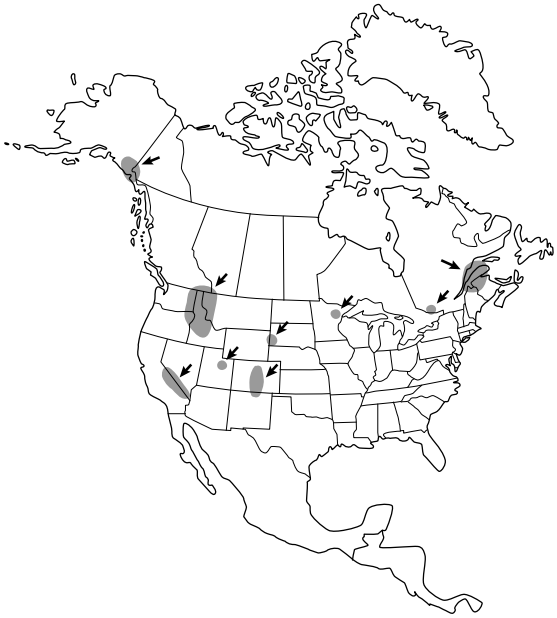
Botrychium campestre var. *lineare*

Family: Ophioglossaceae
Genus: *Botrychium*
Species: *Botrychium campestre* var. *lineare* (W. H. Wagner) Farrar comb. *nov. ined.*
Common name: Slender Moonwort
Ploidy: Diploid



Technical description—A small *Botrychium*, the leaf 6-18 cm long above the upright subterranean stem 0.5-3.0 cm long. Trophophore sessile to stalked up to 1 cm. Blade oblong, 1-pinnate. Pinna pairs 4-6, strongly separated by 2-5 times the width of segments, to moderately ascending, approximately equal in length, except for shorter apical 1-4 pairs of segments. Pinnae mostly straight, slightly upcurved, narrowly linear (narrowest in small plants), often expanded at the truncate apex or rarely linear-spatulate, commonly bifid with linear lobes. Color pale green; texture thick chartaceous, rigid. Sporophore 1-2 times the length of trophophore, with a single major axis. (Wagner and Wagner, 1994 as *B. lineare*)

Taxonomy

Botrychium lineare was described as a new species in 1994 by W. H. Wagner (Wagner and Wagner, American Fern Journal 84:5-10), based on type specimens from Wollowa Co., Oregon. Silhouettes of the species are included in that publication. From a genetic comparison of *B. lineare* and *B. campestre* throughout the range of these two taxa, D. R. Farrar concluded that these taxa are best treated as varieties of a single species, *B. campestre* (see discussion below).

In describing *B. lineare*, Wagner recognized its close relationship to *B. campestre*. Genetic identity, a statistical measure of the allelic similarity between taxa, between these two species at a value 0.8 (see *Moonwort Systematics*) is much higher that that generally expected between species within a genus (usually 0.7 or lower).

Discovery of a large area containing large numbers of both species at Witch Springs prairie in the Black Hills of South Dakota allowed further testing of the relationship between *B. lineare* and *B. campestre* as they occur in the Great Plains and the eastern U.S. Previous analysis of plants in the Black Hills and eastward in South Dakota, Iowa and Minnesota showed the two taxa consistently expressing different alleles at two gene loci, and differing significantly in allele frequency at an additional four loci. Furthermore, very few hybrid individuals had been identified. At Witch Spring, plants of both taxa were collected for genetic analysis in numbers representing their relative frequencies as they occurred throughout approximately 10 acres of west-facing hillside and the drainage basin at its base. Of 56 plants tested, 24 displayed the typical genotype of *B. campestre*, 30 displayed the typical genotype of *B. lineare*. Two displayed combinations of these genotypes, presumably due to hybridization between the two taxa.

Of those plants with position data (this information was lost for 4 plants) 26 of 29 plants of *B. lineare* we collected from the drainage basin, whereas *B. campestre* was more evenly distributed with 9 of 22 plants having been collected from the well-drained hillside. The two plants displaying hybrid genotypes were collected from the drainage area where both taxa occurred.

Undoubtedly the dominant pattern of self-fertilization in gametophytes of *Botrychium* (that has been demonstrated by several researchers) strongly limits the number of hybrids formed. Nevertheless, if hybrid plants are formed, are vigorous and are fertile, as the Witch Springs plants appear to be, they should reproduce their hybrid genotype and disperse as do the parental taxa. A hybridization rate of approximately 4% per generation, as found in this study, should lead, over time, to a greater abundance of hybrids, and the occurrence of some populations composed predominantly or entirely of hybrids. The absence of such populations at Witch Spring and elsewhere where *B. lineare* and *B.*

campestre co-occur suggests that the hybrids do not reproduce and migrate as freely as their parent taxa, i.e., they may have reduced fitness.

The Witch Spring data suggest that *B. campestre* and *B. lineare* have diverged in the Great Plains and eastern U.S. sufficiently to warrant recognition as different taxa.

Genetic and morphological examination of plants in the mountains of the western US and Canada present a different picture from those east of the mountains. Discovery of many large populations at high elevations in Colorado has allowed extensive genetic and morphological testing. Results of these studies indicate that the mountain taxon (variously identified as *B. lineare* or *B. campestre*) not only displays the full range of genetic and morphological variation found in eastern plants, but additional variation as well, and this variation is displayed within large populations. The Colorado populations display the greatest genetic variability of any species of *Botrychium*. They also display a high level of genetic heterozygosity consistent with regular outbreeding.

The great variability of mountain populations and their breeding system suggest that these populations are ancestral to the eastern populations which have diverged into morphologically and genetically distinct “lineare” and “campestre” taxa. In part, this is likely due to adoption, in this region, of sexual reproduction through inbreeding that maintains these differences. But because two taxa cannot be clearly differentiated in the western mountains, and because the eastern populations are genetic and morphological subsets of the mountain populations, maintenance of *B. lineare* and *B. campestre* as distinct species is not warranted. However, because the two can be separated in the Great Plains and eastward it is appropriate to recognize, in this area, var. *lineare* and var. *campestre* as varieties of *B. campestre*. If a varietal name is to be applied to mountain populations, most would fit genetically and morphologically most comfortably into var. lineare. *B*. *campestre* var. *campestre* is the taxon that is most diverged from the mountain populations, especially in its adoption of dry lowland prairies and glades as its principal natural habitat.

Identification

The pinnae of *Botrychium campestre* var. *lineare* have the narrowest pinna span of all moonworts, hence the common name, slender moonwort. They broaden scarcely at all toward their outer margin except as they divide, usually into two lobes diverging at an angle of about 45^{o} or more. The basal pinnae are typically the largest and longest. Only two other taxa approach this morphology. *Botrychium campestre* var. *campestre* has shorter, broader pinnae that are less deeply lobed and a rachis that is broader relative to the total leaf width. In var. *campestre*, the largest and longest pinnae are typically above the basal pair. Narrow-pinnae forms of *B. ascendens* may be similarly lobed, but the lobes

broaden toward an outer margin that is dentate. At maturity the sporophore stalk of *B. ascendens* is usually approximately half the length of the trophophore whereas it is distinctly less than half (usually 1/3 or less) the length of the trophophore in both varieties of B. *campestre*. Variety *lineare* and *B. ascendens* tend to be less glaucous than *B. campestre var. campestre*.

Distribution and Abundance

B. lineare was petitioned for listing as a Candidate Endangered Species in 1999 and became officially listed by the US Fish and Wildlife Service as a candidate species in 2001. This listing initiated extensive field search and study of the species' biology and genetics throughout its range, resulting in a number of new population discoveries, some estimated at more than 500 individuals, and significant range extensions. Based on these discoveries, the Fish and Wildlife Service officially removed *B. lineare* from the list of Candidate Endangered Species in 2007.

Botrychium campestre var. *lineare* is among the less frequently sighted of all moonwort species, but diligent search has shown it to be more common than previously thought. It is now known from Alaska and Yukon south in the Rocky Mountains to Colorado and northern New Mexico and in the Sierra mountains to southern California and southern Nevada. Eastward it is known from the Black Hills of Wyoming and South Dakota to northern Minnesota and western Quebec. It is known historically from eastern Quebec and New Brunswick. likely. Because of its distinctive morphology it is unlikely that *B. campestre* var. *lineare* has been overlooked or misidentified more so than other moonworts. Rather, its sporadic occurrence is probably a true reflection of its relative abundance.

Earlier searches in historic sites in eastern Canada were unsuccessful, however, its recent discovery in eastern Quebec has provided new insight into its preferred eastern habitat. These plants grow under upland stands of *Thuja occidentalis*, suggesting that additional search in this habitat may be more profitable. In Minnesota, *B. campestre* var. *lineare* occurs in herbaceous meadow-like vegetation in iron mine tailings basins.

Most populations of *B. campestre* var*. lineare* are small, ranging from 1 to 100 with most being less than 10. However, a population recently discovered in the Black Hills of South Dakota is estimated to exceed 500, and a number of Colorado populations exceed 100. The breadth of the species' range, the small size of many populations and the general inconspicuousness of the plants suggest that more undetected populations may exist, especially in the mountains of western Canada, the northern Great Plains and northeastern North America .

As with other mountain populations, the morphology and genetics of California and Nevada plants allies them most closely with *B*. *campestre* var.

lineare. On the basis of current knowledge, *B. campestre* var*. lineare* in California must be considered truly rare. In describing *B. lineare*, W. H. Wagner examined California herbarium specimens from Piute Canyon in Fresno County (incorrectly labeled as Inyo Co.) as *B. lineare* and from Monache Creek in Tulare County as *B. campestre*, although the morphology of the Tulare Co. plants is equally consistent with that of *B. lineare*. Both of these sites have been revisited with discovery of single plants at the Monache Creek site in 2005 and 2006, and the Piute Canyon site in 2007. A historic collection from an unlisted county "in a canyon on South Mt., Mill Creek Falls opposite Vivian Creek Falls" is particularly intriguing as it contains a drawing of a plant that must surely have been *B. campestre var. lineare*. The precise location of this site is unknown. In 2008 *B. campestre* var*. lineare* was found at the head of Virginia Canyon in Yosemite National Park in Tuolumne County.

The Monache Creek site appeared to be heavily overgrown by *Salix*. Possibly succession has greatly limited the amount of suitable habitat since the early collections of numerous plants at this site. Brief search of other seep springs in the area disclosed presence of other *Botrychium* species, suggesting that intensive search of these sites (topographic maps indicate many of these high elevation springs in the general area) may be profitable. The discoveries of many rare species (see discussions of *B. paradoxum, B. tunux* and *B. yaaxudakeit)* at the Virginian Canyon site also suggest that search of limestone/marble outcrops in the Sierra Mountains may locate additional populations of *B. campestre* var*. lineare*.

Nevada plants of *B. campestre* var. lineare are located in seep springs in the Spring Mountains near Las Vegas. A few plants have been located at several sites, but historic collections indicate presence of 100's of plants at several sites.

Habitat

Wagner and Wagner (1994) described one of the Quebec *Botrychium campestre* var. *lineare* habitats as "a steep limestone cliff with narrow grassy horizontal terraces" on which the plants were supposed to have grown. The other Quebec site was described by the collector as "a gravelly beach". The New Brunswick collection lacked details of habitat other than "mountain". The habitat of the recently discovered western Quebec plants near Gatineau is on limestone bedrock under stands of *Thuja occidentalis.* Minnesota plants occur among herbaceous and young tree growth on abandoned iron mine tailings and drained settling ponds.

Habitats of western populations of *Botrychium campestre* var. *lineare* are primarily meadows, fen-like seeps and gravelly roadsides. The largest known extant populations in the Black Hills of South Dakota are native prairie remnants and pastured prairies. Additional smaller populations in the Black hills occur on

abandoned grassy logging roads. Most of the populations in the Rocky Mountains from Colorado to Alaska are along roadsides where plants grow in gravelly soil and similar surfaces resulting from past (15 to 50 year old) disturbance. In more natural settings *B. campestre* var*. lineare* grows at high elevations in similarly gravelly meadows resulting from natural weathering, including avalanche meadows. In California and Nevada plants grow on the margins of fen seeps and streams where their roots reach mineral soil and on grassy ledges at the base of calcareous rock outcrops.

The various habitats of *B. campestre* var*. lineare* are not as disparate as they initially appear to be. Nearly all are associated with mid-successional meadow vegetation composed dominantly of perennial herbaceous vegetation developed over calcareous bedrock or soils influenced by calcareous seepage. They are seldom found under dense woody canopies and are not found in recent (less than 10 to 15 years since last disturbance) disturbances such as roadsides, although such disturbances, with sufficient aging, may become supportive of moonwort growth if other conditions of sufficient moisture and appropriate soil chemistry and texture are also met.

Aging of disturbed areas generally results in community succession from weedy annuals to herbaceous perennials to, in areas suitable for tree growth, closed-canopy forests. It may be that the endophytic mycorrhizal fungus required by *Botrychium* species, itself prefers or requires perennial herbaceous vegetation for its carbohydrate source. It is noteworthy, in this regard, that the only forests known to support *B. campestre* var. *lineare* (and and other *Botrychium* species, including *B. campestre* var*. campestre*) are those dominated by Cupressaceae *s.l.* Species of this family are among the few conifers known to be colonized by endophytic mycorrhizal fungi.

There is strong indication that *Botrychium campestre* var*. lineare,* as well as other moonwort species, tend to occur on limestone influenced substrates. The Quebec sites are a limestone, and the two Oregon sites are on either side of a mountain ridge capped with limestone. The large populations in the Black Hills occur on the belt of limestone encircling the igneous core. Colorado populations occur almost exclusively on sites influenced by limestone bedrock. In Virginia Canyon in California *B. campestre* var*. lineare* occurs at the base of cliffs containing rare outcrops of metasedimentary limestone. The other California populations are in sites influenced by hardwater seeps.

Population Genetics

Like other species of *Botrychium*, an overall low genetic variability and a low number of heterozygous plants indicate that the Great Plains and eastern populations of *Botrychium campestre* var. *lineare* reproduce sexually primarily through intragametophytic self-fertilization, thus it is capable of dispersing via

single spores. In addition, both varieties of B. campestre reproduce prolifically by asexual underground gemmae. This asexual propagation further contributes to a high degree of genetic uniformity within populations.

Mountain populations of *B. campestre* var. *lineare* possess greater genetic variability than most moonwort species. Among populations it is polymorphic (has more than one allele) at 45% of its loci and has an average of 1.55 alleles per locus. The averages for all diploid species of moonwort *Botrychium*s for these measures of genetic variability are 28.8% and 1.36 alleles per locus. Other ferns and flowering plants average over 50% polymorphic loci and about 2 alleles per locus (T. A. Ranker, Univ. of Colorado, personal communication and Hamrick and Godt, 1990). The averages for *Botrychium* drop to 23.8% and 1.28 alleles per locus when the highly variable *B. simplex* is omitted from the average. Only *B. simplex* with its several varieties approaches *B. campestre* var. *lineare* in overall genetic variability.

Because mountain populations of B. campestre var. lineare appear to be outbreeding, inbreeding depression in the small populations could be of conservation concern. On the other hand, the elevated genetic variability within larger populations, relative to other moonworts, may give *B. campestre* var. *lineare* a greater adaptability to environmental change than other *Botrychium* species. As with all *Botrychium*, reliance on mycorrhizal fungi for water, mineral and organic nutrition may also make them less sensitive to environmental change.

Phylogenetic Relationships

Recent discovery of many large populations of *B. campestre* var*. lineare* plants at high elevations in Colorado bear relevance to the origins of the complex. Populations of these plants typically contain a range of morphologies and genotypes encompassing those of both *B. campestre* varieties, as well as morphologies and alleles distinct from either. Surprisingly, these populations contrary to all other populations of all other *Botrychium* species that have been studied genetically, express an outcrossing breeding system. The number of heterozygous individuals in these populations, as well as the total amount of genetic variability, is typical of most outbreeding ferns. An important possibility is that this taxon may be retaining the ancestral condition of all diploid *Botrychium* species.

A reasonable phologenetic hypothesis is that mountain populations of B. campestre are ancestral to both eastern varieties*.* Each of the eastern derivative varieties retain a subset of the genetic and morphological variation of the parent mountain species. Possibly this occurred through increasing isolation of mountain populations following a more or less continuum of occurrence along the boundaries of Pleistocene glaciation. It is interesting to note in this regard that

var. *campestre*, southernmost of all Great Plains species, has adopted the earliest of all *Botrychium* phenologies, allowing it to emerge, mature and senesce in early Spring while its habitat mimics that of boreal and mountain habitats, and before the onset of summer drought and heat. Although var. *lineare* and var. *campestre* co-occur in intermediate habitats in the Great Plains and eastward, var. *lineare* more typically occurs in boreal and mountain habitats and has a distinctly later phenology.

Botrychium var. *lineare* is a diploid species (n=45). It may be one of the ancestral parents of *B. ascendens* (along with *B. lunaria* var. *crenulatum*). This is suggested by the tendency of *B. ascendens* to produce bilobed pinnae similar to those of *B. campestre* var. *lineare*. The slender, widely diverging lobes of pinnae of *B. echo* likewise suggest *B. campestre* var. *lineare* as an ancestral parent of that species (along with *B. lanceolatum*). *B. campestre* var. *lineare* also provides the best match for parentage of *B. spathulatum* (along with *B. lunaria*.) However, because most alleles are shared between both varieties of *B. campestre,* on the basis of electrophoretic data it is not possible to distinguish with certainty between these varieties as to the ancestral parents of *B. ascendens*, *B. echo and B. spathulatum*. The best assumption may be that an ancestral species similar to *B. campestre var. lineare* participated in the origin, through allopolyploidy, of these allotetraploid species.

Additional images of *Botrychium campestre* var*. lineare*:

Above photographs by Drake Barton