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Distribution, status, and habitat characteristics of Columbia Quillwort (*Isoetes minima*, Isoetaceae) in Canada

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Abstract

In Canada, the globally rare lycophyte, Columbia Quillwort (*Isoetes minima*), is currently known from four subpopulations, all within a 25-km radius of Castlegar in the Selkirk and Monashee Mountain ranges of southern British Columbia. These constitute just over a quarter of all known subpopulations in Canada and the United States. The species is found in Canada in sloping pocket meadows that are naturally fragmented within a larger forested matrix. The plants grow in spring seepage areas in thin soils that discourage the establishment of larger, more vigorous vascular plant competitors. Long combined within *Isoetes howellii* (*sensu lato*), *I. minima* has only recently been confirmed to be a distinct species, and, in 2019, it was assessed as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). We build on information in the COSEWIC status report by describing the species' morphology and ecology in greater detail and provide a comparison of critical identification features of closely related species as well as a dichotomous key for *Isoetes* species in British Columbia.

Key words: *Isoetes minima*; British Columbia; Endangered; Species at Risk; Canada

Introduction

Quillworts (*Isoetes*, Isoetaceae, Isoetales) are an ancient and widespread lineage of perennial lycophytes with a fossil record that dates from the Late Devonian era (375 million years ago [mya]; Pigg 2001; Larsén and Rydin 2016). Contemporary lineages arose during the Jurassic Period (200–145 mya) and diversified in the mid-Paleogene (Tertiary Period; 45–60 mya; Pigg 2001; Wood *et al.* 2020).

Currently, there are ~200 named taxa (species, subspecies, hybrids) of *Isoetes* worldwide (Troia *et al.* 2016), with perhaps 100 more expected to be recognizable based on morphology (Brunton and Troia 2018). Molecular studies suggest that, in addition, there may be 50 or more morphologically cryptic taxa in North America alone (Schafran 2019). In the last 30 years, two species have been described in Canada (Britton and Goltz 1991; Brunton *et al.* 2019), and an undescribed taxon is suspected to exist along the Pacific coast of British Columbia (BC; D.F.B. unpubl. data).

In Canada, there are 13 described *Isoetes* species, six of which are found in BC (Cody and Britton 1989; Taylor *et al*. 1993, 2003; Brunton *et al.* 2019, 2020). Half of the BC species are aquatic or grow on emergent shores of rivers and ponds, while the other half are terrestrial species found in ephemeral pools and seeps (Klinkenberg 2020).

Isoetes minima A.A. Eaton (*I. howellii* var. *minima* (A.A. Eaton) N. Pfeiffer) is a sexual diploid ($2n = 2x = 22$; Taylor *et al.* 2003) within a complex group of western North American quillworts (Figure 1). In Canada, this group includes Bolander's Quillwort (*Isoetes bolanderi* G. Engelmann), Howell's Quillwort (*Isoetes howellii* G. Engelmann, including *Isoetes melanopoda* var. *californica* A.A. Eaton and *Isoetes underwoodii* L. Henderson), and Nuttall's Quillwort (*Isoetes nuttallii* A. Braun).

Isoetes minima was first collected on 16 May 1889 by W.N. Suksdorf near Waverly, north of Spokane in eastern Washington, United States (*W.N. Suksdorf 2365,* 16 May 1889, WS 119319). It was described by Eaton (1898) on the basis of being the only trilobed species with a partial (up to 75%) velum coverage of the sporangium. Eaton (1898: 30) used these features along with spore ornamentation characteristics, the most distinctive of which he described as

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Figure 1. Mature Columbia Quillwort (*Isoetes minima*) plant; coin (Canadian dime) is 18 mm wide (14 May 2017, Fairview Meadows, British Columbia). Photo: R. Batten.

"by the equator [equatorial ridge], which resembles a ship's wheel with the spinules for handspikes", to distinguish *I. minima* from the morphologically similar Pacific coastal species, *I. nuttallii* and Orcutt's Quillwort (*Isoetes orcuttii* A.A. Eaton), which grow in similar spring ephemeral situations.

Subsequently, however, these distinctions have been misinterpreted (Pfeiffer 1922) or not considered significant by *Isoetes* researchers, and *I. minima* has been synonymized within *I. howellii* (*sensu lato*) (Taylor 1970; Cody and Britton 1989; Taylor *et al.* 1993). On his 1889 collecting trip, Suksdorf collected a number of *I. howellii* plants that looked superficially similar to *I. minima*. Based on the study of this limited material, Pfeiffer (1922) reduced *I. minima* to a variety of *I. howellii*. This treatment was followed by Taylor *et al.* (1993) in the *Flora of North America North of Mexico*. Only recently did DNA analysis of a Canadian subpopulation provide support for the original morphological evidence of the distinct species status of *I. minima* (Taylor *et al.* 2003).

The confined geographic distribution of *I. minima* and its limited dispersal ability combine to make it one of the rarest *Isoetes* species in Canada (COSEWIC 2019a) and North America. This Columbia Region endemic species is restricted in distribution to the Columbia River catchment and has been designated, globally, as Critically Imperilled (G1), with fewer than 15 known subpopulations (Natureserve 2020). It is at the northern limit of its range in Canada; in the United States, it is known from at least three sites in Washington, two in Idaho, and six in Oregon (W. Fertig pers. comm. 10 August 2020; L. Kinter pers. comm. 6 August 2020; S. Vrilakas pers. comm. 12 August 2020; Figure 2). It is assessed as Endangered in Canada (COSEWIC 2019a) and is provincially red-listed and ranked S1S2 (Critically Imperilled–Imperilled; BC Conservation Data Centre 2020).

In light of the limited number of publications detailing its natural history, status, habitat preferences, and distribution in Canada, our study builds on information provided in the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) status report (COSEWIC 2019a) and is the most comprehensive documentation of the morphology, ecology, and key identification characters of this globally rare species. The species' morphology is described in detail with precise spore size measurements and diagnostic scanning electron microscope (SEM) images that show critically important spore morphology. The site ecology is described in detail including the identification of key associates. Critical features for the identification of this challenging species are presented in comparison with previously unreported diagnostic distinguishing features between *I. minima* and its closest generic allies. A dichotomous key for *Isoetes* species in BC is included.

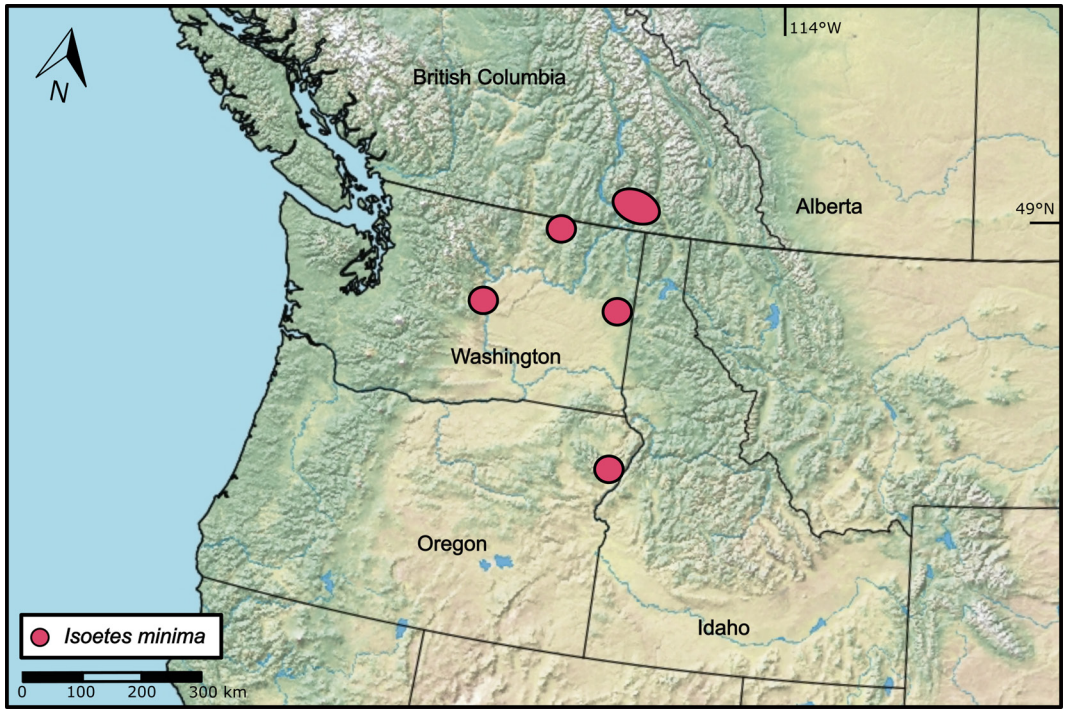

FIGURE 2. Global distribution of Columbia Quillwort (*Isoetes minima*). Basemap from SimpleMappr (Shorthouse 2010).

Methods

These investigations are based on field and herbarium studies of *I. minima* populations in Canada and the United States undertaken by us and others since 2008. In 2017, all three known sites within the Canadian range were surveyed by C.Y.M. and R.B. in the course of preparing the COSEWIC status report (COSEWIC 2019a). Herbarium records in the Canadian Museum of Nature (CAN), Agriculture and Agri-Food Canada (DAO), Daniel Brunton Private Herbarium (DFB), University of Guelph (OAC), University of British Columbia (UBC), Royal British Columbia Museum (V; acronyms of Thiers 2020), and the Consortium of Pacific Northwest Herbaria (https://www.pnwherbaria.org/data.php) were examined to identify known locations. The BC Conservation Data Centre database, the Canadian Wildlife Service (Pacific Region), Parks Canada (Conservation Programs Branch), the Ministry of Environment and Climate Change Strategy (Species Conservation Unit), and local stewardship officers (Research Ecologist, Ministry of Forests, Lands and Natural Resources) were canvassed for their knowledge of additional sites.

The three subpopulations known before 2017 were resurveyed by C.Y.M. and R.B. from 10 to 15 May 2017. *Isoetes minima* is easy to see early in the spring when surrounding vegetation is low in stature. Site details from previous records were consulted in advance to determine coordinates and site descriptors that guided plant searches. Spatial data were collected to help guide the definition of "subpopulation" for each site described in the status report. NatureServe (2004) defines subpopulation for all taxa as a group of occurrences that are separated by <1 km; or, if separated by 1–3 km, with no break in suitable habitat between them exceeding 1 km; or, if separated by 3–10 km, connected by linear water flow and having no break in suitable habitat between them >3 km. Universal transverse mercator (UTM) coordinates were recorded using a hand-held global positioning system unit (GPSMAP 62sc; Garmin Ltd., Olathe, Kansas, USA) at the centre of each cluster. (A "cluster" is a group of plants within a given subpopulation.) Other data collected included a count of the total number of plants by temporarily marking each plant with a wooden skewer (Figure 3). Habitat descriptions included associated species and repeat visits were made to each site later in the season to provide a more comprehensive associated species list as later species developed. Details of the condition of the population including threats and management concerns were described as well as the overall landscape context of the site. BC Conservation Data Centre element occurrence forms were completed for each location.

Figure 3. Columbia Quillwort (*Isoetes minima*) habitat (11 May 2017, Lloyd's Meadow, British Columbia); black sticks indicate position of mature plants and white sticks indicate location of sporelings. Photo: C. Maslovat.

In 2017, new subpopulations were searched for (C.Y.M., R.B., and D.F.B.) in areas beyond the known sites thought to have suitable habitat. Surveys undertaken in other suitable habitat in BC southwest of Rossland, west of Castlegar, east of Christina Lake, west of Creston (R.B. and C.Y.M.), and southeast of Montrose (D.F.B.) did not reveal additional subpopulations. All known suitable habitat in the area between patches of plants within confirmed sites were surveyed, with no new subpopulations found.

Megaspore and microspores of BC *I. minima* plants were examined through dissecting light microscopes and with SEM imagery. The extensive library of SEM spore images of *I. howellii* and related taxa prepared by D.M. Britton before 2007 (using methods described in Brunton and Britton 2006) was reviewed. Additional SEM images were generated by P.C.S. and D.F.B. For these new images, air-dried spores were attached to SEM stubs by means of adhesive carbon discs. These were sputter coated with a gold/palladium alloy and examined with a 2017 model SEM (FEI Apreo ThermoFisher Scientific, Hillsboro, Oregon, USA) at 15 kV and 25 pA, with a working distance of 10 mm and a spot size of 6.

Megaspore sizes reported for individual specimens represent the average width (across the equatorial region) of at least 20 (often 40) spores, as measured (D.F.B.) through a light dissecting microscope (Wild M3B, Leica, Heerbrugg, St-Gallen, Switzerland) at 40× magnification, with the aid of an in-mount graticule (ocular micrometer) for measurements. Comparable microspore measurements are based on the average of 20 (frequently 40) longitudinal measurements taken from SEM images of clusters of spores.

Results

History, distribution, and status in Canada

Isoetes minima is a relatively recent addition to the Canadian flora. The first record is from Beavervale Meadow, discovered by Oldriska and Adolf Ceska (*A. & O. Ceska #30,000*, 5 July 1996, V), and a second site (Lloyd's Meadow) was discovered later that month by Hans Roemer (*H. Roemer 96-164*, 12 July 1996, V). In 2002, a third site (Fairview Meadow) was found during a Botany BC field trip; data for that locality were submitted to the BC Conservation Data Centre by Sharon Hartwell (BC Conservation Data Centre 2017). The fourth subpopulation was found during the 2017 survey ~1 km east of Lloyd's Meadow (V). All subpopulations occur within 25 km of Castlegar, BC (Figure 4).

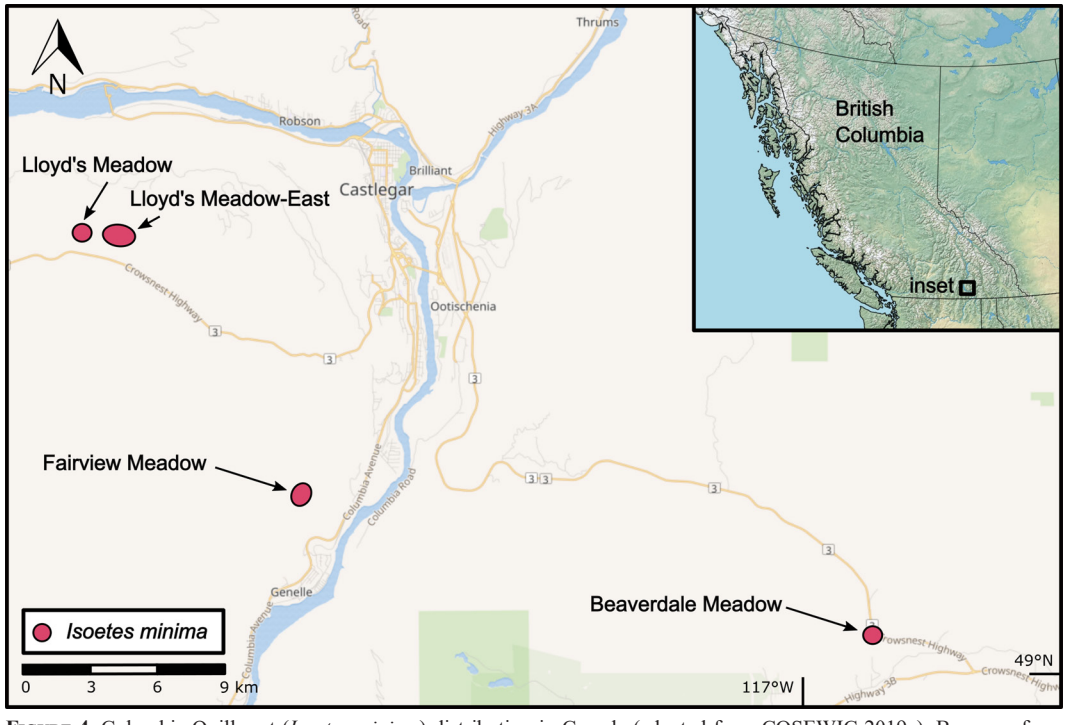

FIGURE 4. Columbia Quillwort (*Isoetes minima*) distribution in Canada (adapted from COSEWIC 2019a). Basemap from SimpleMappr (Shorthouse 2010).

The total population of *I. minima* in Canada in 2017 (COSEWIC 2019a) was 1019 mature individuals capable of reproduction (Table 1). Sporelings (immature plants) were observed at all of the sites.

Globally, *I. minima* is known from fewer than 15 subpopulations. In the United States, the sites are widely dispersed in Washington, Idaho, and Oregon, with distances between sites in Washington being as much as 200 km (Figure 2). The Canadian occurrences are approximately 100 km from the closest known United States sites.

Habitat characteristics

In Canada, the habitat of *I. minima* is confined to discrete forest openings within the Interior Cedar–Hemlock biogeoclimatic zone (BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development 2018). The range is within the Selkirk Foothills Ecosection, a transition zone divided by the BC–Washington border which lies between the Selkirk Mountains to the east and the Okanagan Highlands to the west (Demarchi 2011). The substrate is composed of granitic batholiths and sedimentary rocks, and the landforms have been altered by glaciers which rounded the mountains and left glacial debris on the valley floor, particularly south of Castlegar (Demarchi 2011).

This habitat is rare: it consists of small (170–300 ha), sloping pocket meadows (glades) within a larger forested matrix. It is further restricted by a narrow geographic area and by a limited range of elevation: 700–1160 m in Canada and 1370–2299 m in the United States (COSEWIC 2019a). Within the meadows, characteristic features of places where plants were found include a south-to-east facing aspect free from snow early in the spring; sustained spring (to early summer) seepage over thin soils; and gentle to moderate slopes (up to 20%). Spring seepages are naturally uncommon in the landscape, occurring where ephemeral underground moisture is carried on top of shallow bedrock and generally exhausted by mid-June. The thin (usually 3–7 cm, but infrequently 10–15 cm) soil discourages the establishment of larger, more vigorous vascular plants that would compete for light, moisture, and nutrients (COSEWIC 2019a).

Isoetes minima is found on acidic or circumneutral substrates throughout its range. Most sites are in full sun (Figure 3), but the species has been observed in smaller pocket meadows where there is partial shade from adjacent tree cover (COSEWIC 2019a). In Canadian subpopulations, *I. minima* is usually found on the upslope edge of exposed bedrock (COSEWIC 2019a).

Table 1. Subpopulation size and key associates of Canadian subpopulations of Columbia Quillwort (*Isoetes minima*; COSEWIC 2019a; this study).

Subpopulation	Mature plants (spore-bearing)	Immature plants	Total plants	Associated species
Beavervale Meadow 49.20°N, 117.45°W	57	12	69	False Mermaidweed (*Floerkea proserpinacoides* Willdenow); Dwarf Hesperochiron (*Hesperochiron pumilus* Grisebach); Darkthroat Shootingstar (*Primula pauciflora* (Green) A.R. Mast & Reveal); Buttercup-leaved Susksdorfia (*Suksdorfia ranunculifolia* (Hooker) Engelmann); Oregon Woodsia (*Woodsia oregana* D.C. Eaton); Nuttall's Larkspur (*Delphinium nuttallianum* Pritzel); Yellow Stonecrop (*Sedum stenopetalum* Pursh); Small-flower Blue-eyed Mary (*Collinsia parviflora* Douglas ex Lindley); One-flowered Broomrape (*Aphyllon uniflorum* (L.) Torrey & A. Gray); Spotted Knapweed (*Centaurea stoebe* L.)
East Lloyd's Meadow 49.30°N, 117.76°W	181	11	192	*F. proserpinacoides*; *H. pumilus*; Three-leaved Lewisia (*Lewisia triphylla* (Watson) Robinson); *S. ranunculifolia* (species list incomplete)
Fairview Meadow 49.24°N, 117.69°W	254	46	300	Large-flowered Clarkia (*Clarkia pulchella* Pursh); *F. proserpinacoides*; *H. pumilus*; *P. pauciflora*; *S. ranunculifolia*; *W. oregana*; *D. nuttallianum*; *S. stenopetalum*; *C. parviflora*; *A. uniflorum*; Narrow-leaved Montia (*Montia linearis* (Douglas ex Hooker) Greene); *C. stoebe*
Lloyd's Meadow 49.30°N, 117.78°W	527	57	584	*C. pulchella*; *F. proserpinacoides*; *H. pumilus*; *P. pauciflora*; *S. ranunculifolia*; *W. oregana*; *D. nuttallianum*; *S. stenopetalum*; *C. parviflora*; *A. uniflorum*; *M. linearis*; *C. stoebe*
Total individuals	1019	126	1145	

Immediately adjacent to *I. minima* plants, vegetation is either absent or dominated by thick moss mats (primarily *Philonotis fontana* (Hedwig) Bridel, *Niphotrichum elongatum* (Frisvoll) Bednarek-Ochyra & Ochyra, and *Bryum weigelii* (Biehler) J.R. Spence) and forbs, with a few shrubs infrequently present at the edges of the seeps. Characteristic associated forb species are included in Table 1. In Canada, *I. minima* is also associated with the federally rare plant, Dwarf Hesperochiron (*Hesperochiron pumilus* Grisebach), as well as other species limited to the same habitat, such as Pink-fairies (*Clarkia pulchella* Pursh) and False Mermaid-weed (*Floerkea proserpinacoides* Willdenow; Table 1). Indeed, the only Canadian occurrences of Dwarf Hesperochiron and Hairy Paintbrush (*Castilleja tenuis* (A. Heller) Chuang & Hickard), which have both been assessed by COSEWIC as Endangered, occur in one or more of the meadows where *I. minima* grows (COSEWIC 2019b and 2019c, respectively). The regionally rare False Mermaid-weed is present at all sites.

Identification and physical distinctions of Isoetes minima

Isoetes minima is among the smallest quillwort species in North America (Taylor *et al.* 2003) and the world (Brunton and Troia 2018). Although similar in appearance to dwarfed plants of *I. howellii*, with which it overlaps in range, Larsén and Rydin (2016) provide molecular evidence that it is more closely related to the predominantly coastal *I. orcuttii* and *I. nuttallii*. Regardless, *I. minima* is a relatively distinctive taxon with a unique megaspore ornamentation character that readily separates it from *I. howellii* (Figure 5).

The following describes the most significant physical characteristics of *I. minima*, based on our observations supplemented by the (sparse) literature addressing this and related taxa. The observations of Pfeiffer (1922) are used cautiously, however, as they included misidentified specimens (likely *I. howellii*) in their consideration of *I. minima* characteristics.

Gross form and leaves—The pale green plants emerge as tufts of 6–12, ~0.5–0.75-mm-wide leaves from a corm-like rootstock (Figure 1), which has been described as either three-lobed (Eaton 1898) or two-lobed (Pfeiffer 1922; our study). Freund *et al.* (2018) considered corm lobation to be of significant value in classifying some *Isoetes* taxa into clades, but their study did not include *I. minima*.

Most Canadian plants average 3–6 cm in height, but if there is sufficient available moisture toward the end of the April–June growing season, the plants can

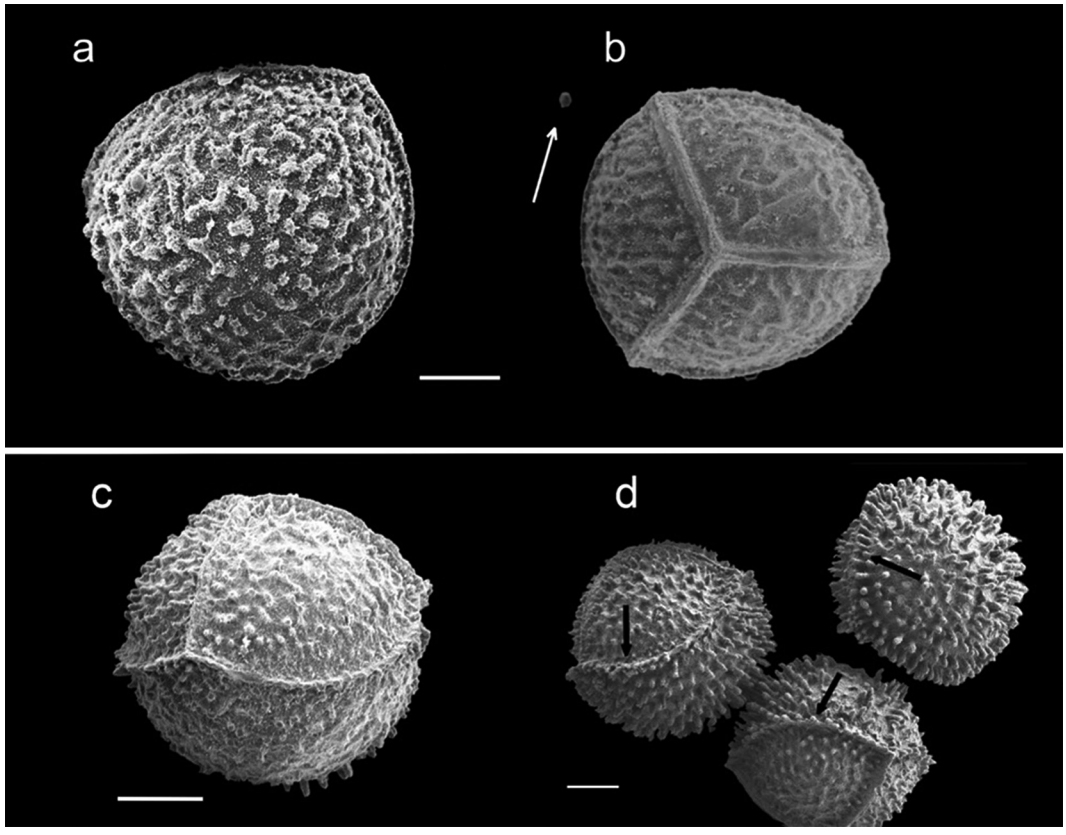

FIGURE 5. Howell's Quillwort (*Isoetes howellii*) and Columbian Quillwort (*Isoetes minima*) megaspores. a and b. *Isoetes howellii* open ornamentation pattern of low, broad, anastomosing muri (walls) with (a) distal side (*L.F. Henderson 2,894*, Moscow, Idaho [MO 200569]) and (b) proximal side with microspore [arrow] (*D.F. Brunton & K.L. McIntosh 10,855*, Akamina-Kishinena Provincial Park, BC [OAC]). c and d. *Isoetes minima* with (c) dense ornamentation pattern of thin tubercles on distal and proximal sides (*D.F. Brunton & K.L. McIntosh 17,243*, Salmo, BC [DFB]) and (d) diagnostic row of tubercles ("spokes of ship's wheel") [arrows] atop the equatorial ridge. Scale bars = 100 µm. Photos: a and b. D.M. Britton, University of Guelph, 1992. Used with permission. Photos: c and d. P. Sokoloff.

become much larger, with maximum height ranging between 11 and 20 cm (COSEWIC 2019a).

Sporangia containing either megaspores or microspores and set into the adaxial side of the swollen leaf bases are covered by a partial velum (tissue membrane). Velum coverage is substantial (Figure 6), ranging from an estimated 60 to 75% (Eaton 1898) and calculated to average 60.4% (n = 27) in a representative Canadian subpopulation (our study). This is substantially greater than the range of velum coverage documented for *I. howellii* (typically 25–40%: Brunton *et al.* 2020) and substantially less than the 100% coverage of *I. nuttallii* and *I. orcuttii* (Pfeiffer 1922; Taylor *et al.* 1993).

Megaspores—The globose, white megaspores (Figure 5c,d) are small, even in comparison with most other North American diploids, ranging from 320 to 420 µm in diameter (Taylor *et al.* 2003) with megaspores of Canadian plants ranging from 384 to 424 µm with an average of 406.9 µm (SD 18.0 µm, n = 80, two subpopulations) in our study. Contrary to the low pattern of broad muri (walls) evident in *I. howellii* (Figure 5a,b), *I. minima* megaspores are more or less uniformly covered by short, blunt, narrow tubercles (Figure 5a–c).

A distinctive—indeed diagnostic—feature of *I. minima* is the megaspore equatorial ridge, which is conspicuously "beset with polished spinules," as stated by Eaton (1898: 30; Figure 5d). No other North American (or global?) *Isoetes* has such a feature (D.F.B. pers. obs. 2020).

Microspores—Eaton (1898) accurately described the minute, white (*en mass*) microspores as ranging from 26 to 31 µm in length (mean 29.67 µm, n = 20, *D.F. Brunton & K.L. McIntosh 17,243*, 28 June 2008, Salmo, BC [DFB]) and being sparsely papillose or

Figure 6. Columbia Quillwort (*Isoetes minima*) inner basal leaves showing partial (~ 65%) coverage of light tan-coloured sporangia; megaspores are evident through unmarked, translucent sporangium walls (29 June 2014, Lloyd's Meadow, British Columbia). Photo: R. Batten.

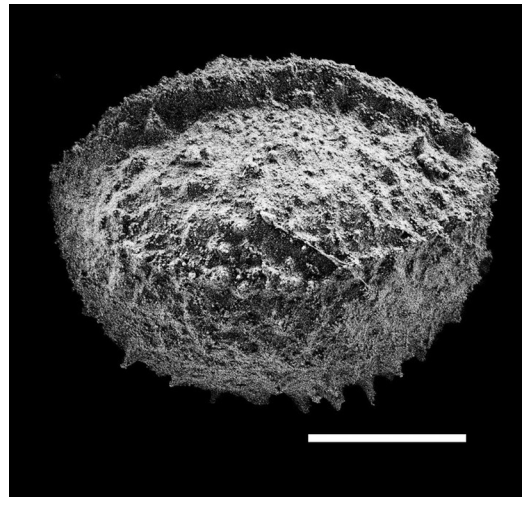

Figure 7. Columbia Quillwort (*Isoetes minima*) microspore (A. *Ceska & O. Ceska 19,754*, 30 June 1985, Ellenburg, Kittatis County, WA [DFB]). Scale bar = 10 µm. Photo: P. Sokoloff, August 2020.

coarsely echinate (Figure 7). Those of *I. howellii* are similar in size and ornamentation, but are somewhat more echinate. An apparently broad and conspicuous dorsal ridge on the latter may present a usefully distinctive feature in comparison to the inconspicuous and narrow dorsal ridge of *I. minima* microspores (Brunton *et al.* 2020), but this possibility requires further study.

Table 2 summarizes the distinctions between *I. minima* and the morphologically most similar taxa found in the Pacific northwest with which there could be confusion. Its most distinctive characteristics are its wet-meadow habitat, diminutive size, and unique megaspore ornamentation (particularly the diagnostic equatorial ridge feature).

Key—The following key to BC *Isoetes* species is based on mature plants with well formed (white, globose) megaspores. Data are from Britton and Brunton (1993, 1995, 1996) and the present study. Hybrids can be expected to occur sparingly in mixed populations of *Isoetes* and several have been described from BC (Britton and Brunton 1993, 1995, 1996). They are identified by spore size, cytology, and morphological features that are intermediate between their on-site putative parents. Sterile hybrids can be distinguished from fertile species (treated in the key) by their misshapened (not globose) form, variable (not uniform) size within a single sporangium, and spore ornamentation that expresses features of two (not a single) species.

1. Spring to early summer (March–late June) maturing plants of ephemeral open vernal pools or seepage areas in upland forest glades or in shore meadows; megaspores 350–450 (500) µm in diameter **2**
– Summer to autumn (July [rarely late June]–September) maturing aquatic plants in permanently shallow to deep oligotrophic water of lakes, ponds, and rivers (or late-season emergent); megaspores 450–600 (720) µm in diameter **4**
2. Velum coverage of sporangia complete (100%); megaspores with ± smooth ornamentation; in vernal pools along the Pacific coast ..... ***Isoetes nuttallii***
– Velum coverage of sporangia partial (25–75%); megaspores with ridged or tuberculate ornamentation; in forest glade seepages or shoreline meadows in interior **3**
3. Short (<7 [rarely 15] cm tall) forest glade seepage plants; velum coverage of sporangia 60–75%; megaspores average 407 µm in diameter, ornamentation prominently densely tuberculate with diagnostic row of spinules along equatorial ridge; matures May and June in Canada ..... ***Isoetes minima***
– Moderately (10–15 [rarely 30] cm) tall shore meadow plants; velum coverage of sporangia 25–40%; megaspores average 450 µm in diameter, ornamentation obscurely mounded or with broken

TABLE 2. Major morphological/ecological characteristics of Columbia Quillwort (*Isoetes minima*) and related taxa (particularly notable features in bold type); data from Cody and Britton (1989), Taylor *et al.* (1993), COSEWIC (2019a), Brunton *et al.* (2020), and the present study.

	Isoetes minima	Howell's Quillwort (*Isoetes howellii* Engelmann)	Orcutt's Quillwort (*Isoetes orcuttii* A.A. Eaton)	Nuttall's Quillwort (*Isoetes nuttallii* A. Braun ex Engelmann)
Habitat	Seasonal **seepage on bedrock slopes** with **unique** floristic associates	**Ephemeral wetlands** with widespread wetland associates	Ephemeral swales with **vernal pool** floristic associates	Ephemeral swales and vernal pools with **vernal pool** floristic associates
Plant size	**Exceptionally small** stature; **3–6 cm** (rarely to 20 cm)	Moderately robust; **10–15 cm** (up to 30 cm) long	Small stature; **<8 cm** long	Small to modest stature; **8–10 cm** (to 25 cm) long
Canadian distribution	Kootenay region	Interior west of Rocky Mountains	n/a	Pacific coast
Leaf form and colour	**Stiffly** erect; **green**	**Weak, arching to reflexed**; pale to **bright green**	Erect, **pliant**, pale green	Erect, **firm**, bright green
Velum coverage	60%	**25–40%**	100%	100%
Megaspores	Average 407 µm in diameter; densely fine-tuberculate pattern; **row of tubercles ("spinules") along equatorial ridge**	Average 450 µm; low, **broad anastomosing muri** or obscure mounds pattern; equatorial ridge plain	Average **320 µm**; smooth to obscurely tuberculate; equatorial ridge plain	Average 421 µm; smooth to tuberculate; equatorial ridge plain
Microspores	Smooth to coarsely low echinate; average ~30 µm long	Coarsely low echinate; average ~28 µm long	Smooth to papillose; 20–30 µm long	Papillose; 20–30 µm long

network of low muri; smooth-crested equatorial ridge; matures July (rarely late June)–August in Canada .... ***Isoetes howellii*** (in part)

4. Early summer (July [rarely late June]–August) maturing plants of shallow ponds and emergent shores; megaspores average 450 µm in diameter, ornamentation obscurely mounded or with broken network of low muri .... ***Isoetes howellii*** (in part)

– Mid-summer to late autumn (late July–September) maturing plants of deep to shallow permanent water (or mixed emergent-aquatic along shores in late season); megaspores >500 µm in diameter, ornamentation with pronounced echinate or ridged pattern **5**

5. Robust plants with thick, evergreen, dark green leaves in shallow to (typically) deep water; megaspores >600 µm in diameter, ornamentation coarsely papillate or with short muri in broken-reticulate pattern .... ***Isoetes occidentalis*** L.F. Henderson

– Plants thin to moderate, deciduous, bright to dull green leaves in shallow water (mixed emergent-aquatic in late season); megaspores <550 µm in diameter, with echinate ornamentation **6**

6. Bright green diploid plants with ascending to recurved leaves; megaspores 420–475 (510) µm in diameter, with densely echinate ornamentation of fine-tipped spines .... ***Isoetes echinospora*** M. Durieu

– Dull green to green tetraploid plants with ascending leaves; megaspores 500–550 (600) µm in diameter, with echinate ornamentation of thin tubercles and blunt-tipped spines .... ***Isoetes maritima*** L. Underwood

Discussion

We confirm that the obscure and long-overlooked lycophyte, *I. minima*, represents a distinct and rare native element of the North American flora. Its short growing season, small size, and isolated occurrences have resulted in limited investigations both in Canada and the United States (the furthest south population being within 800 km of the Canadian border). Accordingly, our understanding of basic aspects of its natural history, such as physical size, limitations to morphological variation, reproductive potential, and distributional vectors, are based on relatively limited data gathered over a short period (several decades). Many of the detailed morphological and ecological characteristics of this rare lycophyte, for example, are documented for the first time in our present study. As well, only preliminary genetic information is available concerning its relationship with associated species. Is it an ancient relict species or a newly

developed evolutionary "experiment"? That and similar origin and relationship questions remain to be answered.

The four known Canadian subpopulations (Figure 4) constitute just over a quarter of the species' total global occurrences. Accordingly, the long-term stability and security of Canadian subpopulations of this Columbia Region endemic is of significant importance to the global survival of the species. Maintaining the rare pocket meadow seepage habitat is fundamental to the sustainability of this species as well as the other Species At Risk and regionally significant taxa that are entirely contained within it. The lack of suitable habitat across the landscape coupled with limited dispersal mechanisms is likely the cause for the rarity of *I. minima*.

Maintaining the ecological integrity of the habitat will require protecting upslope hydrology, limiting encroachment by trees and shrubs into the pocket meadows, and preventing the further spread of invasive, non-native plants. All of the known subpopulations are on provincial Crown land, which precludes impacts associated with land development (COSEWIC 2019a). However, Crown land remains subject to threats associated with recreational activities, logging, invasive species, and fire suppression.

All known subpopulations in Canada are accessible to the public and subject to varying levels of use. Fairview Meadow is a mountain biking area and near active all-terrain vehicle trails; bike tracks were observed within several metres of *I. minima* (COSEWIC 2019a). A new housing development adjacent to the site may increase future recreational impacts. Biking and hiking activities trample plants or can dislodge soil, trails can alter hydrology by diverting water flow from seepage areas, and tires and shoes can introduce and spread invasive non-native plants (COSEWIC 2019a). Recreational activities are more likely to create channels that increase water flow either by flooding or by drying the habitat, rather than expanding the seepage areas. Such activities in small amounts, however, may also create habitat by dispersing spores, decreasing competition from vascular plants, and altering hydrology in ways that could transport megaspores to new habitats.

Logging was planned for at least one of the sites; however, the logging company is now aware of the presence of rare species on the site. The company has agreed to leave a buffer of 30 m beside and below the meadow and any harvesting upslope will involve a detailed drainage plan using Lidar data to ensure that the hydrology of the site is not impacted (G. Cordeiro pers. comm. 18 July 2018). Logging and road building, even in areas adjacent to open meadows, can alter hydrology and erode the thin soils, damaging seepage areas (COSEWIC 2019a). The presence of heavy equipment in nearby areas can also spread invasive plants.

Non-native invasive plants, most notably Spotted Knapweed (*Centaurea stoebe* L.), are present at all sites. Although knapweed plants are small while *I. minima* is producing sporangia, later in the season knapweed dominates all Canadian sites. Knapweed may cause premature drying of seepages, potentially reducing reproductive success and rendering the habitat unsuitable for *I. minima* (COSEWIC 2019a).

The role of wildfire in maintaining the open pocket meadow habitat is unclear (COSEWIC 2019a). Imagery over the last 10 years shows shrub and tree encroachment along the edge of pocket meadow habitat. However, the specialized habitat has likely always been uncommon on the landscape limiting the distribution of *I. minima*. Encroachment may decrease the habitat available to *I. minima* by shading, while tree and shrub roots may absorb seepage flow and alter hydrology. With the increased fuel loading associated with decades of fire suppression, future wildfires may be more severe and may degrade habitat by causing erosion and altering hydrology (COSEWIC 2019a).

Isoetes minima will be impacted by climate change. Climate model projections (Pacific Climate Impacts Consortium's statistically downscaled climate scenarios) for the Kootenay Region suggest temperatures will increase across all seasons and all elevations, with worst-case scenarios predicting an increase in the average annual temperature of 1.6°C in the 2020s, 3.2°C in the 2050s, and 5.3°C by the 2080s (BC Agriculture and Food Climate Action Initiative 2019). Predictions suggest an increase in overall annual precipitation, with more extreme precipitation events, and a decrease in precipitation during the summer season (BC Agriculture and Food Climate Action Initiative 2019). Increased winter precipitation (coupled with warmer temperatures) is predicted to fall as rain rather than snow, resulting in decreased snowpack, more rapid snowmelt, and further reducing spring and summer flows (Province of BC 2016). These climatic changes are likely to reduce flow to seepages and may cause earlier drying of the habitat. If the window between snow melt and seepage drying is compressed, *I. minima* may not have sufficient time to produce mature sporophytes. Changes to flow patterns (decreased flow or extreme rain events) may interfere with effective spore dispersal.

Predicted climate changes may also result in an increased risk of wildfire, larger fluctuations of unpredictable seasonal conditions, and more extreme heat events (Province of British Columbia 2016; BC Agriculture and Food Climate Action Initiative 2019). All of these could potentially have a negative impact on *I.*

minima subpopulations.

From the limited surveys we conducted, at least three of the four Canadian subpopulations of *I. minima* appear to be self-sustaining because previously documented subpopulations have persisted for almost 25 years. A lack of previous rigorous plant counts, however, makes long-term population trends impossible to determine at present.

Although it appears that *I. minima* in Canada is currently self-sustaining, and other subpopulations could be found in the southern interior of BC, subpopulations of the species will always be vulnerable to declines resulting from direct or inadvertent human activity. To minimize the potential harm and loss from such negative impacts and to maximize the potential for recovery and sustainability, more knowledge of the natural history of this species is required. Further research is necessary to determine how *I. minima* and other associated rare species disperse to such isolated habitats within large stretches of unsuitable terrain. More information is required to understand whether *I. minima* is susceptible to impact from invading non-native plants and how invasive plants might alter hydrology. Further surveys and monitoring are essential for determining natural population fluctuations over time and to document other potential occurrences. Further research to understand taxonomic relationships, particularly with the closely related species *I. howellii* and *I. nuttallii,* will also help illuminate the diversity of *Isoetes* species globally and inform our understanding of evolutionary radiation and relationships in the genus.

Author Contributions

Conceptualization: C.Y.M.; Data Gathering & Curation: C.Y.M., R.B., and D.F.B.; Methodology: C.Y.M., D.F.B., R.B., and P.C.S.; Documentation Review: C.Y.M., D.F.B., R.B., and P.C.S.; Data Analysis: C.Y.M., D.F.B., and R.B.; Microscopic Imagery: P.C.S. and D.F.B.; Writing – Original Draft: C.Y.M.; Writing – Revision & Editing: C.Y.M., D.F.B., R.B., and P.C.S.; Cartography: P.C.S.

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