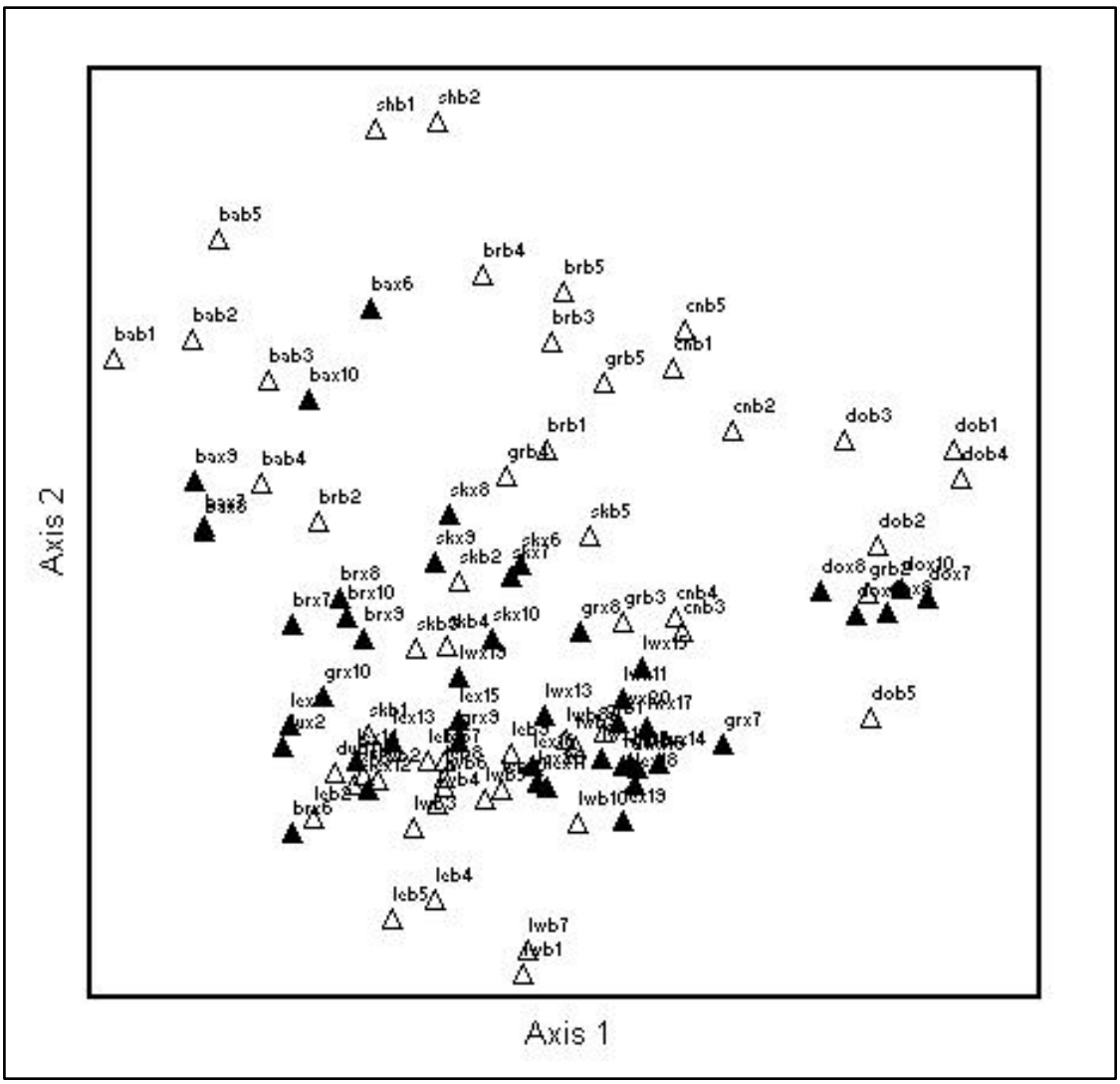

Figure 11. Relativized NMS ordination of all plant associates for all *Botrychium pumicola* in the Cascade Range of central and southern Oregon and northern California. Δ = with *B. pumicola*. ▲ = without *B. pumicola*.

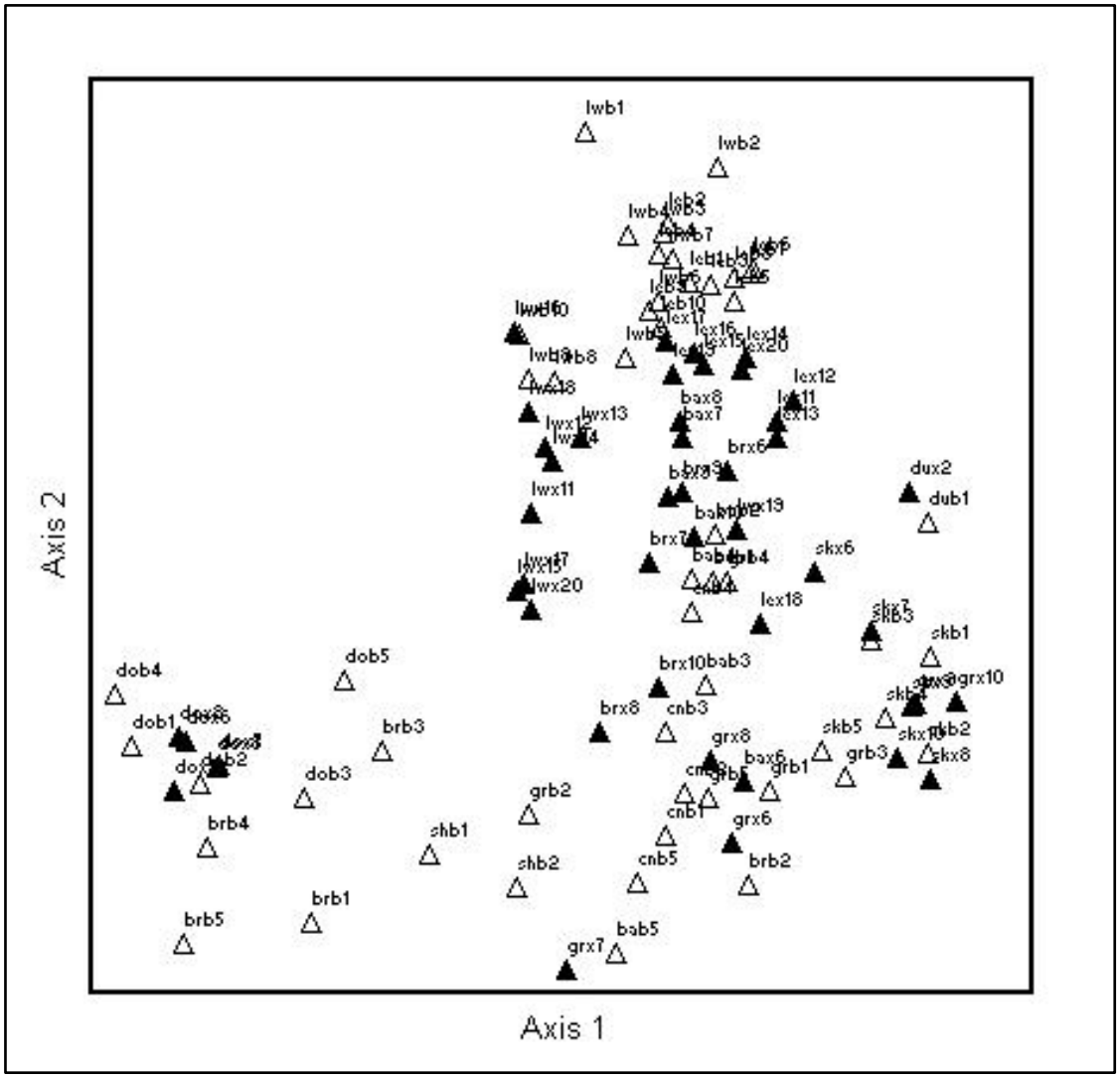

Figure 12. NMS ordination of plant associates in greater than 5% of plots at each *Botrychium pumicola* population in the Cascade Range of central and southern Oregon and northern California. Δ = with *B. pumicola*. ▲ = without *B. pumicola*.

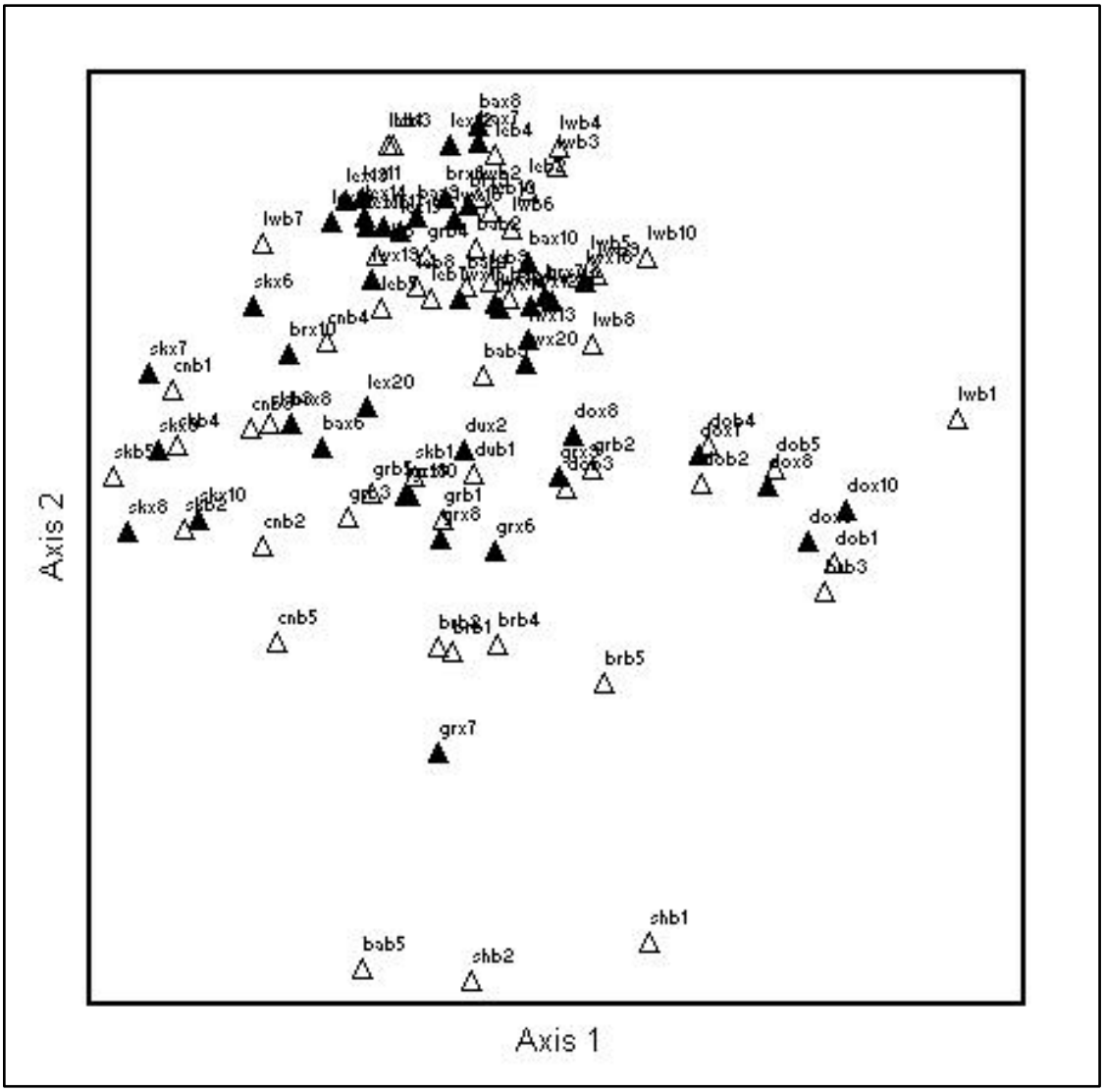

Figure 13. NMS ordination of plant associates in greater than 5% of plots with outlier *Raillardella argentea* removed, for each *B. pumicola* population in the Cascade Range of central and southern Oregon and northern California. Δ = with *B. pumicola*. ▲ = without *B. pumicola*.

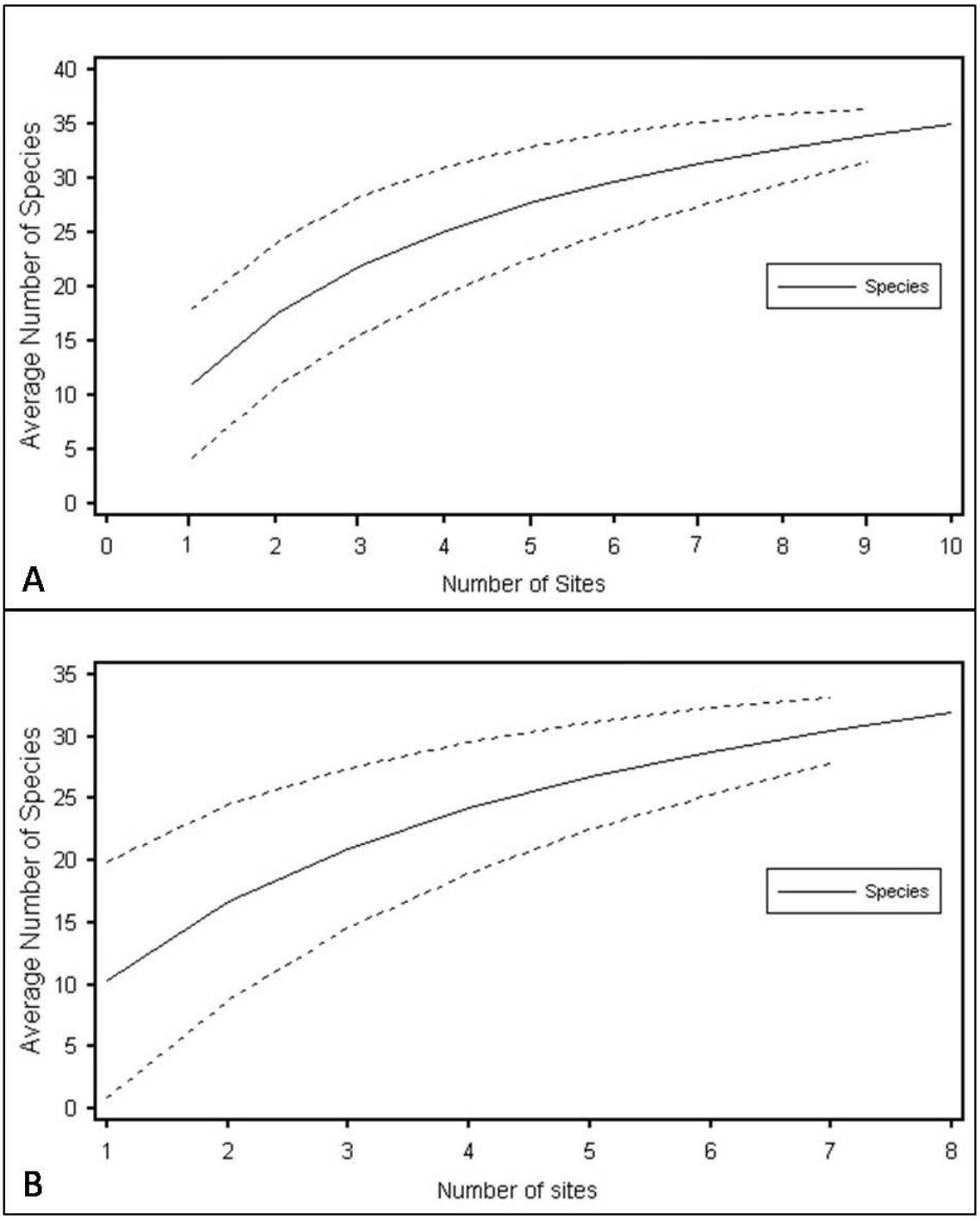

Figure 14 Species area curves showing species richness of associated plant species for plots (A) with *B. pumicola*; (B) without *B. pumicola*, in the Cascade Range of central and southern Oregon and northern California.

Table 5. Nearest Neighbor Analysis for *Botrychium pumicola* plots in the Cascade Range of central and southern Oregon and northern California. Average Nearest Neighbor Index (NNI) given for each population for *B. pumicola* to nearest *B. pumicola* dispersion and *B. pumicola* to nearest plant associate dispersion. Dutton Ridge *B. pumicola* dispersion could not be calculated due to a sample size of one.

Population	*B. pumicola* to nearest *B. pumicola* NNI	*B. pumicola* to nearest plant associate NNI
Dutton Ridge	0.000	0.742
Shastina Cone	0.336	0.095
Broken Top	0.607	2.041
Dome	0.675	0.950
Mt. Bachelor	0.735	0.726
Llao West	1.101	0.692
Grotto Cove	1.145	0.676
Cloud Cap	1.169	0.875
Llao East	1.221	0.656
Skell Head	1.328	0.859

Table 6. *Botrychium pumicola* trophophore and sporophore lengths (mean, sd) for populations in the Cascade Range of central and southern Oregon and northern California.

Population	Trophophore length (cm)	Sporophore length (cm)
Dutton Ridge	1.5 (0.0)	1.5 (0.0)
Cloud Cap N	1.9 (0.9)	2.1 (1.1)
Shastina Cone	2.1 (1.3)	2.1 (1.3)
Skell Head	2.1 (0.7)	2.1 (0.8)
The Dome	2.2 (0.7)	2.0 (0.9)
Llao Rock W	2.4 (0.7)	2.4 (1.0)
Llao Rock E	2.5 (0.7)	3.2 (1.3)
Grotto Cove	2.6 (0.7)	2.5 (0.8)
Broken Top	2.6 (1.2)	3.0 (2.1)
Mt. Bachelor	2.6 (1.4)	2.3 (1.6)

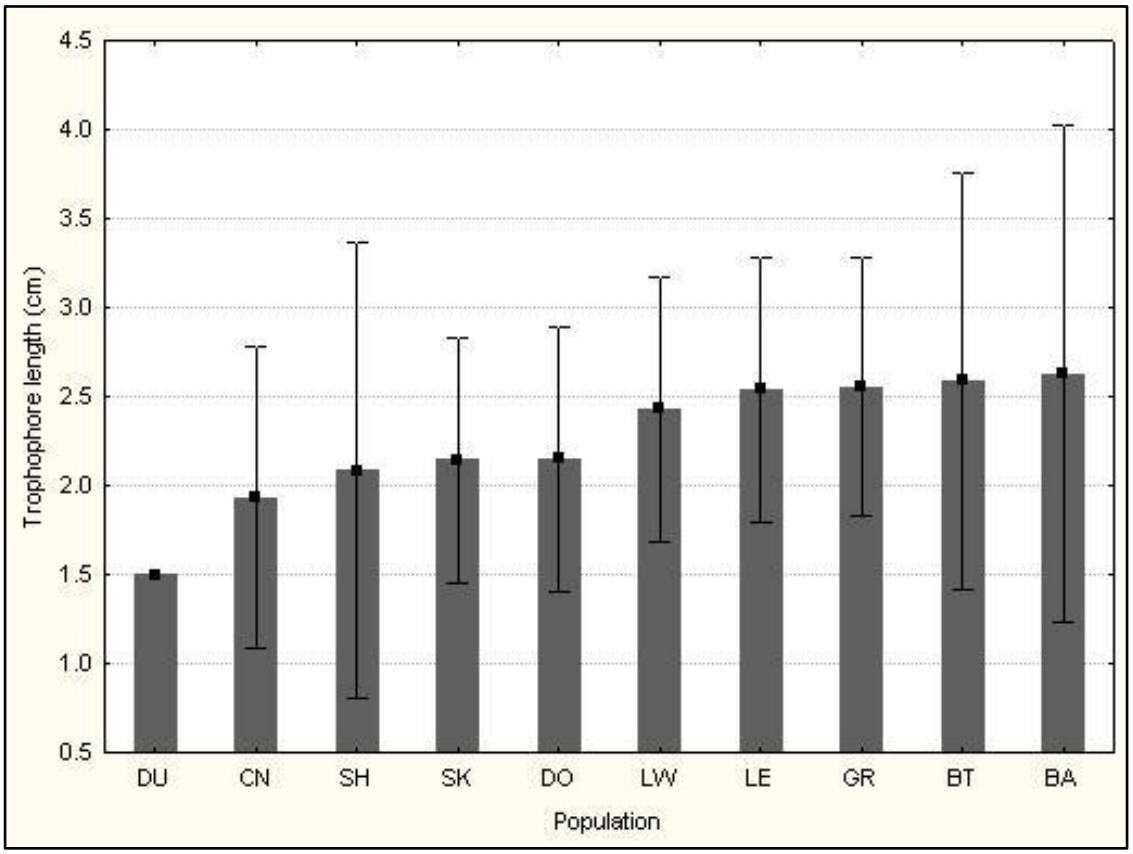

Figure 15. Trophophore length (mean, sd) for each population of *Botrychium pumicola* in the Cascade Range of central and southern Oregon and northern California. DU, Dutton Ridge; CN, Cloud Cap North; SH, Shastina Cone; SK, Skell Head; DO, The Dome; LW, Llao Rock West; LE, Llao Rock East; GR, Grotto Cove; BT, Broken Top; BA, Mt. Bachelor

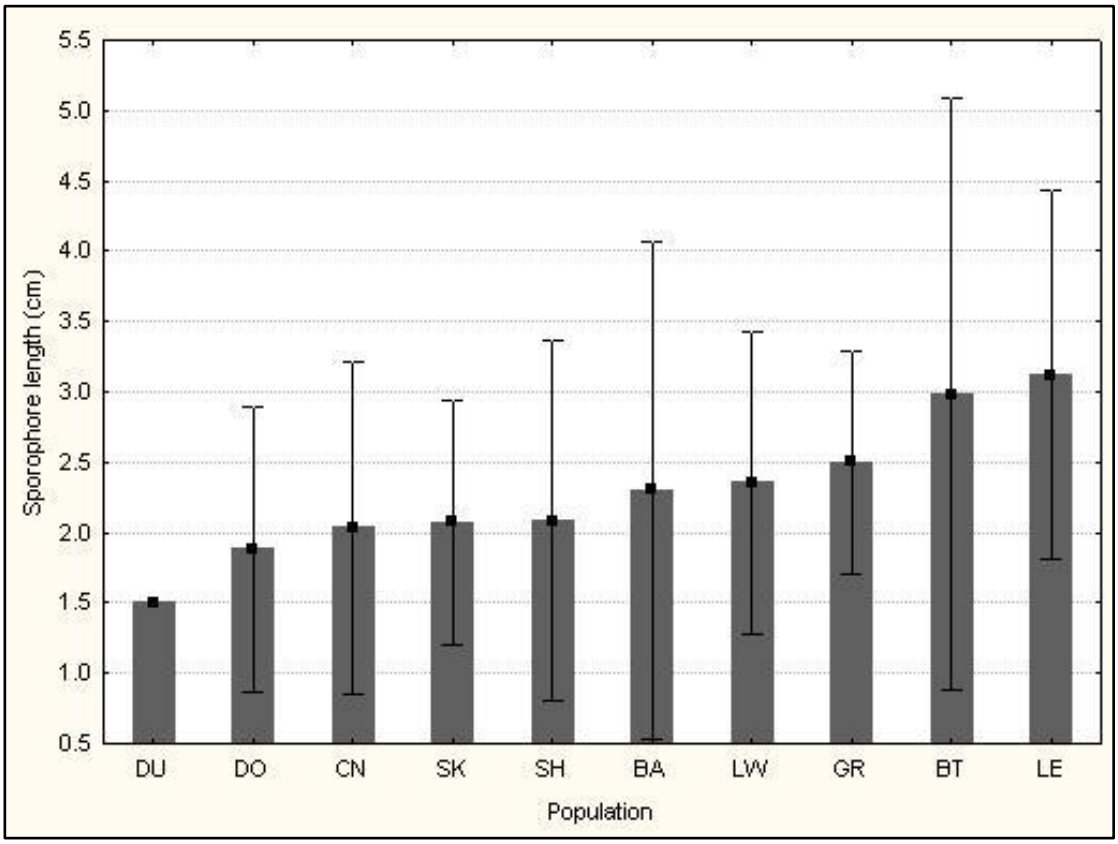

Figure 16. Sporophore length (mean, sd) for each *Botrychium pumicola* population in the Cascade Range of central and southern Oregon and northern California. DU, Dutton Ridge; DO, The Dome; CN, Cloud Cap North; SK, Skell Head; SH, Shastina Cone; BA, Mt. Bachelor; LW, Llao West; GR, Grotto Cove; BT, Broken Top; LE, Llao East.

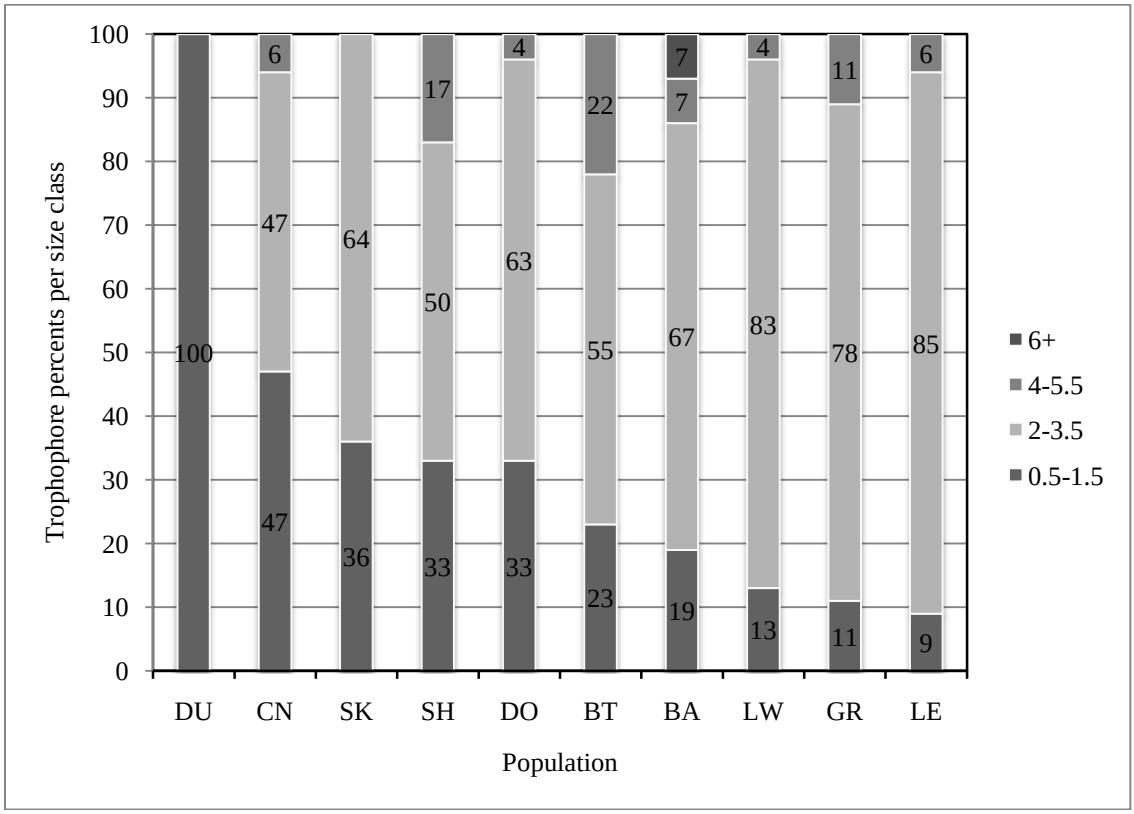

Figure 17. *Botrychium pumicola* trophophore size classes as a percent of the total population for each population in the Cascade Range of central and southern Oregon and northern California. Trophophore size classes: small (0.5-1.5 cm); medium (0.2-3.5 cm); large (0.4-5.5 cm); extra-large (≥0.6 cm). DU, Dutton Ridge; CN, Cloud Cap North; SK, Skell Head; SH, Shastina Cone; DO, The Dome; BT, Broken Top; BA, Mt. Bachelor; LW, Llao Rock West; GR, Grotto Cove; LE, Llao Rock East.

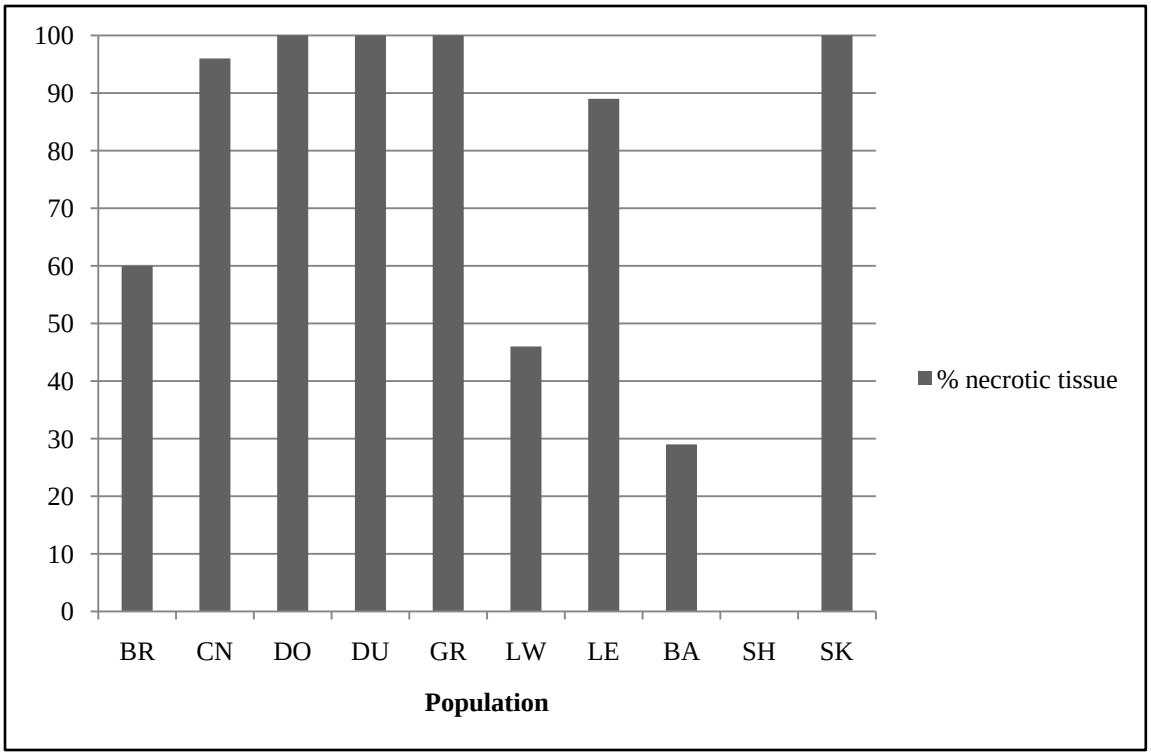

Figure 18. Percent of samples with trophophore brown necrotic tissue for each *Botrychium pumicola* population in the Cascade Range of central and southern Oregon and northern California.

Table 7. Frequency of *Botrychium pumicola* trophophores with brown necrotic tissue. Total *B. pumicola* population, number sampled, and percent of population with brown necrotic tissue for each population in the Cascade Range of central and southern Oregon and northern California.

Population	Total	Number sampled	Trophophores with necrotic tissue	Percent of samples with trophophore necrotic tissue
Grotto Cove	13	9	9	100
Skell Head	37	14	14	100
Dome	75	30	30	100
Broken Top	636	52	31	60
Cloud Cap South	22	0	0	0
Cloud Cap North	115	28	27	96
Llao Rock West	257	118	54	46
Llao Rock East	≥1000	99	88	89
Dutton Ridge	2	1	1	100
Mt. Bachelor	372	28	8	29
Shastina Cone	6	6	0	0

Isolation of AM fungal and *Botrychium pumicola* spores

No AM fungal spores were observed in any of the soil samples (Table 8). *Botrychium pumicola* spores were found in samples from Cloud Cap, Llao Rock, Grotto Cove, Broken Top and Mt. Bachelor (Figures 19 – 21). AM hyphae were observed in the plastic bag containing soil from Grotto Cove site (Figure 19D).

Table 8. Presence and absence of *Botrychium pumicola* and AM fungal spores by population in plots with and without *B. pumicola* from study sites in the Cascade Range of central and southern Oregon.

Population	*B. pumicola* spores	AM Fungal spores
B. pumicola present		
Cloud Cap North	1	0
Cloud Cap South	2	0
Grotto Cove	0	0
Llao Rock West	1	0
Llao Rock East	20+	0
Skell Head	4	0
Dutton Ridge	0	0
Broken Top	1	0
Mt. Bachelor	1	0
B. pumicola absent		
Cloud Cap North	0	0
Cloud Cap South	0	0
Grotto Cove	3	0
Llao Rock West	not collected	not collected
Llao Rock East	0	0
Skell Head	0	0
Dutton Ridge	0	0
Mt. Bachelor	1	0
Broken Top	0	0

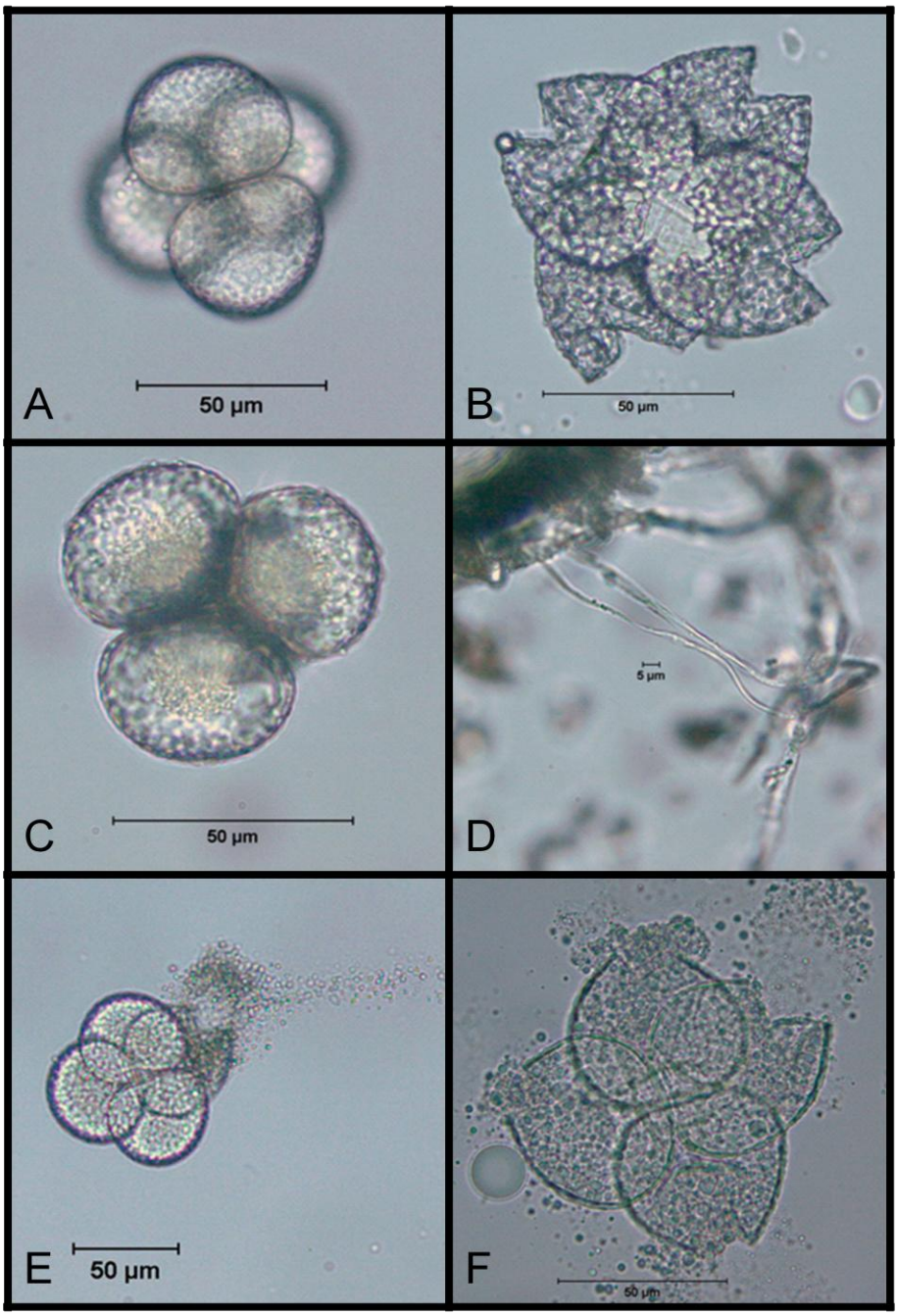

Figure 19. Spores from soils at Grotto Cove (Crater Lake National Park, Oregon): (A) Control *Botrychium pumicola* spores in tetrad; (B) Control *B. pumicola* spores releasing protoplasm; (C) *B. pumicola* spores in tetrad from plot without *B. pumicola*; (D) AM fungal hyphae from plot without *B. pumicola*; (E) *B. pumicola* spore releasing protoplasm; (F) *B. pumicola* spore releasing protoplasm.

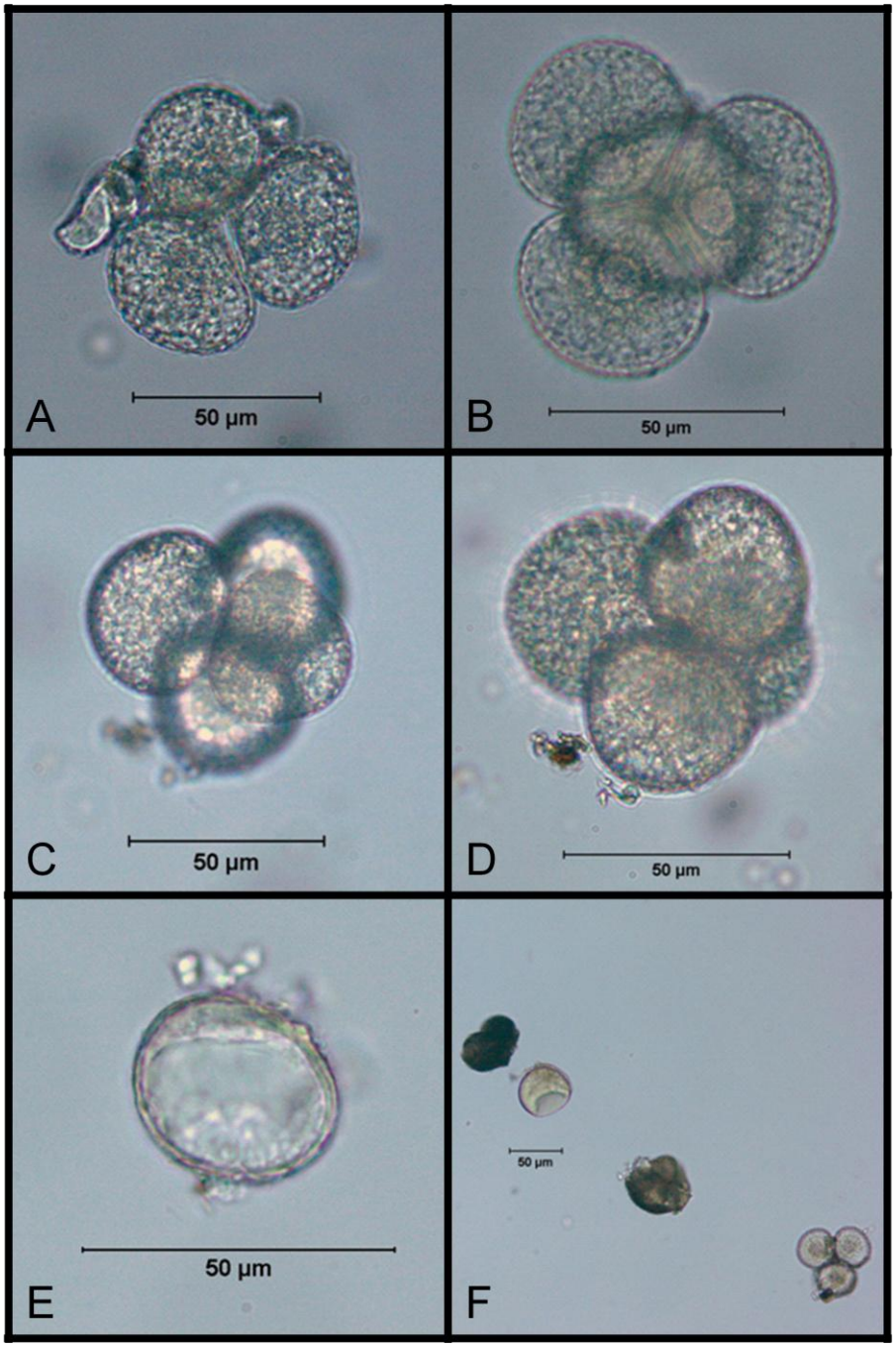

Figure 20. Spores from soils at Crater Lake National Park, Oregon: (A) Cloud Cap North *Botrychium pumicola* spores in tetrad; (B) Cloud Cap South *B. pumicola* spores in tetrad; (C) Llao Rock Pop West *B. pumicola* spores in tetrad; (D) Llao Rock West *B. pumicola* spores in tetrad; (E) Llao Rock East *B. pumicola* spore; (F) Llao Rock East *B. pumicola* spores with conifer pollen.

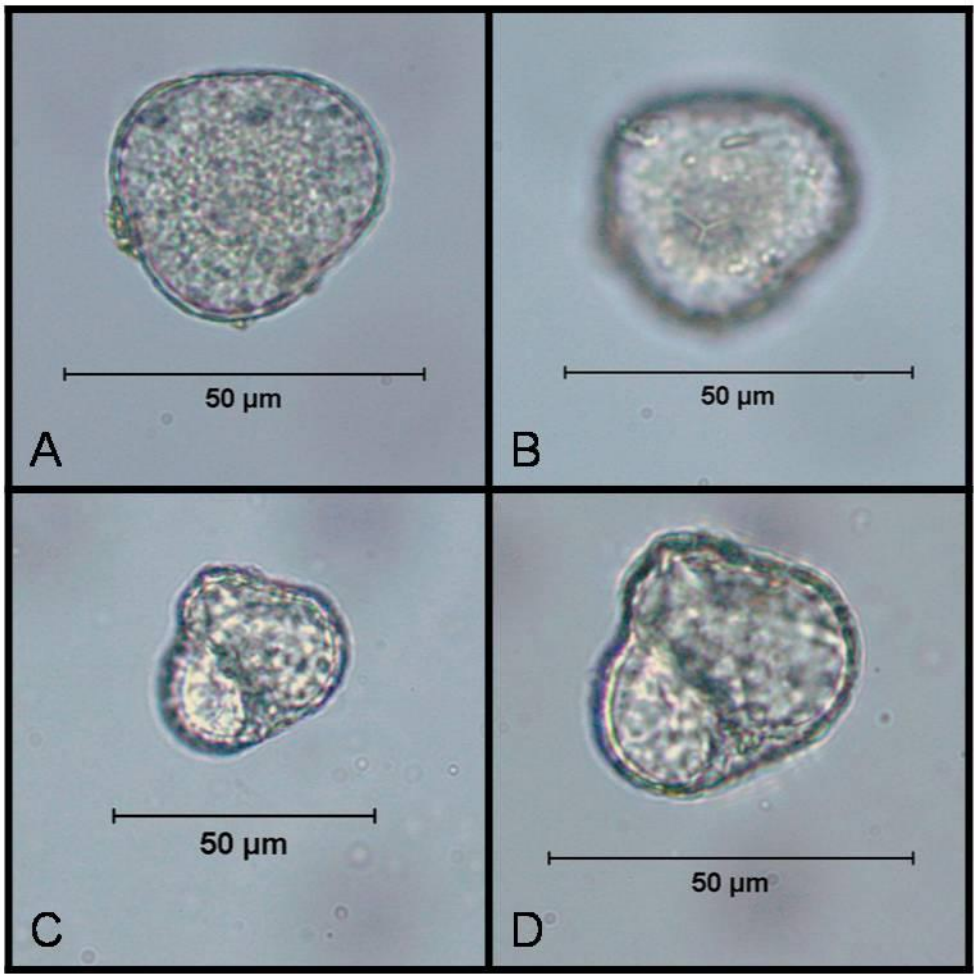

Figure 21. Spores from soils at Deschutes National Forest, Oregon: (A) Broken Top *Botrychium pumicola* spore from plot with *B. pumicola*; (B) Broken Top *B. pumicola* spore from plot with *B. pumicola* showing trilete mark; (C) Mt. Bachelor single *B. pumicola* spore from plot without *B. pumicola*; (D) Mt. Bachelor *B. pumicola* spore from plot without *B. pumicola*.

DISCUSSION

Subalpine and alpine populations of *Botrychium pumicola*, the pumice moonwort, are restricted to select volcanic habitats in the Cascade Range of central and southern Oregon and extreme northern California. *Botrychium pumicola* habitat consists of basalts, rhyolites, dacites, andesites, and other pyroclastics; however, *B. pumicola* is not restricted to pumice substrates as previously reported (Hopkins et al., 2006; FNAEC, 1993). *Botrychium pumicola* tolerates a range of soil nutrient and soil textural properties, and is found in association with a variety of sparse, herbaceous perennial plant species. Ecotypes may have evolved in different subalpine and alpine habitats in response to their particular local environmental condition.

Abiotic Factors

With the Bonferroni correction, no differences in abiotic factors were observed between plots with and without *B. pumicola*. This indicates that *B. pumicola* tolerates a range of soil nutrient levels, soil textural properties and site characteristics.

The Bonferroni correction is conservative, resulting in diminished power to detect differences among paired samples (Narum, 2006). Without the Bonferroni correction, soil potassium (K) levels were significantly higher in plots with *B. pumicola*. Potassium is essential for plant growth, development and regulation of stomata (Larcher, 2002). The essential role of K in photosynthesis and related processes becomes increasingly important with increased atmospheric CO_2 levels (Cakmak, 2005). Plants deficient in K are sensitive to the effects of high light intensity (Cakmak, 2005). A higher K level may

help *B. pumicola* regulate water loss in high elevation habitats where the plants are subject to low summer precipitation, intense UV radiation, temperature extremes, and desiccating winds. Potassium and calcium (Ca) are essential for spore germination in ferns (Miller and Wagner, 1987). Lower K levels may inhibit spore germination and decrease gametophyte success in habitats that look suitable for *B. pumicola*. Low K and Ca levels at some populations, such as Dutton Ridge, may negatively influence spore germination and the ability of gametophytes and sporophytes to survive the harsh conditions, thereby keeping the population very small (two plants).

Higher levels of soil nutrients, including K and N, influence the health of arbuscular mycorrhizal (AM) fungi. Hyphal biomass was significantly lower in phosphorus and nitrogen limited sites and *Glomus* species increased with higher N levels (Treseder and Allen, 2002). *Botrychium pumicola* forms obligate symbioses with AM fungi predominately with *Glomus* (Kovacs et al. 2007; Winther and Friedman, 2007). Higher N levels may increase the incidence of this partnership.

Soil density was higher in plots with *B. pumicola* (without Bonferroni correction). Plots with *B. pumicola* also had higher percentages of silt and clay which contributes to a higher soil density. Silts and clays have better water holding capacity and may provide more water throughout the growing season. Silt and clay soils also have a better capacity to hold K, a highly soluble element easily leached from sandy soils (Barbour et al., 1987). Percent H_2O did not differ between plots with and without *B. pumicola*; however, soil sampling captured a single point in time and did not reflect soil moisture availability over the entire growing season. Soil density with corresponding higher levels of silts and clays may also influence soil temperature which was lower in plots with *B. pumicola*.

Lupinus lepidus, one of the most common associated plant species observed at all sites except Mt. Bachelor and Shastina Cone, may be important due to its ability to fix nitrogen on nitrogen-poor pumice (Wood and del Moral, 1988). Plots with *B. pumicola* had higher ammonium than plots without *B. pumicola.* Ammonium availability may be an important factor for *Botrychium* spore germination (Melan and Whittier, 1990).

Botrychium pumicola populations occurred on flat to steep slopes up to 27°. The steepest slope was at Cloud Cap South. At this site the substrate appeared unstable in some areas which may have limited increase of the population. Slopes greater than 27° may create unstable conditions that sever connections between *B. pumicola* and AM fungal hyphae. For example, at Broken Top no *B. pumicola* occurred on the steeper slopes above and directly below the main population, only where the slope was 15°. The relative stability of the site may allow for mycorrhizal connections between *B. pumicola* and associated plant species. On the steeper slope above the population these fungal connections may be routinely severed as the loose substrate shifts.

Populations of *B. pumicola* occurred on all aspects except northwest, with south, southwest, and west aspects preferred. Aspect may influence snow accumulation, rate of snow melt and accumulation of summer precipitation. Snow distribution, snow pack duration and relief-driven summer precipitation strongly influence moisture availability during the growing season (Körner, 1999). For *B. multifidum* the shortage of precipitation during the summer decreased fertility in subsequent years (Mesipuu et al., 2009). Aspect and topographically influenced accumulations of soil moisture may create patches of suitable habitat that affect the distribution of *B. pumicola* populations.

Broken Top and Llao Rock West had the highest levels of organic matter as well as the highest number of *B. pumicola* individuals. The increased water holding capacity and higher levels of nutrients associated with organic matter may be a factor leading to the high density and frequency of *B. pumicola* at these sites.

Biotic Factors

Botrychium pumicola populations occur with diverse herbaceous perennial plants typical of high elevation pumice fields and volcanic rubble. The most common plant associates, although not at all sites, were *Carex breweri, Eriogonum umbellatum, Lupinus lepidus* and *Elymus elymoides* (Appendix A). These species, along with *Castilleja arachnoidea*, occurred at Llao Rock and Broken Top, the sites with the highest number of *B. pumicola*. No plant associate was identified as a reliable indicator species. At Crater Lake National Park, *Raillardella argentea* is present only at Llao Rock and Dutton Ridge, the sites with the highest (>1300) and lowest (two) number of *B. pumicola* respectively. *Raillardella argentea* is dense at Llao Rock and may be an important mycorrhizal network partner with *B. pumicola*.

A nearest neighbor analysis of *B. pumicola* and its nearest plant associate showed that at all populations, except at Broken Top, the plants had a clumped dispersion. This indicates that the advantage of close proximity to a mycorrhizal plant outweighs competition between *B. pumicola* and its nearest plant associate. The random to uniform dispersion at Broken Top was unusual, along with the high percentage of clay in the soil. This dispersion suggests the ability to maintain a long distance hyphal connection between each *B. pumicola* and a potential mycorrhizal partner.

A nearest neighbor analysis of *B. pumicola* showed a clumped to random dispersion. Clumping suggests vegetative reproduction via gemmae. Alternatively, the clumping of *B. pumicola* may result from the tendency for the sporophore to senesce and fall next to the parent plant, releasing spores. This could facilitate the mycorrhizal inoculation of spores and help maintain the immediate population (Johnson-Groh and Lee, 2002). Random dispersion indicates that some spore dispersal is occurring and that spores are able to find appropriate fungal symbionts required for development.

Some *B. pumicola* individuals did not produce sporophores; of those that did, some did not mature to release spores but wilted before spore release. During drought conditions Muller (1992) found that some *B. matricariifolium* individuals wilted prematurely and did not set spores. A similar response may occur with *B. pumicola* in response to the dry conditions encountered by individuals which emerge later in the season.

Many *B. pumicola* were observed with brown, necrotic tissue which appeared to be frost damage on their otherwise healthy looking pinnae. In this high elevation environment *B. pumicola* individuals must balance early emergence to take advantage of spring snow melt with the risk of frost damage. Johnson-Groh (2002) frequently observed *B. gallicomontanum* with necrotic tissue due to frost damage, a prairie species that, similar to *B. pumicola*, must emerge early to avoid the heat and drought of prairie summers. Many plants, especially those adapted to cold climates, produce cryoprotectant proteins, fatty acids and carbohydrates (Alberdi et al., 2002; Alberdi and Corcuera, 1991). Antifreeze proteins may also be protective where fluctuating temperatures cause freezing and thawing of plant tissues (Griffith et al., 2005). If *B. pumicola* has these

protections, the necrotic tissue may be caused by disease or desiccation rather than frost damage. The high percentage of necrotic tissue (100%) on *B. pumicola* sampled at The Dome, Dutton Ridge, Grotto Cove and Skell Head warrants further investigation.

Johnson-Groh and Lee (2002) reported difficulty in correlating *Botrychium* size with age because comparisons must be made during the same stage of development. In this study, measurements for each population were taken at similar phenological stages, allowing correlations of size with age. The high mean trophophore lengths at some populations such as Llao Rock and Broken Top, suggest that they contain older individuals. Dutton Ridge and Cloud Cap North with the lowest mean trophophores and sporophores, may be young populations. Subsequent monitoring is needed to determine if this is an accurate population size and if the population is expanding or contracting.

Cloud Cap North, Skell Head, Dome and Shastina Cone had the highest percentage of trophophores in the small class size. This suggests new recruitment by either gemmae or spores into these populations. Llao East and West and Grotto Cove had the lowest percentage of small class size trophophores, with most of these populations in the medium trophophore size class. This suggests a higher rate of past recruitment into these populations. Mount Bachelor , with the largest trophophores, indicates this population may be the oldest or healthiest of all populations in this study. Environmental factors may also be influencing trophophore and sporophore size. For *B. multifidum*, drought and herbivory decreased trophophore size in subsequent years (Mesipuu et al., 2009). No herbivory was observed at any of the sites, however further study is needed to determine if other environmental factors are affecting trophophore size.

Mycorrhizae

Plants in association with AM fungi have been shown to take up higher amounts of nutrients (Allen, 2007). Micro-site influences including the health of AM fungal communities may determine distribution of *B. pumicola*. Shifts in biodiversity of AM fungi can influence plant diversity, plant growth and nutritional status (van der Heijden et al., 1998). Different AM fungal genera also produce different influences on soil dynamics (Dodd et al., 2000). AM fungi may affect the efficiency of *B. pumicola* to access nutrients and therefore the suitability of the habitat. In pumice soils AM fungal hyphae bridge gaps across soil pores (Allen, 2007). This may assist *B. pumicola* in obtaining the necessary water to cope with low precipitation during the growing season (late June – mid August) which averages less than 2.5 cm. at Crater Lake National Park (WRCDC, 2010).

Pocket gophers (*Thomomys talpoides)* were found to distribute AM fungal spores in the Pumice Plain area of Mount St. Helens (Allen and MacMahon, 1988). Pocket gopher activity was observed at Dutton Ridge, Cloud Cap, Skell Head, Llao Rock and Grotto Cove. As pocket gophers move about the landscape, they may act as a vector for AM spores through their fossorial behavior (Allen et al., 2005). In southern Oregon, *T. bottae* disperses ectomycorrhizal fungal spores through mycophagy (Taylor et al., 2009). It is possible that *T. mazama* may distribute AM fungal spores in *B. pumicola* habitat and influence the success of *B. pumicola* spores or gemmae to connect with obligate AM fungal symbionts.

The lack of AM fungal spores in this study may indicate a dispersal limitation, seasonality of spore production or spore production at lower soil depth. In California,

AM spore abundance was highest in September (Egerton-Warburton and Allen, 2000). Sampling in this study may have missed peak AM sporulation due to the seasonality of spore abundance. Titus et al. (2007) encouraged the use of trap cultures instead of raw spore counts from pumice soil samples due to the tendency to underestimate AM fungal richness where spore numbers are low, and some AM fungal species do not regularly sporulate. The AM fungal species which inhabit pumice and other volcanic microsites in *B. pumicola* habitat may exhibit similar behavior. This makes spore capture through centrifugation, in this study, an unreliable measure of AM fungal presence.

Botrychium pumicola Spore Bank

Spore bank formation in ferns has been documented by del Ramirez-Trejo et al. (2004) and Dyer (1994). Johnson-Groh (2002) suggested that *Botrychium* may form spore banks, however in this study a low spore density was observed. Only twenty spores were observed in the soil sample from Llao Rock East where the population size is ≥1000 *B. pumicola* individuals. A small number of spores were found in all other populations, even in plots without *B. pumicola*. This suggests that a small spore bank forms and that spores reaching apparently suitable habitat in adjacent areas, fail to produce mature plants. In a spore trap study under a closed canopy, most *B. virginianum* spores fell within 3 m of the source plant (Peck et al., 1990). This was consistent with the findings of Dyer (1994) who found a much reduced spore bank two meters from the source. Subalpine and alpine populations of *B. pumicola* are located in exposed habitats subject to frequent and sustained high winds. Spores may be dispersed away from the population where they may not land in suitable habitat.

Rarity

According to Rabinowitz's (1981) classification, *B. pumicola* falls into the category of a species with both a narrow geographic range and narrow habitat specificity. The rarity of a species may be influenced by the size and number of habitable sites, the carrying capacity of the sites, the length of habitability and the probability of dispersal to those habitats (Harper, 1981). *Botrychium pumicola* appears limited to volcanic substrates with appropriate soil nutrients, soil temperature, soil density and AM fungi. The species may also be limited by spore dispersal into suitable habitat. However, due to the windy nature of existence on exposed volcanic substrates, spores may regularly land in suitable habitat making the potential for colonizing distant habitat seem high. The disjunct distribution of populations suggests that although dispersal opportunities occur, the species encounters dispersal limitations.

Johnson-Groh et al. (2002) found an average mortality rate of 73% between gametophyte and juvenile sporophyte stages and a 93% mortality rate between juvenile sporophyte and emergent sporophyte stages for several *Botrychium* species. Peck et al (1990) also found high mortality during the subterranean stages of *B. hesperium*. If *B. pumicola* spores reach suitable habitat, the potential for failure of spores, gametophytes and juvenile sporophytes is high.

Botrychium pumicola may be a "neo-endemic," a relatively new species that has not had time to expand its range (Fiedler, 2001). It may also be recovering from bottleneck effects due to past volcanic eruptions.

The re-discovery of *B. pumicola* on Shastina Cone (Farrar, 2010) adds a fascinating layer to the ecology of *B. pumicola* and opens a new chapter on the evolution

and distribution of the species thought to be an Oregon endemic. The last eruptions of Shastina Cone occurred 9700-9400 years ago (Christiansen, 1990). It is possible that through long distance spore dispersal the population on Shastina Cone re-colonized newer volcanic substrates in southern Oregon. The prevailing winds on Shastina Cone blow in a N-NE direction (WRCDC, 2010). Further research is needed on *B. pumicola* spore dispersal and the genetic relationship between the *B. pumicola* population on Shastina Cone and those to the north in southern and central Oregon.

Management Implications

The low percentage of *B. pumicola* trophophores in the small size class indicates low recruitment rates at Broken Top, Mt. Bachelor, Llao Rock and Grotto Cove. High percentages of trophophore necrotic tissue should be investigated. The decline of the Dutton Ridge population is of immediate management concern. Long term monitoring of size-age correlations, cycles of emergence and spore dispersal are needed to determine if populations are expanding, contracting or stable. *Botrychium pumicola* is found on select pumice and volcanic substrates which, in this study, showed higher K and soil density and lower soil temperatures. Since the species does not appear to be colonizing new habitat, it is essential to protect known populations.

Future investigations should include the effects of climate change on these high elevation ferns. *Botrychium pumicola* may be affected by increased CO_2, increased maximum temperatures and decreased snow-water equivalent expected with climate change (Mote, 2003). The effects of climate change on *Botrychium* species is a concern of land managers in the western United States (Anderson and Cariveau, 2004). Land managers must consider that predicted increased temperatures and potential decrease in

moisture levels may push the species northward in latitude and up in elevation. Long distance spore dispersal may allow for colonization of new sites. However, this study shows that *B. pumicola* may have specific micro-site habitat requirements that limit colonization. The disjunct distribution of the species and limited suitable habitat may preclude *B. pumicola* from establishing new populations at a pace to match the rate of climate change.

Conclusions

The disjunct distribution of *Botrychium pumicola* may be caused by a variety of abiotic and biotic factors converging to create habitat islands appropriate for the growth and development of *B. pumicola* spores, gametophytes, sporophytes and their AM fungal symbionts. These factors include availability of soil nutrients particularly K, Ca and N, soil textural properties and related water holding capacity, and site characteristics such as slope, aspect and soil temperature. Abiotic and biotic factors in turn affect the health of AM fungal symbionts creating a complex above and below ground community which together form the appropriate habitat islands for *B. pumicola*.

Further research on *B. pumicola* dispersal limitations, AM fungal symbionts, age and status of populations (stability, decline or increase), and factors creating suitable habitat islands is needed. Investigations to determine if subalpine and alpine populations of *B. pumicola* have evolved ecotypes in response to their particular local environments is needed to aid in the understanding and conservation of this rare Cascade Range endemic.

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APPENDIX A

Presence, absence and total number of associated plant species for each *Botrychium pumicola* site (*B. pumicola* present) in the Cascade Range of central and southern Oregon and northern California (2009). 1=present, 0=absent. GR, Grotto Cove; SK, Skell Head; DO, The Dome; BT, Broken Top; CN, Cloud Cap North; LE, Llao Rock East; LW, Llao Rock West; SH, Shastina Cone; BA, Mt. Bachelor.

	Population									
Plant Associate	GR	SK	DO	BT	CN	LE	LW	DU	SH	BA
Achillea millefolium	0	0	1	1	0	0	0	0	0	0
Achnatherum occidentale	1	1	0	0	1	0	0	1	0	0
Agoseris sp.	0	0	1	0	1	1	1	0	0	0
Antennaria alpina var. *media*	0	1	0	0	0	1	1	0	0	0
Antennaria geyeri	1	0	0	0	0	0	0	0	0	0
Antennaria rosea	0	0	0	0	1	0	0	0	0	0
Arabis divaricarpa	0	0	0	0	0	0	0	0	0	1
Arabis lyallii	0	0	0	0	0	1	0	0	0	0
Arabis platysperma	0	0	0	0	0	0	0	0	0	0
Arabis suffrutescens var. *horizontalis*	0	0	0	0	1	0	1	0	0	0
Calyptridium umbellatum	1	1	0	1	0	0	0	0	0	1
Castilleja applegatei	0	0	1	0	0	0	0	0	0	0
Castilleja arachnoidea	0	0	0	1	0	1	1	0	0	0
Carex breweri	1	1	0	1	1	1	1	1	0	1
Carex inops	0	0	0	0	0	0	1	0	0	0
Collinsia parviflora	0	0	1	0	0	0	0	0	0	0
Elymus elymoides	1	1	0	1	1	1	1	0	0	0
Ericameria nauseosa	1	0	0	0	0	0	0	0	0	0
Erigeron compositus	1	0	0	0	1	0	0	0	1	1
Erigeron elegantalus	0	1	0	0	1	0	0	0	0	0
Eriogonum ovalifolium	1	0	0	0	1	1	1	0	0	0
Eriogonum pyrolifolium	0	0	0	0	0	0	0	0	0	0
Eriogonum umbellatum	1	1	1	1	1	1	1	0	0	0
Eriophyllum lanatum	0	1	0	0	0	0	0	0	0	0
Festuca idahoensis	0	0	0	0	1	0	0	0	0	0
Gayophytum diffusum	0	0	1	0	0	0	0	0	0	0
Holodiscus microphyllum	0	0	0	0	0	0	0	0	1	0
Hulsea nana	0	0	0	0	0	0	0	0	0	1
Ipomopsis congesta	1	1	0	0	0	0	0	0	0	0
Linanthus pungens	0	1	0	0	0	0	0	0	0	0
Lomatium martindalei	0	0	0	0	0	1	1	0	0	0
Lupinus lepidus	1	1	1	1	1	1	1	1	0	0
Machaeranthera canescens var. *shastensis*	1	0	0	0	0	1	0	1	0	0
Mimulus nanus	0	1	0	0	0	0	0	0	0	0
Phacelia hastata	1	1	1	0	0	1	0	0	0	0
Phlox diffusa	0	0	0	0	0	1	1	0	0	0
Phlox hoodii	1	0	1	0	1	0	1	0	0	0
Phoenicaulis cheiranthoides	0	0	0	0	1	0	0	0	0	0
Potentilla sp.	0	0	0	0	1	0	0	0	0	0
Penstemon davidsonii	0	1	0	1	0	0	0	0	1	1
Penstemon speciosus	0	0	1	0	0	0	0	0	0	0
Pinus contorta	1	1	1	0	0	0	0	0	0	0
Polygonum newberryi	0	0	0	0	0	0	0	0	0	0
Raillardella argentea	0	0	0	0	0	1	1	1	0	0
Ribes erythrocarpum	1	0	0	0	0	0	0	0	0	0
Senecio canus	1	0	1	1	1	0	0	0	0	1
Trisetum spicatum	0	0	0	0	0	0	0	0	0	1
Total Number of Species	17	15	12	9	16	14	14	5	3	8

APPENDIX B

Presence, absence and total number of associated plant species species for each *Botrychium pumicola* population (*B. pumicola* absent) in the Cascade Range of central and southern Oregon (2009). 1=present, 0=absent. GR, Grotto Cove; SK, Skell Head; DO, The Dome; BT, Broken Top; LE, Llao Rock East; LW, Llao Rock West; BA, Mt. Bachelor.

	Site							
Plant Associate	GR	SK	DO	BT	LE	LW	DU	BA
Achillea millefolium	0	0	1	0	0	0	0	0
Achnatherum occidentale	1	1	0	0	0	0	1	0
Agoseris sp.	1	1	1	0	1	1	0	0
Antennaria alpina var. *media*	0	1	0	0	1	0	0	0
Antennaria geyeri	0	0	0	0	0	0	0	0
Antennaria rosea	1	0	0	0	0	1	0	0
Arabis divaricarpa	0	0	0	0	0	0	0	0
Arabis lyallii	1	0	0	0	0	0	0	0
Arabis platysperma	1	0	0	0	0	0	0	0
Arabis suffrutescens var. *horizontalis*	0	0	0	0	0	1	0	0
Calyptridium umbellatum	1	1	0	0	0	0	0	1
Castilleja applegatei	0	0	1	0	0	0	0	0
Castilleja arachnoidea	0	0	0	1	1	1	0	0
Carex breweri	1	1	0	1	1	1	1	1
Carex inops	0	0	0	0	0	1	0	0
Collinsia parviflora	0	0	1	0	0	0	0	0
Elymus elymoides	1	1	0	1	1	1	0	0
Ericameria nauseosa	0	0	0	0	0	0	0	0
Erigeron compositus	0	0	0	0	0	0	0	1
Erigeron elegantalus	1	1	0	0	0	1	0	0
Eriogonum ovalifolium	0	1	0	0	1	0	0	0
Eriogonum pyrolifolium	0	0	0	1	0	0	0	0
Eriogonum umbellatum	1	1	1	1	1	1	0	0
Eriophyllum lanatum	1	1	0	0	0	0	0	0
Festuca idahoensis	0	0	0	0	0	0	0	0
Gayophytum diffusum	0	0	1	0	0	0	0	0
Holodiscus microphyllum	0	0	0	0	0	0	0	0
Hulsea nana	0	0	0	0	0	0	0	0
Ipomopsis congesta	1	1	0	0	0	0	0	1
Linanthus pungens	0	1	0	0	0	0	0	0
Lomatium martindalei	0	0	0	0	0	0	0	0
Lupinus lepidus	1	1	1	1	1	1	1	0
Machaeranthera canescens var. *shastensis*	1	0	0	0	1	1	0	0
Mimulus nanus	0	1	0	0	0	0	0	0
Phacelia hastata	0	1	1	0	1	1	0	0
Phlox diffusa	0	0	0	0	1	0	0	0
Phlox hoodii	1	0	1	0	0	1	0	0
Phoenicaulis cheiranthoides	0	0	0	0	0	0	0	0
Potentilla sp.	0	0	0	0	0	0	0	0
Penstemon davidsonii	0	1	0	1	0	0	0	1
Penstemon speciosus	0	0	0	0	0	0	0	0
Pinus contorta	0	0	0	0	0	0	0	0
Polygonum newberryi	0	0	0	1	0	0	0	0
Raillardella argentea	0	0	0	0	1	1	1	0
Ribes erythrocarpum	0	0	0	0	0	0	0	0
Senecio canus	0	1	0	0	0	0	0	1
Trisetum spicatum	0	0	0	0	1	0	0	0
Total Number of Species	15	17	9	8	13	14	4	6

APPENDIX C

Plant associates per m² for each *Botrychium pumicola* site (*B. pumicola* present) in the Cascade Range of central and southern Oregon and northern California. GR, Grotto Cove; SK, Skell Head; DO, The Dome; BT, Broken Top; CN, Cloud Cap North; LE, Llao Rock East; LW, Llao Rock West; SH, Shastina Cone; BA, Mt. Bachelor.

	Botrychium pumicola present									
Plant Associate	LW	LE	BT	DO	GC	DU	CN	SK	BA	SH
Achillia millefolium	0	0	34.4	9	0	0	0	0	0	0
Achnatherum occidentale	0	0	0	0	5.2	20	0.2	8	0	0
Agoseris sp.	20.1	6.5	0	1.2	0	0	0.4	0	0	0
Antennaria alpina var. *media*	7.3	4.4	0	0	0	0	0	12.8	0	0
Antennaria geyeri	0	0	0	0	3.6	0	0	0	0	0
Antennaria rosea	0	0	0	0	0	0	15.8	0	0	0
Arabis divaricarpa	0	0	0	0	0	0	0	0	0.2	0
Arabis lyallii	0	0.2	0	0	0	0	0	0	0	0
Arabis platysperma	0	0	0	0	0	0	0	0	0	0
Arabis suffrutescens var. *horizontalis*	0.2	0	0	0	0	0	0.2	0	0	0
Calyptridium umbellatum	0	0	0.2	0	1	0	0	3.6	1.4	0
Castilleja applegatei	0	0	0	12	0	0	0	0	0	0
Castilleja arachnoidea	8.5	4.3	0.4	0	0	0	0	0	0	0
Carex breweri	24	40.2	1.8	0	14.4	10	10.4	6.8	15.2	0
Carex inops	12.4	0	0	0	0	0	0	0	0	0
Collinsia parviflora	0	0	0	3.6	0	0	0	0	0	0
Elymus elymoides	2.5	7	1.8	0	3.2	0	7.6	4.8	0	0
Ericameria nauseosa	0	0	0	0	0.6	0	0	0	0	0
Erigeron compositus	0	0	0	0	1.4	0	1	0	0.2	1
Erigerion elegantalus	0	0	0	0	0	0	24.4	17.4	0	0
Eriogonum ovalifolium	0.1	1.1	0	0	0.2	0	3.6	0	0	0
Eriogonum pyrolifolium	0	0	0	0	0	0	0	0	0	0
Eriogonum umbellatum	4.8	5	0.4	2.4	1	0	2	3.2	0	0
Eryophyllum lanatum	0	0	0	0	0	0	0	0.6	0	0
Festuca idahoensis	0	0	0	0	0	0	2.2	0	0	0
Gayophytum diffusum	0	0	0	3.8	0	0	0	0	0	0
Holodiscus microphyllum	0	0	0	0	0	0	0	0	0	1.5
Hulsea nana	0	0	0	0	0	0	0	0	0.2	0
Ipomopsis congesta	0	0	0	0	1.6	0	0	2.4	0	0
Linanthus pungens	0	0	0	0	0	0	0	3.8	0	0
Lomatium martindalei	1	0.2	0	0	0	0	0	0	0	0
Lupinus lepidus	1.3	4.9	1.4	0.6	7.6	8	7.6	14.6	0	0
Machaeranthera canescens var. *shastensis*	0	0.3	0	0	1.2	1	0	0	0	0
Mimulus nanus	0	0	0	0	0	0	0	0.8	0	0
Phacelia hastata	0	0.8	0	4	3.6	0	0	3.6	0	0
Phlox diffusa	1.3	3.3	0	0	0	0	0	0	0	0
Phlox hoodii	8.6	0	0	37.8	3.4	0	7.2	0	0	0
Phoenicaulis cheiranthoides	0	0	0	0	0	0	0.6	0	0	0
Potentilla sp.	0	0	0	0	0	0	8.4	0	0	0
Penstemon davidsonii	0	0	8	0	0	0	0	0.2	15	3
Penstemon speciosus	0	0	0	0.6	0	0	0	0	0	0
Pinus contorta	0	0	0	0.2	0.2	0	0	0.2	0	0
Polygonum newberryi	0	0	0	0	0	0	0	0	0	0
Raillardella argentea	94.5	113.5	0	0	0	15	0	0	0	0
Ribes erythrocarpum	0	0	0	0	0.2	0	0	0	0	0
Senecio canus	0	0	1.2	0.4	0.2	0	6	0	0.8	0
Trisetum spicatum	0	0	0	0	0	0	0	0	1.4	0

APPENDIX D

Plant associates per m² for each *Botrychium pumicola* site (*B. pumicola* absent) in the Cascade Range of central and southern Oregon and northern California. GR, Grotto Cove; SK, Skell Head; DO, The Dome; BT, Broken Top; LE, Llao Rock East; LW, Llao Rock West; BA, Mt. Bachelor.

	Botrychium pumicola absent							
Plant Associate	LW	LE	BT	DO	GC	DU	DK	BA
Achillia millefolium	0	0	0	11.2	0	0	0	0
Achnatherum occidentale	0	0	0	0	6.6	20	0.8	0
Agoseris sp.	8.8	4.7	0	2.2	2.4	0	0.6	0
Antennaria alpina var. *media*	0	0.3	0	0	0	0	13.8	0
Antennaria geyeri	0	0	0	0	0	0	0	0
Antennaria rosea	10.6	0	0	0	2.4	0	0	0
Arabis divaricarpa	0	0	0	0	0	0	0	0
Arabis lyallii	0	0	0	0	0.2	0	0	0
Arabis platysperma	0	0	0	0	0.4	0	0	0
Arabis suffrutescens var. *horizontalis*	0.1	0	0	0	0	0	0	0
Calyptridium umbellatum	0	0	0	0	1	0	5.2	0.4
Castilleja applegatei	0	0	0	6.8	0	0	0	0
Castilleja arachnoidea	3	0.6	4.4	0	0	0	0	0
Carex breweri	20.7	35.7	24.4	0	6.4	12	9.2	29.2
Carex inops	2.1	0	0	0	0	0	0	0
Collinsia parviflora	0	0	0	0.2	0	0	0	0
Elymus elymoides	8.9	14.4	1.4	0	3.2	0	6.2	0
Ericameria nauseosa	0	0	0	0	0	0	0	0
Erigeron compositus	0	0	0	0	0	0	0	0.8
Erigerion elegantalus	0.9	0	0	0	0.6	0	25	0
Eriogonum ovalifolium	0	5.4	0	0	0	0	1.2	0
Eriogonum pyrolifolium	0	0	0.6	0	0	0	0	0
Eriogonum umbellatum	7.7	8.9	4	0.6	0.8	0	2	0
Eryophyllum lanatum	0	0	0	0	0.2	0	1.2	0
Festuca idahoensis	0	0	0	0	0	0	0	0
Gayophytum diffusum	0	0	0	0.8	0	0	0	0
Holodiscus microphyllum	0	0	0	0	0	0	0	0
Hulsea nana	0	0	0	0	0	0	0	0
Ipomopsis congesta	0	0	0	0	0.8	0	3.2	0.4
Linanthus pungens	0	0	0	0	0	0	5.8	0
Lomatium martindalei	0	0	0	0	0	0	0	0
Lupinus lepidus	1.9	9.5	0.2	0.4	21	10	5.8	0
Machaeranthera canescens var. *shastensis*	2	1.2	0	0	1.2	0	0	0
Mimulus nanus	0	0	0	0	0	0	0.6	0
Phacelia hastata	1.1	7.5	0	1.4	0	0	6.6	0
Phlox diffusa	0	7.4	0	0	0	0	0	0
Phlox hoodii	17.5	0	0	25.4	2.2	0	0	0
Phoenicaulis cheiranthoides	0	0	0	0	0	0	0	0
Potentilla sp.	0	0	0	0	0	0	0	0
Penstemon davidsonii	0	0	3.6	0	0	0	0.2	30
Penstemon speciosus	0	0	0	0	0	0	0	0
Pinus contorta	0	0	0	0	0	0	0	0
Polygonum newberryi	0	0	0.2	0	0	0	0	0
Raillardella argentea	26.8	43.8	0	0	0	20	0	0
Ribes erythrocarpum	0	0	0	0	0	0	0	0
Senecio canus	0	0	0	0	0	0	2.8	2.4
Trisetum spicatum	0	0.1	0	0	0	0	0	0

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