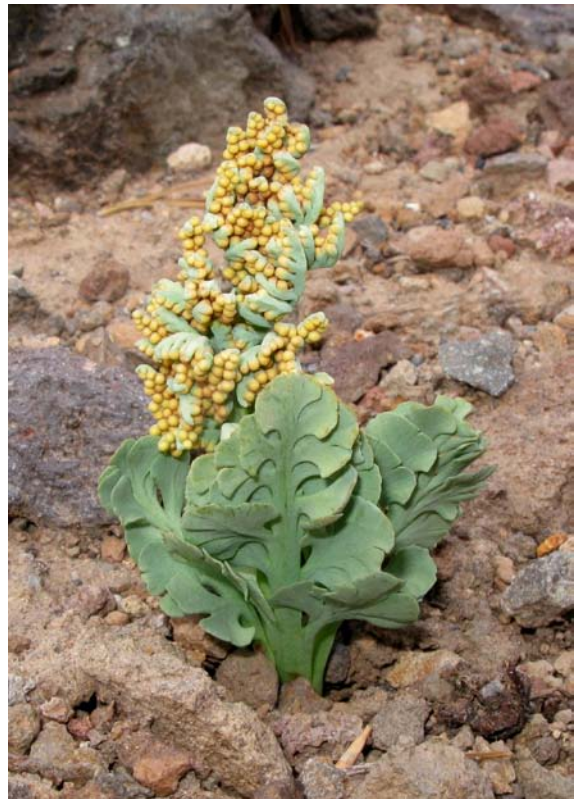


Botrychium pumicola

Family:	Ophioglossaceae
Genus:	<i>Botrychium</i>
Species:	<i>Botrychium pumicola</i> Colville
Common Name:	Pumice Moonwort
Ploidy:	Diploid



Technical description.—Trophophore stalk 0-10 mm, 0.1-0.5 times length of trophophore rachis; blade dull, glaucous, whitish green, deltate, 2-pinnate, 4 x 6 cm, thickly leathery. Pinnae to 6 pairs, overlapping, strongly ascending, distance between 1st and 2nd pinnae not or slightly more than between 2nd and 3rd pairs, asymmetrically cuneate, basal pinna pair often divided into 2 unequal parts, lobed to tip, margins entire, sinuate to shallowly crenate, apex rounded to truncate, venation pinnate. Sporophores 1-3-pinnate, 1-1.5 times length of trophophore, $2n = 90$. (W. H. Wagner, 1993).

Taxonomy

Botrychium pumicola was described by F. V. Colville in 1900 in "Our Native Ferns" by Underwood, and in the Bulletin of the Torrey Botanical Club 28:109 and plate 7, based on specimens collected from Crater Lake, Klamath County, Oregon. Clausen included *B. pumicola* as one of the six distinct species of moonworts he recognized in 1938.

Identification

Botrychium pumicola is the only moonwort other than *B. simplex* that, in large plants, regularly has the basal pinnae elongated and pinnately divided but with the 2nd and higher pinnae remaining simple and entire to palmately lobed. [In *B. lanceolatum* and its allopolyploid derivatives, the middle pinnae are also elongated and pinnately lobed or divided.] *B. pumicola* also shares with *B. simplex* its basic pinna shape, broadly decurrent attachment to the rachis and broadly rounded trophophore apices. Despite these similarities to *B. simplex*, *B. pumicola* is highly distinct morphologically and genetically and clearly warrants its recognition at the species level.

B. pumicola is easily differentiated from *B. simplex* by its sessile to very short-stalked trophophore and short sporophore stalk that at maturity does not exceed the length of the trophophore. It also differs from *B. simplex* in having 2 to 4 veins entering the pinna stalk (due to the principal pinna vein dividing in the rachis well before entering the pinna stalk) vs 1 to 2 veins entering the pinna stalk in *B. simplex* (the principal pinna vein divides at the point of entering the pinna stalk) and production of underground gemmae. Other important differences are in spore size, those of *B. pumicola* averaging about 34 micrometers as compared to the unusually large spores of *B. simplex* averaging 46 micrometers (Farrar and Johnson-Groh 1991), and the manner of their release in tetrads (tetrahedral groups of four) (see Population Genetics), whereas *B. simplex* and all other moonwort species release spores individually.

Smaller plants of *B. pumicola* that do not have the basal pinnae enlarged can be differentiated from other species with simple fan-shaped pinnae by their broad, decurrent bases with multiple veins as well as by their short-stalked and dense sporophores that release spores in tetrads.

Distribution and Abundance

Until recently, *B. pumicola* has been considered to be limited in distribution to Crater Lake and surrounding area in southwestern Oregon. It was estimated in 1997 to consist of 118 populations, 60% of which contained less than 20 individuals, and a total number of plants less than 15,000 (NatureServe Explorer

2002). The NatureServe website states that “a California report (Mt. Shasta) is no longer accepted”, and neither Flora North America (1993) nor the Jepson Manual of Higher Plants of California (Hickman 1993) listed its occurrence on Mt. Shasta in Siskiyou Co. CA (but see discussion below).

William Bridge Cooke, in 1941, found two plants he concluded to be *B. pumicola* on Shastina, a secondary cone of Mt. Shasta in northern California, and described their occurrence as “in a basin near the spur on the south bank of Diller Canyon, west side of Shastina, near timberline” at an elevation of 9027 feet. Subsequent annotations of the specimen by R. T. Clausen and D. Wagner affirmed Cooke’s identification, as well as hand-written notes presumed to be those of W. H. Wagner stating “juvenile specimen” and “spores in tetrads”. No annotations have contradicted Cooke’s identification.

D. R. Farrar re-examined the Cooke specimen in preparation of a revised treatment of *Botrychium* for the 2011 edition of the Jepson Flora. He found this specimen to conform in all respects to *B. pumicola*. In addition to characters of decurrent, multiveined pinna stalks, nearly sessile trophophore, short-stalked and dense sporophore and spores shed in tetrads, spores of the Cooke specimen average 35 micrometers—a size consistent with the published size of *B. pumicola* (33-36 μ) (Clausen 1938) and much smaller than those of *B. simplex*. Cooke’s careful description of the site left no doubt that the plant was collected on Mt. Shasta in a habitat similar to that of mountain populations in Oregon. From the combined evidence, there appeared no reason to discount this historic occurrence of *B. pumicola* in California.

Based on the above evidence, a U. S. Forest Service, Mt. Shasta Ranger Station team led by Eric White scouted the presumed Cooke site in early August of 2009. Near the presumed Cooke site, Mellon Colberg, botanist for the Mt. Shasta Ranger Station, found two small populations of *B. pumicola*. A second trip to the site a week approximately a week later included botrychium specialists, D. R. Farrar, J. Game, and S. Roe-Anderson. This group found a total of 6 plants in the two populations found by Colberg, but found no additional populations. In 2010, two additional trips to the site led by Eric White found only one plant in the 2009 populations, but located two additional populations nearby, each composed of two plants. Plants and sites were photographed both years. A single plant was collected in 2009 by D. R. Farrar to serve as a herbarium voucher. A small sample was taken from the common stalk of this plant to compare genetically with plants from Oregon.

Habitat

In high mountain sites, *Botrychium pumicola* typically grows in unconsolidated volcanic pumice or in glacial till containing pumice, usually on exposed ridge crests. At lower elevations, *B. pumicola* occurs in basins or

drainages that retain subsurface moisture into late summer. These habitats are generally sparsely vegetated with herbs, shrubs and scattered conifers.

A single plant was excavated from an Oregon population in a lowland basin in pumice by Farrar in 2009. The common stalk of the leaf extended approximately 8 cm below the soil to where it attached to the stem apex. From this point, the stem and roots extended another 10 cm into the soil to where the stem was broken off in excavation from the remainder of the stem. The recovered 10 cm of stem supported 19 leaf scars, 20 roots and 15 gemmae. The entire length of the recovered portion, including the above ground trophophore, was approximately 25 cm. The number of leaf scars (produced one per year) indicate that the plant was more than 20 years old and had started growth more than 18 cm below the soil surface. The soil at this depth was saturated.

The habitat on Mt. Shasta in California was described by Cooke as “in a basin near the spur on the south bank of Diller Canyon, west side of Shastina near timberline”. Plants found here in 2009 were in a boulder field on a windy exposed lip at the end of the glacial channel (basin of Cooke?) where the glacial lobe had spilled over the mountainside. Plants were in gravelly soil at the bases of boulders.

Possibly the windswept exposures characteristic of mountain populations of *B. pumicola* provide early emergence from snow cover that could ameliorate the short growing season at high elevations.

Population Genetics

Farrar conducted a genetic assessment of *B. pumicola* via enzyme electrophoresis examining 88 plants from 15 populations across the species' range in Oregon. Scoring of 21 gene loci revealed allelic variation at only 3 loci, this variation was limited to single plants for two loci (PGI-2 and PGM-2) and three plants for one locus (MDH-4), the latter all from the same population. Because these variant individuals are rare and each novel genotype is limited to one population, the data is of limited value in determining relationships among populations. Occurrence of the novel MDH-4 allele in 3 of 8 individuals on one population and its absence in high frequencies elsewhere supports a hypothesis of limited spore dispersal. Larger sample sizes (average sample size was 5.2 individuals per population) could increase confidence in this conclusion.

The extremely limited genetic variability in *B. pumicola* suggests a recent bottleneck in the species' history. Because of its preferred habitat on volcanic pumice, it is easy to envision such bottlenecks as having occurred repeatedly. Mt. Mazama produced the current substrate of Oregon *B. pumicola* in its last major eruption (creating Crater Lake) slightly less than 7,000 years ago (Harris 2005). It and other volcanoes in the Cascade Range have produced similar

ejecta many times. Mt. Shasta was active for hundreds of thousands of years prior to its last pumice ejecting eruption about 9,000 years ago (Harris 2005). Reduction of the species to one or a few remnant populations could have occurred many times through burial by new eruptions, as well as gradual modification of the pumice substrate to a more typical soil but one less supportive of *B. pumicola* (change in composition of the vegetation community would likely accompany soil development). In either case, population recovery on new substrate (pumice of appropriate post-eruption age) could have often been from one or a few individuals of a single genotype.

The enormous pumice and ash fall from Mt Mazama's last eruption deeply blanketed the Cascade Mountains north to British Columbia. Any Oregon plants existing prior to the eruption could not have survived in place. Recolonization of Mt. Mazama pumice must have occurred by spores transported from elsewhere. The Mazama ejecta did not travel far to the south. It covered only a small section in the northeast corner of California, well to the east of Mt. Shasta and Mt. Lassen. Mt. Lassen's last major eruption in 1914-1915 would likely have obliterated populations there, but the last pumice-producing eruption of Mt. Shasta predates the last eruption of Mt. Mazama. Mt. Shasta (and possibly Mt. Lassen) could have supported populations of *B. pumicola* that survived the Mt. Masama eruption and could have been the source of spores recolonizing Oregon when substrates there became suitable.

The single Mt. Shasta plant genetically analyzed in 2009 possessed a genotype identical to the common genotype of Oregon plants. While this result is not proof of a Mt. Shasta origin of the Oregon plants, it does make that hypothesis plausible. Demonstration of greater genetic variability in Mt. Shasta plants than exists in Oregon would lend stronger support to the hypothesis of a Mt. Shasta origin of the Oregon populations. It seems unlikely that the currently known populations on Mt. Shasta, do to their small numbers, could have survived over a long period of time. More likely, these populations are supported by larger populations existing elsewhere on Mt. Shasta. Continued search in similar habitats is likely to reveal such populations.

The presence of plants of *B. pumicola* on Mt. Shasta in both northern California and in the Crater Lake area of Oregon indicates a capacity for long-range dispersability of *B. pumicola* despite its adaptation for precinctiveness through release of spores in tetrad groups. However, if spore dispersal is more limited than in other *Botrychium* species, the populations colonizing newly appropriate volcanic substrate could be from a very few, or possibly a single, founding spore, and thus of a single genotype. The window of time when the pumice substrate is supportive of *B. pumicola* may be too short for appreciable genetic variation to arise through new mutation, as seems to be the case for the current Oregon populations.

The absence of genetic variability in *B. pumicola* should not be of conservation concern relative to inbreeding depression. Because *Botrychium* species reproduce primarily by intragametophytic selfing (union of sperm and egg from the same haploid gametophyte) spore-producing plants are strict homozygotes with no shielded deleterious alleles. Consequently expression of heterozygously shielded deleterious alleles (inbreeding depression) is not a possibility. In addition to sexual reproduction, *B. pumicola* reproduces locally by vegetative gemmae produced on the underground stem (Camacho and Liston, 2001). Both methods propagate the single widespread genotype, apparently well-adapted to its current habitats.

B. pumicola may be less genetically adaptable to habitat or climate change than other species, but its genetic inflexibility is tempered by its derivation of water, minerals, and photosynthate from its mycorrhizae partner. Indications from other moonworts are that this fungal partner is one or more of the common mycorrhizal fungi that occur in a wide range of habitats. As long as the genetic basis for attracting the fungus is maintained, *B. pumicola* and other moonworts may be less sensitive to environmental change than most plant species.

Phylogenetic Relationships

B. pumicola is genetically most similar to *B. simplex* with which it also shares a number of morphological characteristics. However, it is highly distinct from *B. simplex* (Genetic Identity = .67) and clearly warrants species recognition. *B. pumicola* is not known to have participated in the formation of any allopolyploid species.

Additional images of *Botrychium pumicola*

