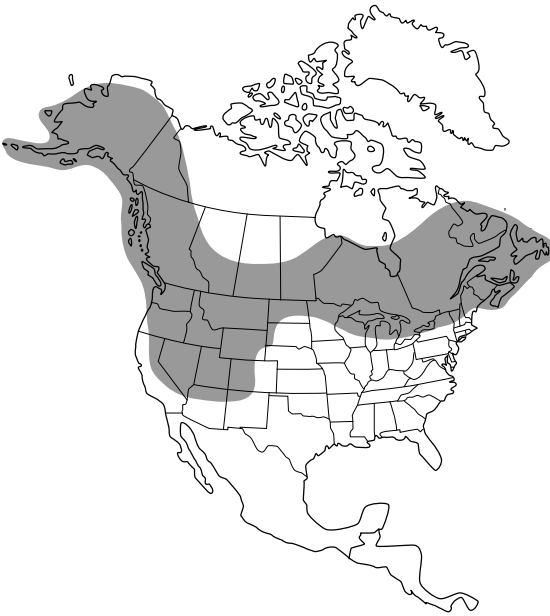


## ***Botrychium minganense***

<b>Family:</b>	Ophioglossaceae
<b>Genus:</b>	<i>Botrychium</i>
<b>Species:</b>	<i>Botrychium minganense</i> Victorin
<b>Synonym:</b>	<i>Botrychium lunaria</i> Sw. var. <i>minganense</i> (Vict.) Dole
<b>Common name:</b>	Mingan moonwort
<b>Ploidy:</b>	Tetraploid



Technical description—Trophophore stalk 0—2 cm, 0 to 1/5 length of trophophore rachis; blade dull green, oblong to linear, 1-pinnate, to 10 x 2.5 cm, firm to herbaceous. Pinnae to 10 pairs, horizontal to ascending, approximate to remote, distance between first and second pinnae not or slightly more than between second and third pairs, basal pinna pair approximately equal in size and cutting to adjacent pair, occasionally basal pinnae and/or some distal pinnae elongate, lobed to tip, nearly circular, fan-shaped or ovate, sides somewhat concave, margins nearly entire, shallowly crenate, occasionally pinnately lobed or divided, apex rounded, venation like ribs of a fan with short midrib. Sporophores 1-pinnate, 2-pinnate in very large, robust plants, 1.5-2.5 times length of trophophore. 2n = 180" (from Wagner and Wagner 1993a).

## Taxonomy

*Botrychium minganense* was described as a new species in 1927 by Frere Marie Victorin based on specimens from the Mingan Islands in the Gulf of St. Lawrence (Trans. Roy. Canad. Inst. Ser. 3, 21 (sect.5):319-340). Because of its seeming intergradation with *B. lunaria*, Victorin's new species was not accepted by Clausen (1938), and reduced by him to a variety of *B. lunaria*. Clausen recognized that the spores of *B. minganense* were larger than those of *B. lunaria* but felt that this was an unreliable character. He further pointed out that the two taxa grow together throughout most of their equally broad North American ranges.

Botanists accepted Clausen's conclusion until 1956 when a study by Wagner and Lord listed 14 characters differentiating *B. minganense* from *B. lunaria*, and most importantly, were able to show that *B. minganense* had twice the number of chromosomes ( $n = 90$ ) present in *B. lunaria* ( $n = 45$ ). Thus *B. minganense* was likely an allotetraploid with *B. lunaria* having been one of its ancestral parents. Allelic patterns derived through enzyme electrophoresis have confirmed this conclusion (Farrar, 2001).

In 1990 Wagner and Wagner drew attention to the possibility of two different taxa within what was then considered *B. minganense* in the Great Lakes area. With further study they (Wagner and Wagner, 1990b) concluded that this was the case and published the description of *Botrychium spathulatum* Wagner, applying this name to specimens with an overall similarity to *B. minganense*, but differing consistently in 24 different characters.

Comparison of allozyme patterns derived from starch-gel enzyme electrophoresis (Farrar and Wendel, 1997) confirmed the distinction between *B. minganense* and *B. spathulatum* and showed furthermore that the origin of their differences most likely was that the non-lunaria parent of *B. spathulatum* was *B. campestre* whereas the non-lunaria parent of *B. minganense* was a species similar genetically to *B. pallidum*. With recognition of the differences between American and European "lunaria" (Stensvold 2008), it became possible to designate European *B. lunaria* as the non-campestre parent of *B. spathulatum* and *B. neolunaria* as the non-pallidum-like parent of *B. minganense*.

## Identification

Because *B. neolunaria* is one of the ancestral parents of *B. minganense*, and because the morphology of both species varies greatly with size and habitat, especially sun vs shade, there is a great deal of morphological overlap between them. In particular, less well developed shade forms of *B. neolunaria* can be difficult to distinguish from *B. minganense*. Of several, usually reliable characters, the most dependable for separating these species is the pinna span of the lowermost pinnae.

In *B. neolunaria* the span is usually 150° to 180° whereas in *B. minganense* it is usually considerably less than 120°. Although the basal pinnae of *B. neolunaria* can be shallowly cleft, the outer margins of the segments circumscribe a uniform arc. By contrast, the outer margin of the basal pinnae of *B. minganense* often appears lobed.

The trophophore of *B. minganense* is almost always distinctly stalked, with the stalk length equal to or greater than the distance between pinnae. If the trophophore of *B. neolunaria* is at all stalked (usually it is sessile), the stalk length is less than the distance between pinnae. Another useful distinction between *B. neolunaria* and *B. minganense* is dull to yellowish green color of the latter as opposed to the deep green of the former. This color difference is likely due to the other ancestral diploid parent of *B. minganense* which is most probably *B. pallidum* or an unknown relative (see discussion under Phylogenetic Relationships).

*Botrychium minganense* can also be difficult to distinguish from *B. pallidum*, particularly after collection of the latter. When fresh, plants of *B. pallidum* can be distinguished by their light gray-green “pallid” color, but this character fades quickly following collection, especially if plants are kept in plastic bags. Plants of *B. pallidum* are usually smaller than those of *B. minganense* with fewer pairs of pinnae (3-5 vs 5-7). The pinnae of *B. pallidum* are shorter and with broader attachment to the rachis and the basal pinnae are often bilobed with the upper lobe larger and the two lobes distinctly spreading. Underground, *B. pallidum* produces numerous gemmae whereas *B. minganense* produces only a few.

*B. spathulatum* differs most clearly from *B. minganense* in having a sessile or nearly sessile trophophore with basal pinnae often larger than the upper pinnae. Its spathulate pinnae are entire or irregularly divided whereas those of *B. minganense* are more uniformly fan-shaped. Comparison silhouettes of *B. minganense* and *B. spathulatum* are presented in the Wagner and Wagner paper (1990b) describing *B. spathulatum*.

David Wagner (1992, 1996) and others have intensively studied the morphological variation within *B. minganense* in western North America and shown it possible to arrange plants into a series of types with subtle morphological differences. Both David Wagner and Herb Wagner have occasionally given provisional names to some of these morphotypes pending further study, thus names such as “sublunaria” and “Colorado” have entered the informal literature on *B. minganense*. Our studies using enzyme electrophoresis have found no consistent genetic correlations to support recognizing these or any other entities as distinct from typical *B. minganense* (Farrar, 2001). Swartz (2002) likewise found that RAPD DNA markers showed no distinct clusters within *B. minganense* that could be correlated with its morphological variability. [see discussion under Population Genetics]

## Distribution and Abundance

*Botrychium minganense* is among the most widespread and abundant moonworts, having been reported from 19 states and 12 Canadian provinces. Like other moonwort species, it occurs primarily in northern latitudes and at high elevations to the south. It is known from Labrador to New England westward across Canada and the northernmost tier of states to Alaska and south in the western mountains to California, Arizona and New Mexico. Recently it has been found in Iceland, the only known occurrence outside of North America.

## Habitat

The habitat of *B. minganense* varies widely from dense forest to open meadow and from summer-dry meadows to permanently saturated fens and seeps. When in meadows, plants may stand in open sun or under dense herbaceous cover. The species is often found in association with old (>10 year) disturbances such as logging roads and road shoulders. It may be locally abundant and sometimes is the only moonwort present in a particular site, but often it occurs with other *Botrychium* species as scattered individuals. In Summit County, Colorado it has been estimated to be the third most common moonwort (after *B. neolunaria* and *B. lanceolatum*) with approximately 1854 plants observed (Kolb and Spribille, 2001). *B. minganense* may be less closely associated with calcareous soils than most moonworts. It is the species most commonly found on basaltic soils of the Blue Mountains of northeast Oregon.

## Population Genetics

*Botrychium minganense* is at the same time genetically one of the most variable and one of the least variable of moonworts. It is almost invariable in its basic alleles inherited from its two ancestral parents. That is, at most loci it has potentially two and only two alleles, one contributed by each of its parental species. However, as often as not, in a given population at a given locus, one of these alleles is not expressed. The gene for that allele has undergone mutation so that it no longer codes for a functional enzyme, it has been “silenced”. Thus a wide array of genotypes is generated. [This is not unusual in polyploid species since they have a redundancy of genes. See discussion in *Moonwort Systematics* under *Speciation and Evolution, Gene Silencing*.]

Because most *Botrychium* species reproduce primarily by intragametophytic self fertilization, reproduction, even though involving gametic union, results in the overall equivalent of asexual reproduction and clone formation [see *Moonwort Systematics* under *Population Genetics, Breeding System*]. Thus gene silencing at a particular locus, once it occurs, is faithfully propagated through the subsequent line

of descendants. At the same time, at other loci and in other plants, other independent silencing mutations occur creating multiple lines of descendants, each with its particular pattern of gene silencing. Since silencing can occur at virtually every locus, an infinite array of genotypes is generated, all differing by which alleles have been silenced alleles at which loci.

To illustrate the effect of silencing, at one of the gene loci for triosephosphate isomerase, *B. neolunaria* produces an allelic form of the enzyme that migrates more slowly in electrophoresis than that from any other moonwort species. Throughout south coastal Alaska that allele (as well as a faster allele contributed by the non-neolunaria parent) is expressed in *B. minganense* except in one large population at Anka Beach near Yakutat. In this population only the faster allele contributed by the non-neolunaria parent is expressed. Morphologically, plants from all sites are indistinguishable. In interior south Alaska different populations of *B. minganense* display all three possible conditions of expressing only the neolunaria allele, only the non-neolunaria allele, or expressing both.

Often all the plants of a given population display the same genotype, i.e., have the same pattern of silencing. Most likely this results from these populations having been founded by individual spores or having gone through a bottleneck in population size where only one genotype survived, subsequently repopulating the area by clone-like reproduction via intragametophytic self fertilization. When such a population also carries a morphological peculiarity we may see an accidental correlation of morphotype and genotype. However when viewed over a larger geographic area, such correlations do not hold (Farrar, 2001).

Genetic variability of *B. minganense* is thus generated by silencing at each locus of one or the other of the two genes that it received in its initial formation through allopolyploid speciation. Only rarely is a third allele present, but this is the case at a very unusual population of *B. minganense* in the Umatilla National Forest in northeastern Oregon. Here plants grow in a circular, naturally treeless area within the forest. These plants exhibit unusual morphological forms as well as a gene allele for malate dehydrogenase not present in any other moonwort species. Possibly the soil conditions that have prevented trees from growing in this area and caused morphological peculiarities have also induced new mutations into this population.

*Botrychium minganense* is one of the most widespread and abundant of tetraploid moonwort species. It also occupies a wide range of habitats. The increased genetic variation inherent in allopolyploids may have contributed to the success of this and other such species (more than 60% of moonwort species are allopolyploids). It is also possible that the genetic variability generated by gene silencing may have enhanced the species' ability to exploit a wide diversity of habitats across its broad range.

## Phylogenetic Relationships

*Botrychium minganense* is a tetraploid species ( $n = 90$ ), containing twice the number of chromosomes of *B. neolunaria* and other diploid moonworts. Both its morphology and its allozyme patterns leave no doubt that one of its ancestral parents was *B. neolunaria*. Allelic matches for the other parent are imperfect, but the currently known diploid species containing the greatest number of non-*neolunaria* alleles found in *B. minganense* is *B. pallidum*. This diploid or a similar, possibly extinct relative also has morphological characters expressed in the color, pinna lobing, and presence of gemmae found in *B. minganense*. This non-*neolunaria* parent is currently listed as *B. "X"* in the table of allotetraploid species in *Moonwort Systematics*.

Additional images of broad- and narrow-pinnae forms of *Botrychium minganense*:

