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Distribution and status of Howell's Quillwort (*Isoetes howellii*, Isoetaceae) in Canada and its relation to Bolander's Quillwort (*Isoetes bolanderi*)

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Abstract

The sparsely documented lycophyte, Howell's Quillwort (*Isoetes howellii*), occurs in Canada in four distinct areas of British Columbia in a variety of microhabitats. Before 2010, two areas of occurrence were known in Canada. Two additional clusters of occurrences have been discovered in the last decade. In Canada, *I. howellii* is found in open, ephemeral wet swales, shallow ponds, and periodically flooded shorelines, channels, and back beach meadows. Habitat rarity may be the primary reason for the large gaps between areas of occurrence. The current viability of the Canadian population is dependent on maintaining the recently discovered large number of individuals in the North Thompson River Region. *Isoetes howellii* shares many similarities with fellow diploid, Bolander's Quillwort (*Isoetes bolanderi*). The possibility that it represents a low-elevation subspecies of *I. bolanderi* requires further investigation. *Isoetes howellii* is rare in British Columbia and warrants consideration as a species at risk in Canada.

Key words: Howell's Quillwort; *Isoetes howellii*; Bolander's Quillwort; *Isoetes bolanderi*; British Columbia; species at risk; Canada

Introduction

Morphologically simple, mostly aquatic quillworts (*Isoetes*, Isoetaceae) are among the oldest vascular plants on earth, originating in the Late Devonian era (375 million years ago; Larsen and Rydin 2016; Troia *et al.* 2019). Contemporary species are thought to have diversified relatively recently, in mid-Paleogene (Tertiary) times (45–60 million years ago; Wood *et al.* 2019). Approximately 200 current taxa (species, subspecies, hybrids) have been described worldwide (Troia *et al.* 2016). However, molecular investigations indicate continued and possibly rapid diversification, with many distinctive genetic traits detected among the recognized flora (Hoot *et al.* 2004; Schafran *et al.* 2018). This has led some to conclude that undetected cryptic species are numerous—at least 50 in North America alone (Schafran 2019). Although the taxonomic implications of DNA findings remain unresolved, *Isoetes* is clearly a taxonomically dynamic genus, even at the northern limits of its continental distribution. A conservative estimate is that 250+ species have been detected globally, with perhaps 100 more expected to be recognized (Brunton and Troia 2018; Troia *et al.* 2019).

Half of the dozen known Canadian *Isoetes* species are found in British Columbia (BC; Cody and Britton 1989; Taylor *et al.* 1993). Most of these grow in acidic or circum-neutral silty sand and gravel substrate on emergent shores of rivers and ponds or in the finer, peaty sand substrate of ephemeral pools and seeps in woodland glades. Two Canadian species have been described in the last 30 years (Britton and Goltz 1991; Brunton *et al.* 2019), and at least one additional undescribed taxon is suspected in western Canada (D.F.B. unpubl. data). It is also likely that some Canadian *Isoetes* occurrences represent additional taxa currently believed to occur only in the United States, Europe, or Asia.

Howell's Quillwort (*Isoetes howellii* G. Engelmann, including *Isoetes melanopoda* J. Gay & M. Durieu var. *californica* A.A. Eaton and *Isoetes underwoodii* L. Henderson; Figure 1) is a diploid ($2n = 2x$

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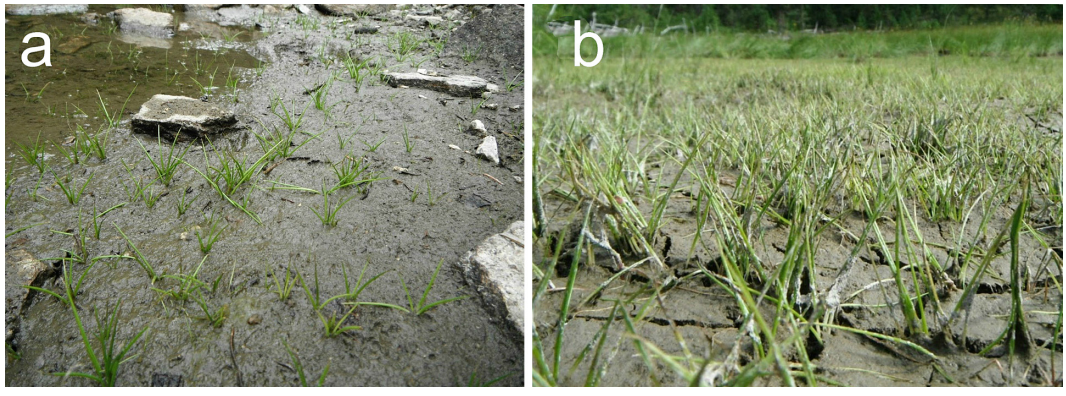

FIGURE 1. Howell's Quillwort (*Isoetes howellii*). a. Amphibious plants, initially submerged and ultimately terrestrial with seasonal pond downdraw (North Thompson River Pond 5, British Columbia [BC], 23 August 2012). b. Dense occurrence of plants in profile (North Thompson River Pond 1, BC, 24 August 2012). Photos: R.S. Krichbaum.

= 22) component of a particularly complex group of western North American quillworts that, in Canada, also includes the aquatic Bolander's Quillwort (*Isoetes bolanderi* G. Engelmann; COSEWIC 2006) and the ephemeral seepage associate, Columbia Quillwort (*Isoetes minima* A.A. Eaton [*I. howellii* var. *minima* N. Pfeiffer]; Taylor *et al.* 2003; COSEWIC 2019). *Isoetes bolanderi* and *I. minima* have been assessed as nationally Threatened and Endangered, respectively (COSEWIC 2006, 2019) with *I. bolanderi* listed as Threatened under the *Species at Risk Act* (SARA Registry 2019).

The limited geographic distribution of *I. howellii*, the apparent reduction in the number of known occurrences through taxonomic reconfiguration (e.g., the determination that some occurrences are *I. minima*; Ceska 2001), and the destruction of apparently suitable quillwort habitat (COSEWIC 2019) suggest that *I. howellii* may be a rare and declining Canadian taxon and worthy of consideration for designation as a national species at risk. However, the recent, unexpected discovery of a new and substantial cluster of occurrences, relatively far from the species' long understood distribution in Canada, complicates that assessment. It raises questions about the potential existence of other occurrences in BC, while also enhancing our understanding of the habitat requirements and limits of this species in Canada. In addition, the difficulty of separating some northern (Canadian) occurrences of *I. howellii* from the closely related *I. bolanderi* raises questions about its appropriate level of taxonomic designation.

There is almost no existing literature addressing the ecology, morphological variation, or taxonomic relationships of *I. howellii* in North America. An apparent exception might be Jon Keely's use of *I. howellii* plants for an investigation of anaerobic metabolism in aquatic plants in southern California, when he made his classic discovery of crassulacean acid metabolism (CAM) photosynthesis in *Isoetes* in the early 1980s (Keely 2014); however, those investigations were focussed almost exclusively on plant chemistry. Accordingly, an examination of the current status, habitat requirements, and distributional limitations of the species in Canada can potentially provide insights into important biological characteristics of this taxon across its range. Our study undertook such an examination and also addressed the potential taxonomic implications of ecological and morphological variability in the Canadian population. Understanding the taxonomy and evolution of basic diploid *Isoetes* is fundamental to understanding the systematics of this genus for which alloploidy is the primary engine of diversity (Hickey *et al*. 1989; Britton and Goltz 1991; Troia *et al*. 2019).

Methods

These investigations are based on field and herbarium studies of *Isoetes* occurrences and specimens from throughout the range of both *I. howellii* and *I. bolanderi* in western Canada and the western United States, as described in Taylor *et al.* (1993). Field investigations of occurrences were conducted in Canada and the United States by D.F.B. between 1992 and 2011 and by M.A.K. and R.S.K. in 2012 and 2019. Voucher specimens of *I. howellii* (80+), *I. bolanderi* (60+), and associated species from the following institutions have been examined: ASU (Arizona State University, Tempe, Arizona); CAN (Canadian Museum of Nature, Ottawa, Ontario [ON]); DAO (Agriculture and Agri-Food Canada, Ottawa, ON); DFB (D.F. Brunton herbarium, Ottawa, ON); DUKE (Duke University Herbarium, Durham, North Carolina); FSU (Florida State University, Tallahas-

see, Florida); Glacier National Park herbarium, Montana; IDAHO (Stillinger Herbarium, University of Idaho, Moscow, Idaho); MICH (University of Michigan, Ann Arbor, Michigan); MO (Missouri Botanical Garden, St. Louis, Missouri); NY (New York Botanical Garden, Bronx, New York); OAC (University of Guelph, Guelph, ON); OSC (Oregon State University, Corvallis, Oregon [OR]); PH (University of Pennsylvania, Philadelphia, Pennsylvania); RM (Rocky Mountain Herbarium, University of Wyoming, Laramie, Wyoming ([WY]); SUNY (Oswego) herbarium, New York; UNLV (University of Nevada, Las Vegas Nevada); V (Royal British Columbia Museum, Victoria, BC); VPI (Virginia Polytechnical Institute and State University, Blacksburg, Virginia); and WTU (University of Washington, Seattle, Washington; acronyms according to Thiers 2020). These included type specimens of both *I. bolanderi* and *I. howellii*. The morphological characteristics of living *I. bolanderi* plants from WY and living *I. howellii* plants from BC were also observed in cultivation in 2019 and 2020.

Spore features (size, ornamentation, shape) are critical to the identification of Canadian *Isoetes* (Cody and Britton 1986; Taylor *et al.* 1993). The spores of 550–600 individual plants from over 140 *I. howellii* and *I. bolanderi* herbarium specimens were examined visually and/or with a scanning electron microscope (SEM). Although most megaspore ornamentation features are readily discernible under 30–40× magnification, microspores are too minute for low-power visual examination. However, as they often provide valuable supporting identification and taxonomic information, we examined the size, ornamentation, and structure of microspores from Canadian occurrences with SEM imagery.

All microscopic features of the vouchers were studied (and measured) 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. Megaspore sizes reported for individual specimens represent the average width (across the equatorial region) of at least 20 (often 40) spores. Comparable microspore measurements are based on the average of 20 (frequently 40) longitudinal measurements taken from SEM images of clusters of spores.

We reviewed an extensive library of SEM spore images of *I. howellii*, *I. bolanderi,* and related taxa prepared by D.M. Britton before 2007 (using the methods described in Brunton and Britton 2006). Additional SEM images were produced 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 SEM, ThermoFisher Scientific, Hillsboro, Oregon, USA) at 15 kV and 25 pA, with a working distance of 10 mm and a spot size of 6.

In our study, we employ the term "population" to refer to the total number of plants known in Canada. "Occurrence" is used to represent distinct groups of plants separated from any other site by more than 100 m of unoccupied habitat. The term "patch" refers to groups of plants separated by less than 100 m of unoccupied habitat.

Results and Discussion

Distribution and status in Canada

Isoetes howellii records in Canada date from shortly after its description (Engelmann 1882), as it was first collected on 29 June 1889 at Kamloops, BC (*John Macoun s.n.,* CAN 5535). Macoun identified this collection as *I. bolanderi*, a determination unchanged by subsequent investigators for over a century (Taylor 1970; Scoggan 1978). In 1991, the specimen was determined by D.M. Britton and D.F.B. to be immature *I. howellii*, based on spore morphology and site ecology. The Kamloops occurrence was apparently situated along the shore of a shallow, semi-permanent sedge-lined pond, but now appears to be extirpated (D.F.B. pers. obs. 29 June 2008). An occurrence 30 km west (downstream) at Kamloops Lake (*A. and O. Ceska 4,547*, 28 August 1980, V) indicates that *I. howellii* persisted in the local area, at a site where apparently suitable emergent silty-gravel shore habitat remains (Google Earth imagery, 29 September 2019).

Several weeks after his Kamloops collection, John Macoun collected a second BC *Isoetes* that he identified as *I. bolanderi*. That specimen (*John Macoun s.n., 17 July 1889*, by the railway bridge, Sicamous, CAN 582996) was revised by D.F.B. to *I. ×marensis* D.M. Britton and D.F. Brunton, the sterile triploid ($2n = 3x = 33$) hybrid between *I. howellii* and tetraploid ($2n = 4x = 44$) Maritime Quillwort (*Isoetes maritima* L. Underwood; Britton and Brunton 1995). Although this strongly suggests that *I. howellii* was also present, its occurrence at the site was not confirmed until 2013 (*F. Lomer 2013*, 21 August 2013, in finely gritty white sand at raised moist margin of green sward, 1.7 km due northwest of Sicamous, V241023).

The third Canadian *I. howellii* record was reported from Akamina Pass near the Alberta (AB) border in southeastern BC and is represented by a collection from a shallow, ephemeral wetland swale at 1783 m above sea level (asl) in an Engelmann Spruce (*Picea engelmannii* C. Parry), Lodgepole Pine (*Pinus contorta* D. Douglas) forest glade (*David Polster s.n., 24 August 1976*, V94753). This is in the ESSFdk1 biogeoclimatic

subzone (Engelmann Spruce–Subalpine Fir dry cool Elk Variant; BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development 2018). This quillwort was also initially identified as *I. bolanderi*. Further collections from the Akamina Pass site (e.g., *D.F. Brunton and K. L. McIntosh 10,855*, 16 August 1991, DFB, MIL, OAC, VPI, WIS) resulted in the redetermination of that occurrence as *I. howellii*. Abundance was observed to be large at this site (~7500 individuals), with concentrations of plants both in the open central portion of the wetland and along the vegetated edge of an ephemeral Wheat Sedge (*Carex atherodes* K. Sprengel) marsh (Figure 2a). *Isoetes howellii* remained abundant at the site until at least 2 September 2012, with thousands of amphibious plants evident in two separate patches in distinct ephemeral wetland swales over an approximate total area of 2.5 ha (M.A.K and R.S.K. pers. obs.). This occurrence was impacted by the severe Kenow wildfire of 2017 (Parks Canada 2018). The fire was very hot and the sedge mat adjacent to the *I. howellii* site burned intensely, leaving ~5 cm of charcoal above unburned sedge peat. Some *Isoetes* plants survived, however, and normal-sized plants were found to be common at the site on 28 September 2020 (P. Achuff pers. comm. 21 October 2020).

To date, no Canadian occurrences of true *I. bolanderi* have been confirmed outside of Waterton Lakes National Park, southwestern AB (COSEWIC 2006).

The first Canadian records of *I. howellii* to be correctly identified at the time of collection were those of Adolf and Oldriska Ceska in 1980 in the Shuswap Lake–South Thompson River Region of south-central BC (e.g., Kamloops Lake, Savona, *A. and O. Ceska 4,548*, 28 August 1980, V, and View Point Motel beach, Salmon Arm, *A. and O. Ceska 4,664*, 29 August 1980, V). Some of these sites were re-examined in the early 1990s by D.F.B. (Figure 2b). Other collections were made 15 km south of Sicamous, BC at Mara Lake, which drains into Shuswap Lake (Figure 3). These collections were secured during fieldwork associated with the description of *I.* ×*marensis* (*I. howellii* × *I. maritima*; Britton and Brunton 1995).

The cluster of occurrences in the Shuswap Lake–South Thompson River Region are scattered across 2400 km^2, all at ~350 m asl (Figure 3). The circumscription of this cluster includes the total area considered to potentially include suitable *I. howellii* sites. *Isoetes howellii* plants in the Shuswap Lake–South Thompson River area are amphibious, growing along lakeshores in coarse sand and gravel substrate in sparsely vegetated wet swales that are often dry by late summer. The total number of individuals in all five extant occurrences is estimated to be <1000. Other *Isoetes* species, notably polyploids *I. maritima* and, less commonly, Western Quillwort (*Isoetes occidentalis* L. Henderson), grow with or near the Shuswap Lake–South Thompson River *I. howellii* plants. An apparent hybrid between *I. howellii* and *I. occidentalis* from Shuswap Lake is being investigated separately.

Before 2010, only the Shuswap Lake–South Thompson River and Akamina Pass occurrences represented the known distribution of *I. howellii* in BC and Canada (Klinkenberg 2019). In 2010, however, *I. howellii* was collected in and around the town of Castlegar in southern interior BC by Frank Lomer. He reported three small occurrences separated by ~1 km, centred on the confluence of the Columbia and Kootenay Rivers: Columbia River at Waldie Island Heron reserve, *F. Lomer 7,487*, 7 September 2010 (UBC); Columbia River north of Zuckerberg Island, *F. Lomer 7,478*, 7 September 2010 (UBC); and Kootenay River by Selkirk College, *F. Lomer 7,498*, 8 September 2010 (UBC). Each occurrence consisted of one or more patches of fewer than 50 individuals scattered over an estimated 1–300 m^2 area. The *I. howellii* plants were

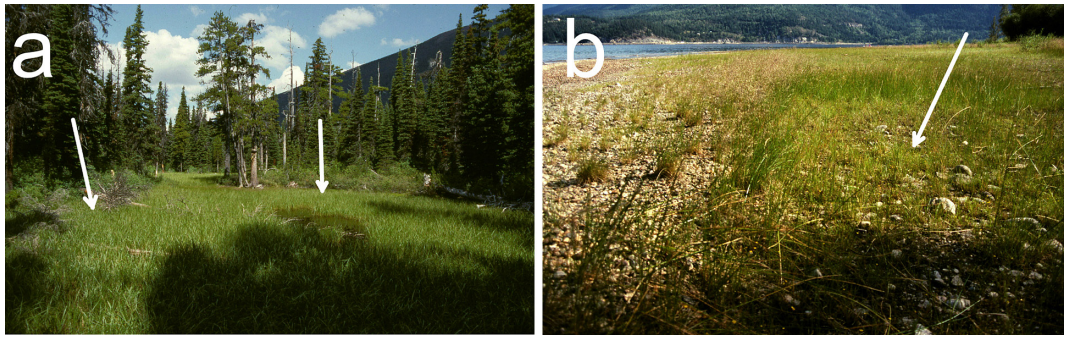

FIGURE 2. Howell's Quillwort (*Isoetes howellii*) habitat. a. Seasonally flooded sedge swale (Akamina Pass, British Columbia [BC], 16 August 1991). b. Back-beach depression (Shuswap Lake, BC, 14 August 1992). Arrows indicate concentrations of *Isoetes* plants. Photos: D.F. Brunton.

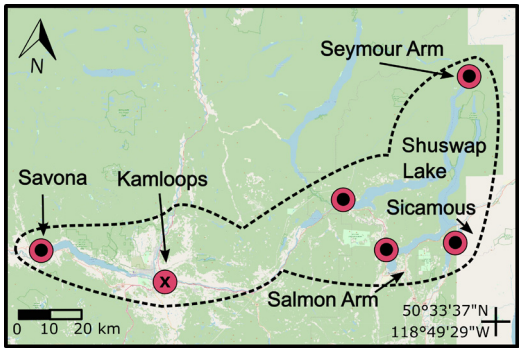

FIGURE 3. Occurrences of Howell's Quillwort (*Isoetes howellii*) at Shuswap Lake and the South Thompson River, British Columbia. Polygon outlines local area of occurrence; dots = approximate location of individual sites; × = extirpated occurrence. Map data: Open Street Map contributors via QGIS3.

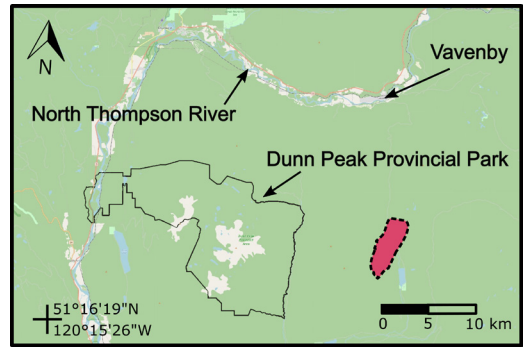

FIGURE 4. Occurrences of Howell's Quillwort (*Isoetes howellii*) in the North Thompson River Region, British Columbia (polygon outlines local area of occurrence; individual occurrences not shown for conservation/protection purposes). Map data: Open Street Map contributors via QGIS3.

growing amphibiously on exposed muddy river channels and on wet, pebbly river shoreline at 415–420 m asl. Associated vascular plant species included Water Mudwort (*Limosella aquatica* L.), Water Pygmyweed (*Crassula aquatica* (L.) Schoenlein), and Clammy Hedge-hyssop (*Gratiola neglecta* J. Torrey). This cluster of occurrences is ~190 km southeast of the Shuswap Lake–South Thompson River Region, and ~260 km west of Akamina Pass.

The most recent and significant *I. howellii* cluster in BC and Canada, which is located south of Vavenby in the North Thompson River Region, arose initially from a discovery by Curtis Björk (*Björk 25 087,* 8 September 2011, UBC). Like previous BC records, this was first thought to be *I. bolanderi*. Subsequent investigations by M.A.K. and R.S.K. in 2012 and 2019 documented four additional occurrences in the vicinity, for a total of five distinct *I. howellii* occurrences scattered across 1.4 km^2 at an elevation of 1770 m asl (*M. & R. Krichbaum 15275–15279, 23–25 August 2012*, DFB, UBC, *15301–15303*, 18 September 2019, DFB, UBC, *15304–15306*, 18 September 2019, DFB; Figure 4). This cluster of occurrences (Table 1) is ~70 km north of the next nearest known *I. howellii* in the Shuswap Lake–South Thompson River cluster at an elevation of ~350 m asl.

The five occurrences of *I. howellii* in the North Thompson River Region are located in a forested basin (~1770 m asl), the centre of which contains an intricate wetland, glade, and forest (*Picea engelmannii*/Subalpine Fir [*Abies lasiocarpa* (Hooker) Nuttall]) complex (Figure 5). This is in the ESSFwc2 biogeoclimatic zone (Engelmann Spruce–Subalpine Fir wet cold Northern Monashee Variant; BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development 2018). Large portions of the basin have been logged in the past. The *I. howellii* plants occur in silty soils in and around shallow ponds, two of which were drawn down to open wet swales and even dry glades in 2012. Four of the five occurrences are surrounded by relatively undisturbed glade and forest habitat; however, the southernmost site (Pond 2) is located next to a road track in a previously logged area. In 2012, evidence of active cattle use (dung and hoofprints) was observed at four of the five ponds; no evidence of cattle activity was observed in the area in 2019 by M.A.K. and R.S.K.

TABLE 1. Summary of occurrences of Howells' Quillwort (*Isoetes howellii*) in the North Thompson River Region, British Columbia, Canada.

Site	Occupied area, m^2	Estimated number of individuals*	Elevation, m a.s.l.
Pond 1	195	487 500	1759
Pond 2	334	835 000	1800
Pond 3	127	317 500	1759
Pond 4	309	772 500	1771
Pond 5	280	700 000	1785
Total	1245	3 112 500	

Note: a.s.l. = above sea level.
*Number of rooted, apparently spore-bearing individuals based on an estimated average density of 25 plants/100 cm^2.

At all five North Thompson River Region occurrences, *I. howellii* plants were found growing amphibiously, with the majority submersed and the remainder emergent to terrestrial, according to seasonal conditions (Figures 1 and 5). In both 2012 and 2019, *I. howellii* plants were also observed growing in dense concentrations at all five sites. In 2019, thousands of sporelings (newly emergent sporophytes) were also observed mixed with non-vascular vegetation floating

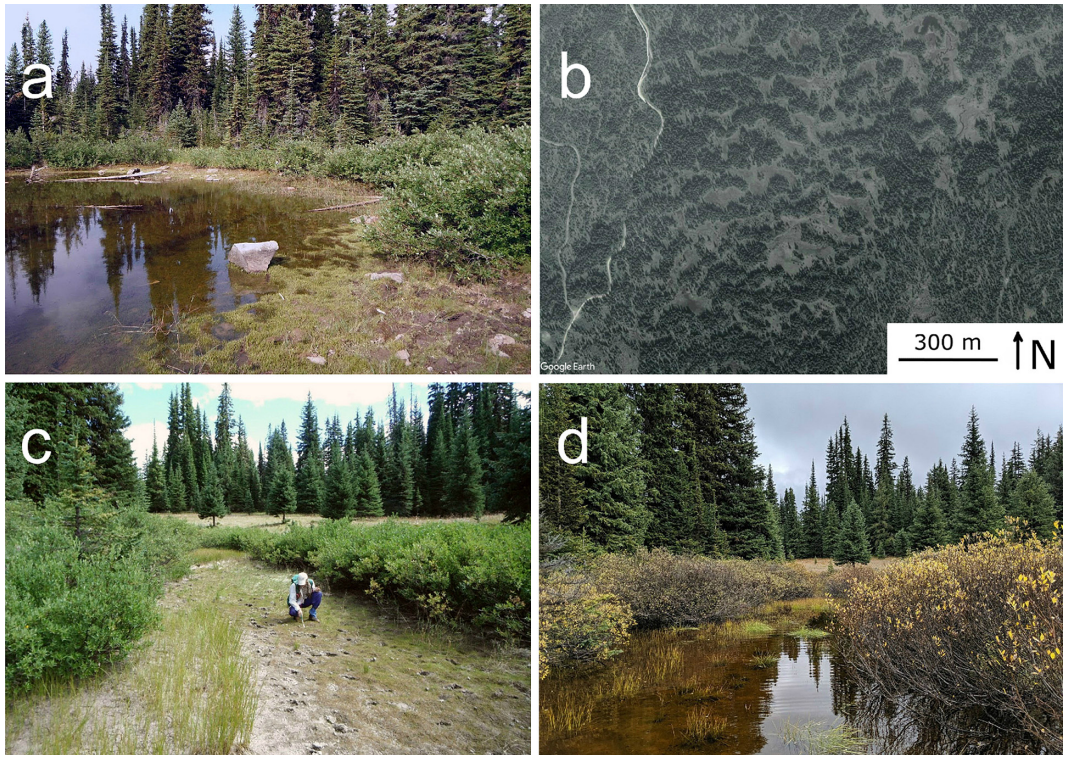

FIGURE 5. Howell's Quillwort (*Isoetes howellii*) habitat in the North Thompson River Region, British Columbia. a. Seasonally emergent shore of Pond 2 (24 August 2012). b. North Thompson River Region forest glades (28 August 2019). c. Pond 3 in desiccated condition (25 August 2012). d. Pond 3 in flooded condition (18 September 2019). Photos: a, c, d: by R.S. Krichbaum. Photo b: GoogleEarth, 28 August 2019.

in the water of some of the ponds. Associated vascular plant species included Lesser Spearwort (*Ranunculus flammula* L.), Russet Sedge (*Carex saxatilis* L.), Few-flowered False-mannagrass (*Torreyochloa pallida* var. *pauciflora* (J. Presl) J.I. Davis), and Barclay's Willow (*Salix barclayi* N.J. Andersson).

Isoetes howellii, then, occupies four distinct and mostly widely separated areas in southern BC (Figure 6). The largest number of occurrences are scattered across an extensive area of the Shuswap Lake–South Thompson River Region. However, these apparently support only several hundred plants in total. Similarly, fewer than 150 plants were reported from the cluster of Castlegar occurrences. The Akamina Pass occurrence supported thousands of plants until at least 2012; the long-term impact of the severe 2017 forest fire at the site has yet to be determined.

By far the largest number of individual plants are found in the recently discovered North Thompson River cluster of occurrences where an estimated 3 000 000+ plants occupy five distinct sites (Table 1), all located within a kilometre of each other. Clearly, the long-term security of *I. howellii* in Canada is largely dependent on the sustainability of this cluster.

Despite the recent discovery of an immense number of plants in a small cluster of occurrences north of its previously known range limit, *I. howellii* will likely continue to be recognized as a locally abundant but nationally rare species. The discovery of other occurrences in southern BC between the Okanagan Valley and the Columbia River Valley (excluding the largely limestone-based Rocky Mountain range adjacent to the AB border) is to be expected. However, these would most likely consist of small numbers of individuals at geographically small sites, such as those in the Castlegar or Shuswap Lake–South Thompson River areas.

Why there are such wide gaps in the occurrence of *I. howellii* in BC is not immediately obvious. The most likely explanation is that the habitat, structure, and substrate type of ephemeral wetlands between these areas is unsuitable within this physiographically very complex cordilleran landscape. Few data exist on the *in situ* requirements and distinctions between *I. howellii* and *I. bolanderi* in terms of substrate chemistry, habitat structure, phytosociology, etc. (Achuff *et al.* 2011).

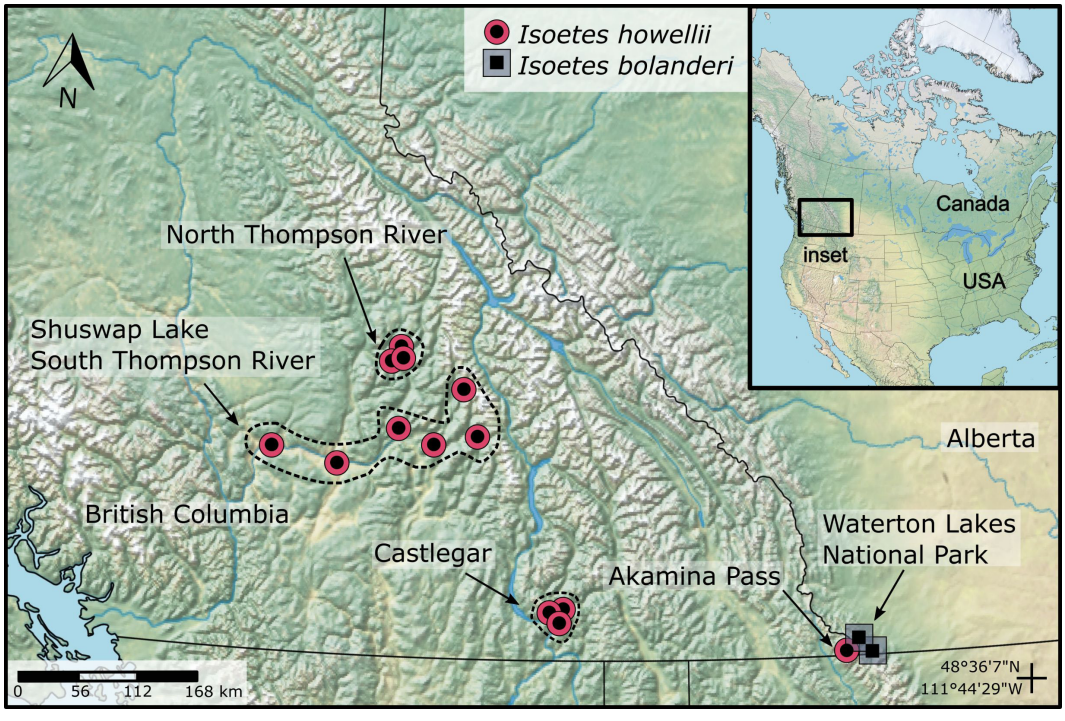

FIGURE 6. Occurrences of Howell's Quillwort (*Isoetes howellii*; dots) and Bolander's Quillwort (*Isoetes bolanderi*; squares) in Canada. Basemap from SimpleMappr (Shorthouse 2010).

The distributional data we present suggest that *I. howellii* may qualify as a species of Special Concern: "a wildlife species that may become threatened or endangered because of a combination of biological characteristics and identified threats" (COSEWIC 2020). Based on the data reported here and at our request, the BC Conservation Data Centre conducted an element subnational ranking of *I. howellii* and concluded that it would rank as provincially rare (S3; J. Penny pers. comm. 11 May 2020). Depending on the determination of contemporary and future threats in the Akamina Pass and (especially) the North Thompson River areas, however, a Threatened status could be more appropriate. That is particularly pertinent because more than 99% of the Canadian population occurs in the North Thompson River cluster, and this area is potentially at risk from a proposed large-scale open-pit mineral extraction operation (Weymark 2020).

Identification of Isoetes howellii *and* I. bolanderi

Several general, but nonetheless useful, distinctions have been documented to assist in separating *I. howellii* and *I. bolanderi*. A striking ecological distinction is noted in Taylor *et al.* (1993), who describe *I. howellii* as a plant of lower (montane) elevation ephemeral pools and wet swales and *I. bolanderi* as typically found in permanent lakes and streams at high (subalpine) elevations. Inconsistencies in this habitat segregation have been observed, however. For example, a substantial satellite occurrence of *I. bolanderi* in Waterton Lakes National Park, AB, is located at a subalpine elevation of 2050 m asl in atypically *I. howellii*-like ephemeral pools close to that species' typical fully aquatic site at Summit Lake (Achuff *et al.* 2011). In the same way, the North Thompson River *I. howellii* occurrences are situated at more typical lower elevations, but in semi-permanent ponds in a Subalpine Fir wet cold Northern Monashee ecosystem (BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development 2018). In addition, plants believed to be *I. bolanderi*, but with *I. howellii*-like spore morphology, are known from high elevation (subalpine) sites in semi-permanent wetland areas in WY, e.g., Mary Lake (among) Lodgepole Pines, former wetland now at 2680 m asl, Yellowstone National Park *C.E. Hellquist, Z. Haake & K. Mott 1919-16, 22 July 2016*, SUNY–Oswego herbarium, and near Coulter Bay Maintenance Area (elevation 2065 m asl), Lake Jackson, Grand Teton National Park *Jack States s. n., August 1970*, RMH 288404. Although ecological distinctions between these two taxa seem to apply clearly to many occurrences, substantial contradictions are evident in others.

A set of morphological distinctions at both the gross and microscopic levels are employed to separate these two taxa. The morphological analysis provided here is derived from the examination of an extensive number of herbarium specimens (see Methods) as well as from our experience in the field with both species in Canada and the United States. As with virtually all other *Isoetes* globally (Pfeiffer 1922; Taylor *et al.* 1993; Chinnock 1998; Troia 2001; Brunton and Troia 2018) spore size, shape, and ornamentation are the most critical and effective features for the separation of *I. howellii* from *I. bolanderi*.

Canadian *I. bolanderi* plants have erect, stiff, and brittle leaves, readily breaking off from the corm with only slight physical impact (e.g., the action of passing or browsing ungulates). Thousands of *I. bolanderi* leaves are commonly seen floating on the surface and washed up along the shore (Figure 7a) of Summit Lake in Waterton Lakes National Park, AB (COSEWIC 2006). In contrast, *I. howellii* observed in the present study had more strongly adherent, reflected, almost flaccid leaves (Figure 7b). This distinction is also apparent in cultivated material, with the leaves of *I. bolanderi* from WY remaining stiffly upright and slightly recurved while BC (North Thompson River Region) *I. howellii* plants are weakly ascending and flaccid (D.F.B. pers. obs.). This is not a universal trait of the species, however, as a number of completely aquatic occurrences of *I. bolanderi* in WY were observed to also have slightly recurved and substantially adherent leaves more like those of *I. howellii* (D.F.B. pers. obs.).

The difference in leaf-base colour reported by Taylor *et al.* (1993), with *I. howellii* being pale brown to even lustrous black, was not conspicuous in our study. Although some *I. howellii* plants from the Akamina and Shuswap Lake, BC, occurrences had slightly darker (uniform brown) leaf bases, no black-coloured bases were observed. Similarly, the cultivated WY *I. bolanderi* and North Thompson River Region *I. howellii* plants all had white to whiteish-tan leaf bases.

Table 2 summarizes the key features we found to differentiate *I. howellii* from *I. bolanderi* most convincingly. The identification of individual specimens relies, not on a single or even a few stand-alone diagnostic feature(s), but the accumulated weight of a suite of characters pointing most convincingly to one or the other taxon. Although megaspore size and ornamentation are valued most greatly for this purpose, the relative weight of any particular identification feature has not been formally determined for these taxa (indeed, for any *Isoetes* taxon). However, typical *I. howellii* megaspores are usually larger than those of *I. bolanderi*, particularly in occurrences in the southern portion of the range; e.g., 430.7 µm (n = 30) from Ellery Lake, Mono County, California, *J.D. Montgomery and D.G. Huttleston 88-78*, 8 August 1988 (DFB). They are also more conspicuously ornamented with low, broad walls (muri; Figure 8a) or obscure mounds (Figure 8b) distributed across the spore surface (perispore) in widely varying configurations and densities. *Isoetes bolanderi* megaspore ornamentation is typically papillate to echinate-tuberculate (Figure 8c) with a dense pattern of short, narrow tubercles. When muri are present (as with some specimens of *I. bolanderi*), they are usually obscure, short (rugulate), and narrower (Figure 8d) than typically seen in *I. howellii*.

Although *I. howellii* microspores tend to have more coarsely echinate ornamentation (Figure 9a) than the more finely echinate to spinulose ornamentation of *I. bolanderi* microspores (Figure 9b), contradictions and intermediate expressions of this pattern are evident

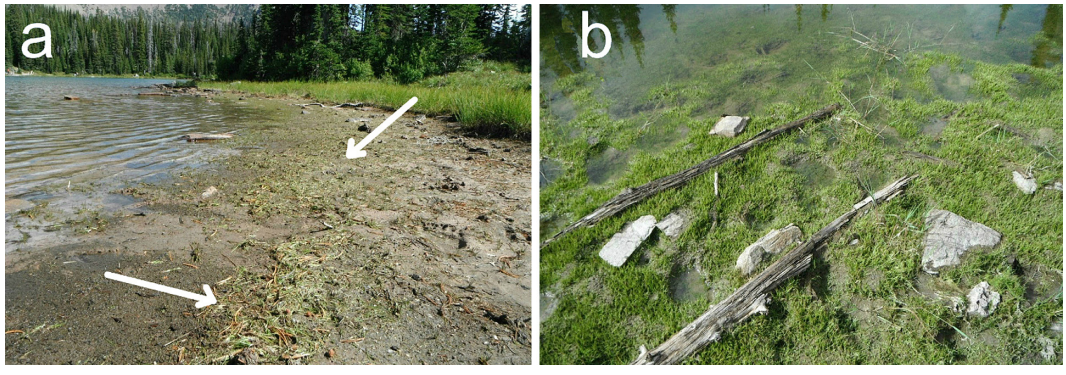

FIGURE 7. Durability of Canadian Howell's Quillwort (*Isoetes howellii*) and Bolander's Quillwort (*Isoetes bolanderi*) plants. a. Thousands of broken-off leaves of *I. bolanderi* forming drift along lakeshore (Summit Lake, Alberta, 2 September 2012). b. Intact *I. howellii* plants despite intensive cattle impact (North Thompson River area Pond 2, British Columbia, 24 August 2012). Photos: R.S. Krichbaum.

TABLE 2. Morphological and ecological distinctions between Howell's Quillwort (*Isoetes howellii*) and Bolander's Quillwort (*Isoetes bolanderi*) with particularly notable features in bold type.

	Isoetes howellii	*Isoetes bolanderi*
Habitat/habit	**Amphibious**, occurring in **lower elevation** (350–1780 m asl), typically montane **ephemeral wetlands**	**Aquatic,** occurring in **higher elevation** (1945–2200 m asl), typically subalpine **permanent lakes** and streams
Leaves	10–15 cm long (rarely to 30 cm)	5–10 cm long
	Weak (flaccid), **reflexed**	Stiff, **erect**
	± **Persistent** on corm, despite physical contact	Readily **deciduous** from corm by physical contact
	Tapering to **blunt** tip	Abruptly **sharp-pointed** tip
Velum coverage of sporangium	25–40%	15–25%
Megaspores	Mealy perispore	Smooth perispore
	Typically **rugose** or with **low, broad muri** in partial to densely **reticulate pattern,** with low, obscure **mounds**, or with short, thick**,** isolated muri and **tubercles**	Typically **densely papillate** (small, low tubercles) to **echinate-tuberculate**; also with sparsely thin-rugulate muri
	>425 µm diameter (mean 450.3 µm, *n* = 190)*	**<425 µm diameter** (mean 400.8 µm, *n* = 110)†
Microspore	Coarsely low echinate; >26 µm length (mean 27.55 µm, *n* = 160)‡	Low echinate to spinulose; <26 µm length (mean 25.72 µm, *n* = 62)§

Note: a.s.l. = above sea level.
*Six occurrences (British Columbia, California, Oregon).
†Four occurrences (Alberta, California, Wyoming).
‡Eight occurrences, (British Columbia, California, Oregon).
§Four occurrences (California, Wyoming).

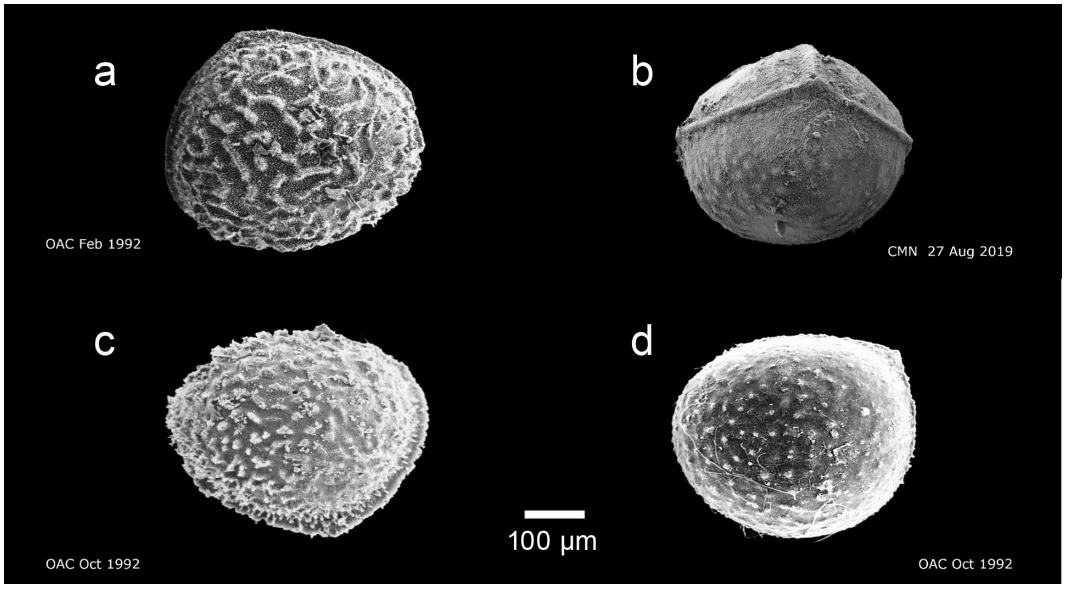

FIGURE 8. Megaspores of Howell's Quillwort (*Isoetes howellii*) and Bolander's Quillwort (*Isoetes bolanderi*). a. *I. howellii* megaspore, distal ornamentation of low muri in semi-reticulate pattern (The Dalles, Columbia River, Oregon, *J. and T. Howell s. n., 1 August 1880*, NY- Isotype). b. *I. howellii* megaspore, lateral ornamentation of low, broad mounds (North Thompson River Pond 1, British Columbia, *M. & R. Krichbaum* 15275, 24 August 2012, DFB, UBC). c. *I. bolanderi* megaspore with abundant echinate-tuberculate ornamentation (Sylvan Lake, Yellowstone National Park, Wyoming, *D.F. Brunton & K.L. McIntosh 10,841*, 13 August 1991, DFB, MU, OAC). d. *I. bolanderi* megaspore with finely papillate to obscurely rugulate muri (Meadowlark Lake, Bighorn National Forest, Wyoming, *D.F. Brunton & K.L. McIntosh 10,833*, 13 August 1991, BM, CAN, DAO, DFB, MIL, OAC, PH, TRT). Photos a, c, d: D.M. Britton. Photo b: P.C. Sokoloff and D.F. Brunton.

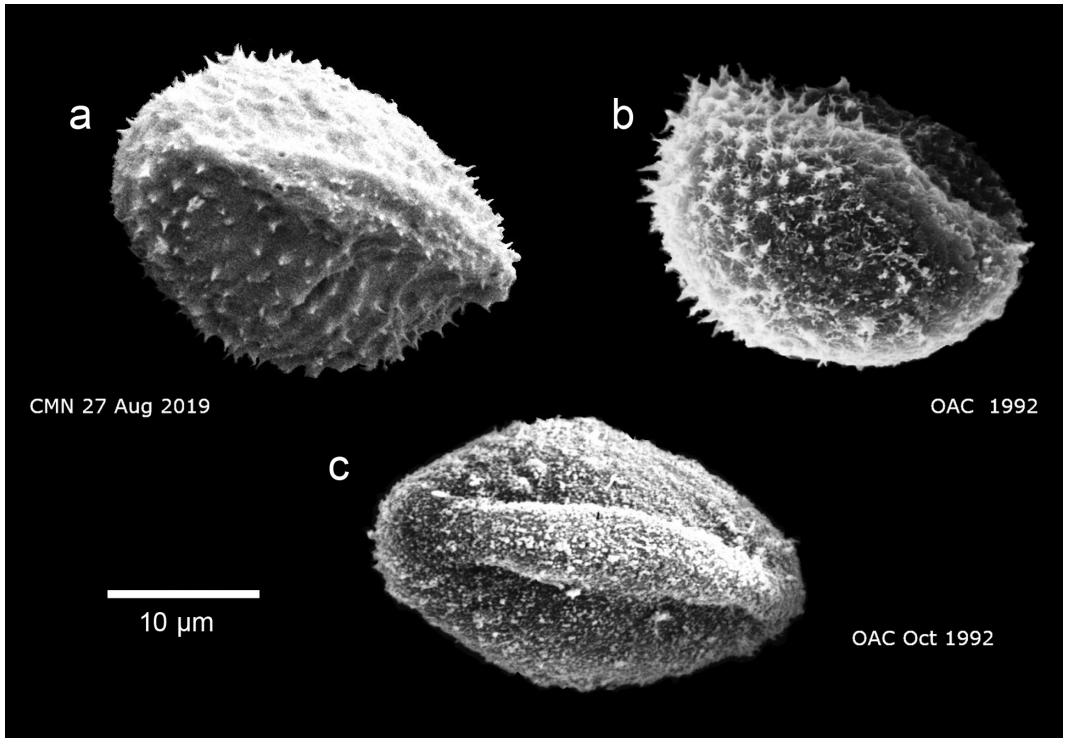

FIGURE 9. Microspores of Howell's Quillwort (*Isoetes howellii*) and Bolander's Quillwort (I*soetes bolanderi*). a. *I. howellii* microspore with echinate perispore pattern (North Thompson River Pond 1, British Columbia [BC], *M. & R. Krichbaum* 15275*, 24 August 2012*, DFB, UBC). b. *I. bolanderi* microspore with spinulose-tufted perispore pattern (Meadowlark Lake, Bighorn National Forest, Wyoming, *D.F. Brunton & K.L. McIntosh 10833*, 13 August 1991, BM, CAN, DAO, DFB, MIL, OAC, PH, TRT). c. *I. howellii* microspore with broad dorsal crest commonly expressed in *I. howellii* and *I. bolanderi* (Little Shuswap Lake, BC, *D.F. Brunton & K.L. McIntosh 11,242*, 14 August 1992, DAO, DFB, OAC). Photo a: P.C. Sokoloff and D.F. Brunton. Photos b, c: D.M. Britton.

among Canadian specimens. Microspore differences are slight between these two diploids, both being approximately the same size (Table 2) and sharing a dorsal crest (Figure 9c) that is wider than that of other western Canadian *Isoetes*.

Taxonomic status of Isoetes howellii

Although "typical" plants of *I. howellii* and *I. bolanderi* can be distinctive, ambiguous expressions of significant distinguishing features appears to be the rule rather than the exception with these two taxa in much of their substantially sympatric ranges (Figure 10). That ambiguity is exacerbated in Canada by the century of confusion over the appropriate identification of such material.

It is notable that no hybrids between *Isoetes howellii* and *I. bolanderi* have been documented even though both species are known to hybridize with other *Isoetes* species (e.g., Britton and Brunton 1995; Taylor 2002). If that absence of known hybrids reflects reality in the wild, it may also indicate that a significant genetic barrier does not exist between the two taxa.

Although being at the northern limit of range may amplify differences in the Canadian population relative to range-wide norms, the possibility that *I. howellii* and *I. bolanderi* represent infraspecific taxa within a single species is a viable possibility. The lack of any single diagnostic distinction and the apparent absence of sterile hybrids supports that interpretation. However, a taxonomic realignment of these taxa would not affect their conservation status, which could apply equally to either a species or subspecies.

It should be noted that the molecular analysis of Larsen and Rydin (2016) contradicts the physical and ecological lines of evidence for the taxonomic similarity of *I. howellii* and *I. bolanderi* that we describe. Genetic investigations of *Isoetes* taxa have been very useful in detecting and qualifying patterns of speciation and evolution in the genus (e.g., Hoot *et al.* 2004; Schafran *et al.* 2018; Wood *et al.* 2019), but in particular cases it has also resulted in unintuitive taxonomic conclusions that contradict most or all other lines of evidence (e.g., P. Schafran pers. comm. 20 February

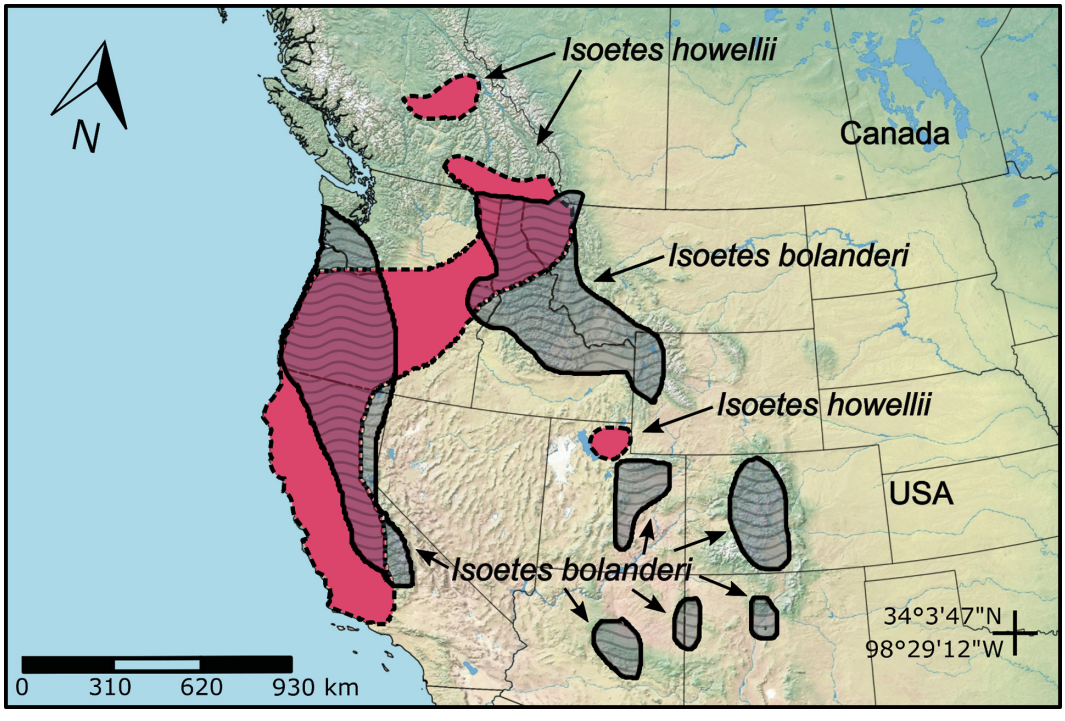

Figure 10. North American distribution of Howell's Quillwort (*Isoetes howellii*), indicated by red polygons with dashed outlines, and Bolander's Quillwort (*Isoetes bolanderi*), indicated by stippled polygons with solid outlines. Basemap from SimpleMappr (Shorthouse 2010).

2019, regarding St. Lawrence Quillwort [*Isoetes laurentiana* D.F. Brunton], as cited in Brunton *et al.* 2019). It was found most recently, however, that *I. howellii* and *I. bolanderi* were "difficult to distinguish molecularly" (J. Suissa pers. comm. 17 November 2020). This underscores the need for taxonomic investigations to be employing systematic DNA sequencing across the range of these sister taxa in concert with rigourous reliability testing for various seemingly significant distinguishing morphological and ecological features.

Author Contributions

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

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