Slender Moonwort, *Botrychium lineare* (Ophioglossaceae), Rediscovered in Quebec

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Slender Moonwort (*Botrychium lineare*), described in 1994, is a very rare fern in eastern North America. It was known in Quebec, Canada, from only two sites in the Gaspé Peninsula but has not been relocated since its discovery at these sites in 1902 and 1942. An ongoing study of *B. lineare* and its recent discovery in northern Minnesota prompted a re-examination of a 1972 collection made in western Quebec, in Gatineau Park, previously identified under various names, including *B. campestre*. A recent visit (2008) to the Gatineau Park site disclosed continued presence of similar plants. Based on morphological characters, spore size, genotype (allozyme electrophoresis), and appropriate habitat features, we conclude that the newly discovered plants and those of the 1972 collection are *B. lineare*. *B. lineare* appears on the Quebec government list of plant species likely to be designated threatened or vulnerable.

Key Words: Slender Moonwort, *Botrychium lineare*, Prairie Moonwort, *Botrychium campestre*, Ophioglossaceae, spores, rare plants, Quebec.

Slender Moonwort (also called Narrowleaf Grapefern), *Botrychium lineare* W.H. Wagner (Figure 1), was described in 1994 (Wagner and Wagner 1994) just one year after the publication of the moonwort family in Volume 2 of the *Flora of North America* (Wagner and Wagner 1993). The Wagners stated that they had begun to recognize the species as distinct about 1978 and finally gathered enough material and evidence to describe it. The species was initially considered to be very rare in North America. In addition to the type collection from Oregon, the species had been found in the United States in California, Idaho, and Montana, and in Canada in New Brunswick and Quebec (Wagner and Wagner 1994). The largest populations numbered 45 and 15 individuals, and a total of only 85 specimens had been seen by the Wagners at the time they described the species.

Today there is better knowledge of this little fern and its habitat, with the addition of substantial collections in western North America, including the Yukon Territory (Farrar 2006*). Although some of the earliest known populations seem to have disappeared, DRF has observed perhaps 100 or more sites, some with hundreds of plants, between South Dakota and California and southern Nevada to Alaska and Yukon. On this basis, the US Fish and Wildlife Service removed Slender Moonwort from its list of Candidate Endangered Species.

However, the situation is very different in eastern North America, where it is one of the rarest species. Only five eastern populations have been recorded: two recently discovered sites in Minnesota and the three

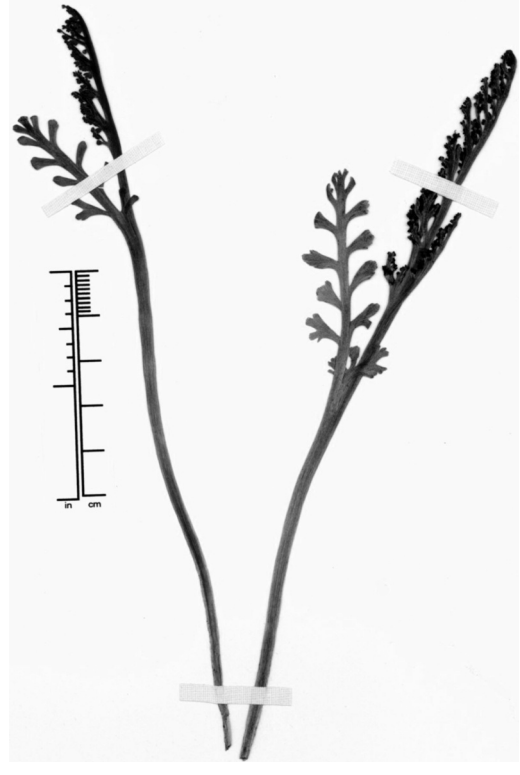

FIGURE 1. *Botrychium lineare* (Mont Commis, Quebec, *E. Lepage 3395*, QFA 95938).

sites cited by the Wagners (one in New Brunswick and two in Quebec; Wagner and Wagner 1994). Attempts by Herb and Florence Wagner to relocate the population observed in 1904 in New Brunswick failed (Wagner and Wagner 1990*).

In Quebec, the two previously located sites are in the Gaspé Peninsula, one at Paspébiac (Bonaventure County: Paspébiac Lighthouse, gravelly beach, 27 July 1902, *E.F. Williams & M.L. Fernald s.n.*, Gray Herbarium, Harvard University (GH)) and the other at Mont Commis, east of Rimouski (Rimouski County: St. Donat, Mont Commis, tablette herbeuse au pied de la muraille, 2 July 1942, *E. Lepage 3395*, Louis-Marie Herbarium, Université Laval (QFA) (Figure 1), Marie-Victorin Herbarium, Institut de recherche en biologie végétale and Université de Montréal (MT)). To our knowledge, the Paspébiac population has not been observed recently. However, serious attempts were made to examine the Mont Commis population. In July 1990, the Wagners, with the help of Pierre Morisset of Université Laval, Quebec City, his wife Catherine, and a colleague, and with guidance from the staff of the Louis-Marie Herbarium, found the site where Quebec botanist Ernest Lepage had discovered Slender Moonwort in 1942 (Figure 1) (Wagner and Wagner 1990*). Climbing on various exposed ledges, the group carefully explored the north-facing limestone cliff at the top of a wooded talus. Despite considerable effort, they found only the Mingan Moonwort (*Botrychium minganense* Victorin), which had also been collected by Lepage along with the then unknown *B. lineare*. The Wagners attributed the failure to locate the population either to its extirpation or simply to the fact that it did not appear that year (Wagner and Wagner 1994).

In July 2000, JC, accompanied by botanists Frédéric Coursol, Martine Jean, and Jacques Labrecque, made another attempt to find *Botrychium lineare* at the Mont Commis site, but we were also unsuccessful. Given the lack of confirmed locations, the Slender Moonwort has been considered to be very rare in Quebec, with a ranking of SH (Possibly extirpated (historical)) (Labrecque and Lavoie 2002*), and it was still similarly ranked in the most recent edition of the Quebec list of species likely to be designated threatened or vulnerable (CDPNQ 2008*).

The claim that the species has been rediscovered in Quebec, based on a re-identified collection made in Gatineau Park in 1972 (Figure 2) and on a specimen collected in the same area in 2008 (Figure 5), requires justification. Moonwort classification and identification are very complex (Farrar 2006*; unpublished data). Identifications based on undeveloped specimens are challenging even for specialists. Recent isozyme and molecular marker techniques are now very useful tools that are assisting in moonwort identification and taxonomy.

The 1972 collection in Gatineau Park (Quebec, Gatineau County: ½ mile east of the junction of Mountain

FIGURE 2. *Botrychium lineare* collected in 1972 in Gatineau Park, Quebec (*J.D. Lafontaine s.n.*, DAO 653584).

Road and Bailey Road, base of escarpment, dense cedar woods, 4 July 1972, *J.D. Lafontaine s.n.*, DAO 653584) received four different identifications before it was finally determined to be *Botrychium lineare* (Figure 2). The specimens, at the early sporulating stage, were first identified as Least Moonwort (*Botrychium simplex* E. Hitchcock) and its variety *tenebrosum* (A.A. Eaton) Clausen. They were next identified as Daisy-leaved Moonwort (*B. matricariifolium* (Döll) A. Brown) preceding the recognition of the two following taxa. The collection was next identified as Prairie Moonwort (*B. campestre* W.H. Wagner & Farrar) by the two specialists who described that species as new (Wagner and Wagner 1986), and that identification was at first confirmed earlier in 2008 by DRF.

Botrychium simplex var. *tenebrosum* and *B. matricariifolium* superficially resemble *B. lineare*, but *B. campestre* is a closer relative (Wagner and Wagner 1994; Farrar 2006*). *Botrychium simplex* var. *tenebrosum* differs from *B. lineare* by the trophophore (refers to the sterile lamina or blade of a botrychium leaf) having medial segments (pinnae) wider than long, mostly rhombic, ovate or elliptical, and frequently decurrent along the rachis, and by the length of the stalk (unbranched portion) of the sporophore (the sporangium-bearing half of a botrychium leaf) exceeding or nearly

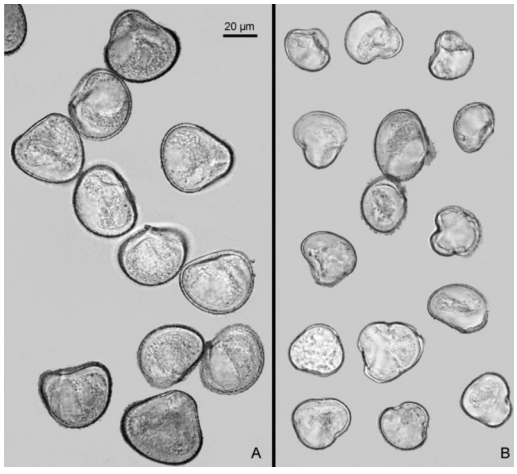

FIGURE 3. Spores of *Botrychium*: a) *B.* cf. *matricariifolium* (*J. Cayouette C9614*, DAO); b) *B. lineare* (*J.D. Lafontaine s.n.*, DAO).

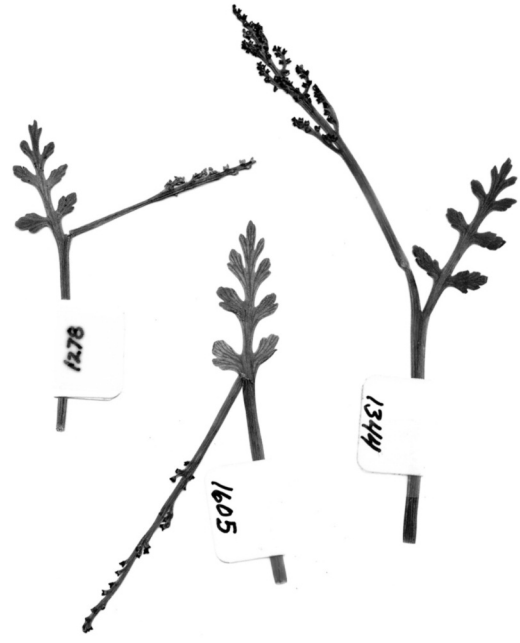

FIGURE 4. Shade specimens of *Botrychium matricariifolium* collected in the Ottawa National Forest, Gogebic County, Michigan, USA (D.R. Farrar, various numbers).

equal to the entire length of the trophophore. In *B. lineare*, the medial trophophore segments are longer than wide, mostly linear, not decurrent on the rachis, and the sporophore stalk is very short, only one-quarter to one-half the length of the trophophore (Figures 1, 5 and 6).

Botrychium matricariifolium differs from *B. lineare* by the trophophore segments above the proximal pair, ovate to elliptic, frequently strongly pinnately divided or at least clearly lobed (Figure 4), with pinnate nervation (the central one enlarged), by proximal pair not so different from the adjacent one (Figure 4), by a long sporophore stalk, and by relatively large spores (Figure 3a). On the other hand, *B. lineare* has the trophophore segments above the proximal pair, linear and sometimes palmately lobed or cleft toward the apex (Figures 1 and 6), with palmate nervation, a shorter sporophore stalk, and smaller spores (Figure 3b).

Slender Moonwort is a close relative of *Botrychium campestre*. They are both diploid species, rather short, with very short sporophore stalks, and with trophophore segments narrow, linear or spatulate, relatively undivided (or, if divided, palmately cleft into two to four segments) and frequently well spaced and separated (Farrar 2006*).

The main differences are related to their morphology, ecology and phenology (Wagner and Wagner 1994; Farrar 2006*). At the morphological level, the trophophore rachis of *Botrychium campestre* is fleshy and broad, up to one-third as wide as the whole trophophore, dull and whitish green, their segments linear to spatulate, commonly overlapping or fused, clearly enlarged at the apex, up to 3-5 lobed, the longest segments toward the upper portion of the trophophore; the entire sporophore length is 1.0–1.5 times that of

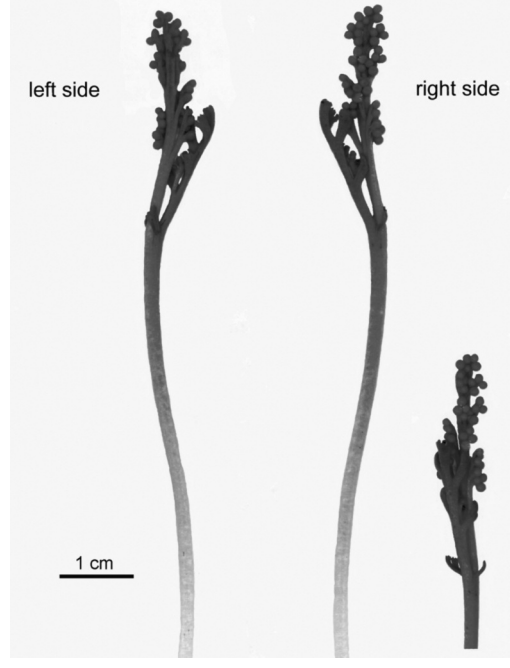

FIGURE 5. *Botrychium lineare*, same specimen viewed from different sides (Gatineau Park, Quebec, *J. Cayouette C9615*, DAO).

the trophophore. On the other hand, the trophophore rachis of *B. lineare* is more herbaceous and narrower, up to one-fifth as wide as the whole trophophore, pale green, their segments more linear or slightly spatulate, separated, slightly enlarged at the truncate apex, with the longest ones, located at the base of the trophophore, sometimes two or more times bifid; the sporophore is up to 2.0 times the trophophore in length. These differences are more easily observed in well-developed specimens.

Botrychium campestre is rather a prairie species of open habitat, where it is also frequently found under Ground Juniper bushes (*Juniperus communis* L. var. *depressa* Pursh) and even close to stands of Eastern Red Cedar (*J. virginiana* L.). This species appears very early. In Iowa, the trophophores are out in April and have disappeared in mid-June (Farrar 2006*). Spores start to mature in May. By contrast, *B. lineare* has been found in various types of habitats, but in eastern North America it occurs in open forests (Wagner and Wagner 1990*, 1994; Farrar 2006*). It appears later, and spores mature from late June to July. Both species seem to prefer calcareous sites.

Based on the previous identification of the 1972 Gatineau Park specimen to *Botrychium campestre* (Figure 2) and on the presence of another collection so identified from the Mingan Islands area, this species was earlier included in the flora of Quebec and in a previous list of rare species (Labrecque and Lavoie 2002*). However, due to recent revisions of the Mingan Islands specimens to Upswept Moonwort (*B. ascendens* W.H. Wagner)—the populations recently relocated and identification confirmed by Labrecque (2007*)—and to the uncertainty of the Gatineau Park identification, *B. campestre* was removed from the flora of Quebec and as part of the rare plant list (CDPNQ 2008*). The current redetermination of the 1972 specimen to *B. lineare* confirms this decision.

At the end of June 2008, DRF examined the 1972 specimen in the Agriculture and Agri-Food Canada National Collection of Vascular Plants, Ottawa (DAO) (Figure 2), and found that the small size of the spores (Figure 3b) was consistent with the species being diploid, with the result that the name of *B. campestre* would be appropriate. On 1 July 2008, in an attempt to relocate the site in Gatineau Park, the authors found four small specimens of *Botrychium* with larger spores (Figure 3a), which better fit the *B. matricariifolium* group, and one plant that appeared to be *B. lineare*. The next day, JC found another site close by with a few more specimens, one of which appeared to be *B. lineare* (Figure 5). The sporophore is somewhat longer than the trophophore, and the trophophore segments are well spaced, narrow, linear, just slightly enlarged at the apex, and sometimes lobed. The above-ground leaf of the specimen (Quebec, Gatineau, Parc de la Gatineau, chemin de la Montagne, sous-bois de *Thuja occidentalis* sur rochers de marbre, au pied d'un escarpement, 2 July 2008, *J. Cayouette C9615*, DAO) was sent to DRF for genotype analysis via enzyme electrophoresis. This analysis revealed the plant to be *B. lineare*. Owing to the failure to rediscover this rare species in the Gaspé Peninsula and in New Brunswick, the Gatineau specimen becomes the easternmost known extant population of this species in North America.

FIGURE 6. *Botrychium lineare* collected in 2009 in Gatineau Park, Quebec. Photo: J. Labrecque.

This site is situated at the base of the Eardley escarpment, where stands of White Cedars (*Thuja occidentalis* L.) indicate the presence of Precambrian marble. The plant diversity seems to be very limited where *Botrychium lineare* was found. Apart from White Cedars, only shrubs of Mountain Maple (*Acer spicatum* Lam.) and Red-Osier Dogwood (*Cornus sericea* L.) were observed, along with a few herbaceous plants such as Marginal Wood Fern (*Dryopteris marginalis* (L.) Gray), Woodland Strawberry (*Fragaria vesca* L.), and Rock Polypody (*Polypodium virginianum* L.).

The southern exposure of the habitats of the Eardley escarpment are conducive to the presence of some of the rarest plants of the Quebec flora. In 1971 and 1972, botanists Daniel Brunton and Donald Lafontaine made substantial discoveries of rare plants close to the site of *Botrychium lineare*, such as Sicklepod (*Boechera canadensis* (L.) Al-Shehbaz), Purple-Stem Cliff-Brake (*Pellaea atropurpurea* (L.) Link), and Blunt-Lobed Woodsia (*Woodsia obtusa* (Spreng.) Torrey) (Lafontaine

1973; Brunton and Lafontaine 1974). Later and still in the same area, Daniel Gagnon discovered the rare Eastern Few-fruited Sedge (*Carex oligocarpa* Willd.) (Hay and Gagnon 1986). Up to now, these are the only sites in Quebec for *Boechera canadensis* and *Carex oligocarpa* (CDPNQ 2008*). This is now also the case for *Botrychium lineare*.

We hope the information presented here on *Botrychium lineare*, including its spore size, habitat preference for rocky calcareous sites, and phenology, will aid in the search for new sites of this extremely rare fern in eastern North America.

Note

After the completion of this paper, additional information became available on the same Gatineau Park population of *Botrychium lineare.* In July 2009, an inventory of the site was conducted (Cayouette and Labrecque 2010*). Forty-four individuals of *Botrychium lineare* (Figure 6) were discovered and their habitat evaluated. The *B. lineare* population was found to be slightly more widespread than expected. A few individuals of two other species of *Botrychium* were discovered on the site: *B. matricariifolium* (probable) and *B. minganense*. Further surveys are planned in order to follow the development of the population.

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