# New records for Eastern Mosquito Fern (*Azolla cristata*, Salviniaceae) in Canada

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Brunton, D.F., and H.J. Bickerton. 2018. New records for Eastern Mosquito Fern (*Azolla cristata*, Salviniaceae) in Canada. Canadian Field-Naturalist 132(4): 350–359. https://doi.org/10.22621/cfn.v132i4.2033

## Abstract

We report a cluster of Eastern Mosquito Fern (*Azolla cristata,* Salviniaceae) populations in five watersheds within a 56-km$^2$ area of Leeds and Grenville County, Ontario. Some of the recently discovered populations were immense, one containing over two million individuals in 2016. These eastern Ontario populations are persistent, having been observed *in situ* continuously for four years. One population was confirmed after an apparent absence of at least 30 years and another was reported as present (or at least recurring) for approximately 50 years. We observed that Canadian *A. cristata* is capable, at least experimentally, of overwinter dormancy and subsequent renewal. *Azolla cristata* in eastern Ontario and western Quebec appears to represent naturally (if sporadically) occurring populations, likely transported from adjacent northern New York populations by migratory waterfowl. These natural occurrences are expected to be more frequent as climate change continues to reduce environmental barriers to the northward establishment of this and other southern taxa.

Key words: *Azolla cristata*; Eastern Mosquito Fern; climate change; native biodiversity; pteridophyte; Frontenac Axis; Ontario; Quebec

## Introduction

The Salviniaceae is a small, cosmopolitan, mainly tropical family of aquatic, heterosporous, free-floating ferns (Svenson 1944; Lumpkin 1993). *Azolla*, the larger of the two genera in the family (traditionally seen as the distinct family, Azollaceae), is characterized by small, dichotomously branching, free-floating plants with lobed fronds (Figure 1) and short thread-like roots extending into the water (Cody and Britton 1989). *Azolla* is ephemeral (Evrard and Van Hove 2004), with populations experiencing brief periods of explosive growth followed by long periods of apparent absence or obscurity that can last for years, as found in this study. Populations frequently form mats several hectares in size that can extend more or less continuously for kilometres (Figure 2; Darbyshire 2002; Darbyshire and Thomson 2004).

All three species of *Azolla* occurring in Canada (Macoun 1890; Brunton 1986; Cody and Britton 1989) are rare here, and many populations have been considered to be non-native. The cosmopolitan Large Mosquito Fern (*Azolla filiculoides* J.-B. Lamark) is native in parts of western North America and is probably introduced in southern British Columbia (BC; Douglas *et al.* 2000; F. Lomer pers. comm. 4 December 2017). A sterile *Azolla* specimen from Brantford, Ontario (ON; *C. J. Rothfels and S. R. Spisani 795*, 24 September 2003, HAM, D.F.B. personal herbarium) is believed to be *A. filiculoides* (based on the morphological distinctions described in Methods). It is reported elsewhere in the Great Lakes Region from the Niagara Frontier area of western New York (NY; Eckel 2005, although not repeated in Weldy *et al.* 2018).

Occurrences of Mexican Mosquito Fern (*Azolla mexicana* Schlechtendal & Chamisso ex C. Presl), a widespread native species in western North America (Lumpkin 1993), are scattered through interior southern BC (Brunton 1986; Goward 1994). It is of conservation concern and assessed (COSEWIC 2008) and listed as threatened in Canada (SARA 2019). *Azolla mexicana* has also been discovered recently in coastal BC as an adventive beyond its natural range (Klinkenberg 2017).

Eastern Mosquito Fern (*Azolla cristata* G.-F. Kaulfuss (*A. caroliniana* auct., non C.L. Willdenow)); is found irregularly across much of the eastern United States and southward into South America (Svenson 1944; Wherry 1961; Lumpkin 1993; Crow and Hellquist 2000; Pereira *et al*. 2011). It is considered a secure species on a global scale (G5) but uncommon to rare in some northern portions of its North American range and critically imperilled (S1S2) in Canada in ON and BC (NatureServe 2019). The Canadian status of *A. cristata,* however, is ambiguous and it has not been assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Possible native populations have been reported in the western Lake Ontario area (both historical and contemporary at Hamilton and the Niagara Peninsula), near Ivy Lea (Leeds and Grenville County, hereafter, L & G County), and from York County (Pryer 1987; Eckel 2005; Oldham and Brinker 2009). Populations found along the Rideau and Ottawa Rivers in ON and Quebec (QU) were reported to represent non-native introductions (Darbyshire 2002; Darbyshire and Thom-

A contribution towards the cost of this publication has been provided by the Thomas Manning Memorial Fund of the Ottawa Field-Naturalists' Club.

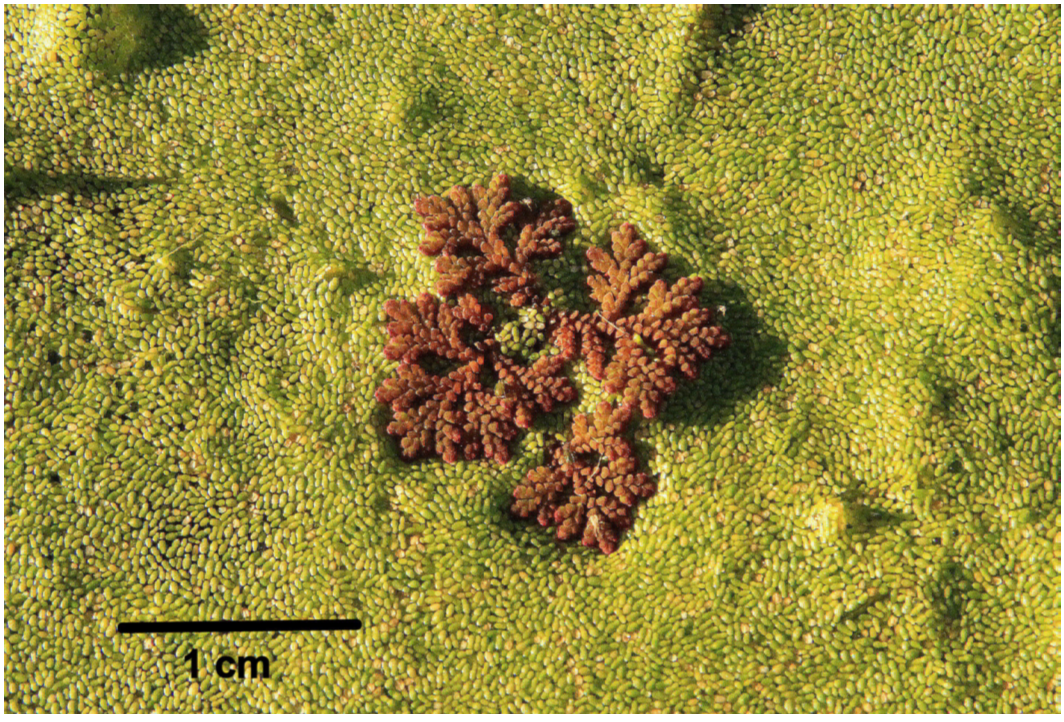


**FIGURE 1.** Single Eastern Mosquito Fern (*Azolla cristata*) plant in a watermeal (*Wolffia* spp.) mat at Maple Grove, Gananoque River, Leeds and Grenville County, Ontario. Photo: D.F. Brunton, 21 September 2016.

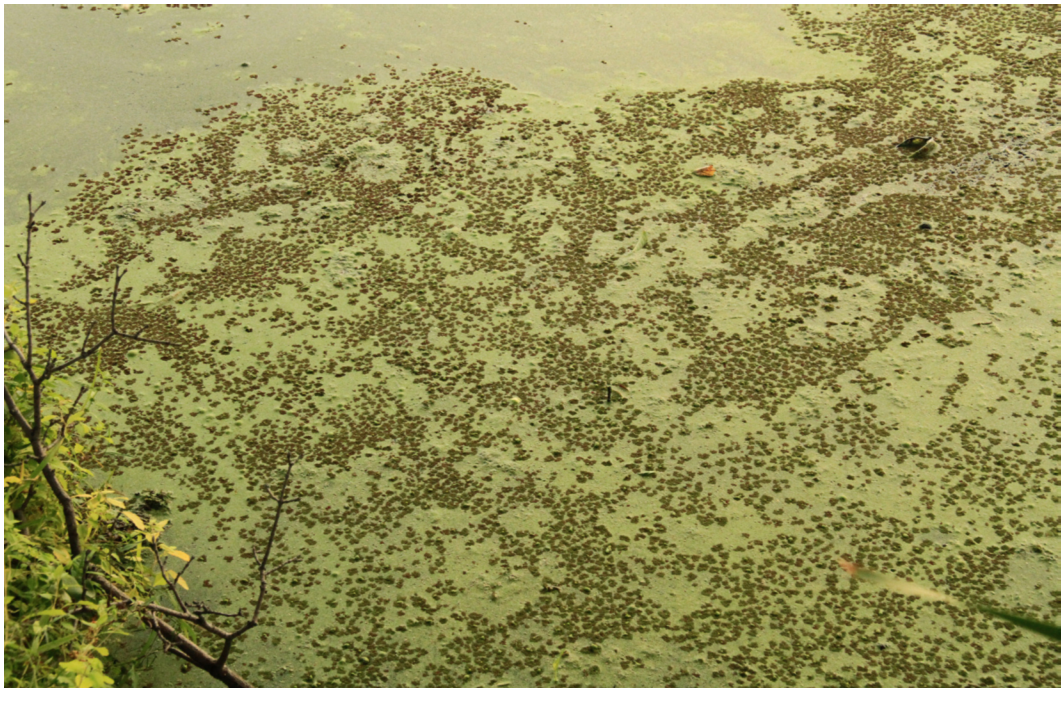

**FIGURE 2**. Large population of Eastern Mosquito Fern (*Azolla cristata*; darker plants) atop a floating mat of watermeal (*Wolffia* spp.), Star Duckweed (*Lemna trisulca*), and Great Duckweed (*Spirodella polyrhiza*) at Kinsman Park, Gananoque, Leeds and Grenville County, Ontario. Photo: D.F. Brunton, 27 September 2016.

son 2004). Recent occurrences in urban areas in southern BC are reported as being introduced (Douglas *et al.* 2000; Klinkenberg 2017). Eastern Canadian *A. cristata* populations have been considered incapable of persisting in the wild for more than one or two seasons (Darbyshire 2002), presumably constrained by Canadian winter conditions. It has been suggested that introduced Canadian populations likely resulted from the dumping of the contents of home aquaria into local waterways (Darbyshire 2002; Darbyshire and Thomson 2004; Klinkenberg 2017).

The discovery and rediscovery of vast and long-persisting populations of *A. cristata* in eastern ON are reported here and the implications of those discoveries are reviewed.

## Methods

Following the September 2014 opportunistic discovery of *A. cristata* near Gananoque (L & G County, ON), ground- and water-based field surveys were undertaken from September through November 2014–2017 in the southern Frontenac Axis area. We investigated potentially suitable still, protected, open water sites in an approximately 1500-km² area within 25 km of the north shore of the St. Lawrence River between Kingston and Brockville. Several L & G County site visits were also conducted in July and early August 2015. These were unsuccessful, although *Azolla* plants were detected at those sites later in the autumn of both 2014 and 2015 (Figures 1, 2, and 3; Table 1). Accordingly, mid-summer searches were not undertaken thereafter.

At each location, we conducted binocular-assisted visual surveys of creek and pond surfaces within 50–300 m of public roadways. Boat surveys also were conducted in September 2014 and 2016 along lower portions of the Gananoque River between Gananoque Lake and its outlet into the St. Lawrence River. Based on the strong association of *Azolla* populations with large mats of the aquatic Columbia Watermeal (*Wolffia columbiana* H. Karsten) and Northern Watermeal (*Wolffia borealis* (Engelmann) Landolt & Wildi ex Gandhi, Wiersema & Brouillet), we searched 15 large *Wolffia* mats evident from satellite imagery (GoogleEarth) on 8 November 2016 (Figure 4).

We collected voucher specimens for all distinct *A. cristata* populations discovered. These are deposited in herbariums at Agriculture and Agri-Food Canada (DAO), the Canadian Museum of Nature (CAN), the University of Guelph (OAC), the University of Manitoba (WIN), and/or D.F.B.'s personal herbarium (DFB). We reviewed the *Azolla* populations annually to determine their persistence. We also reviewed earlier herbarium voucher specimens in DAO, CAN, and the Royal Botanical Gardens (HAM) for additional records. In 2015 and 2016, we conducted informal interviews on

**FIGURE 3.** Dense, free-floating mat of brick-red Eastern Mosquito Fern (*Azolla cristata*) plants at Maple Grove, Gananoque River, Leeds and Grenville County, Ontario. Photo: D.F. Brunton, 5 October 2014.

**Table 1**. Summary of observations of Eastern Mosquito Fern (*Azolla cristata*) in Leeds and Grenville County, Ontario, Canada.

| Year | Gananoque River (St. Lawrence River through Maple Grove to Marble Rock) | | Sucker Brook, Maple Grove | Landon's Bay, St. Lawrence River | Knight's Creek, Ivy Lea | Gray's Creek, Front of Leeds and Gananoque Township |
|---|---|---|---|---|---|---|
| | Lower 10.8 km | Marble Rock | | | | |
| 2014 | Abundant (deep drifts of plants at river mouth) | Abundant | —* | — | — | — |
| 2015 | None | Rare | — | — | — | — |
| 2016 | Abundant (no drifts) | Common | Common | Common | Abundant (forming drifts) | Rare |
| 2017 | Abundant (no drifts) | Common | None | — | Abundant | None |

Note: Abundant = continuous mat; Common = scattered patches 0.5–2 m across; Rare = individual plants or small patches <30 cm wide.
*Not searched.

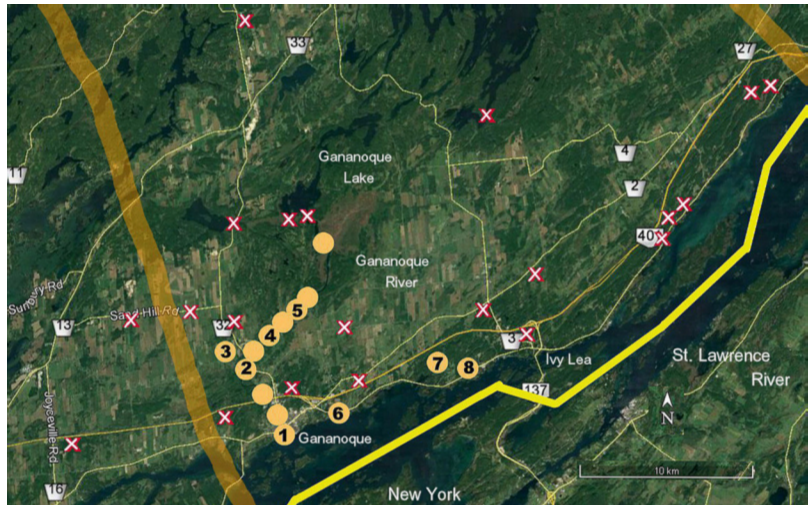


**FIGURE 4**. Locations of Eastern Mosquito Fern (*Azolla cristata*) in Leeds and Grenville County, Ontario. Circles = *Azolla* population (2014–2017); X = site where *A. cristata* was searched for but not found in suitable *Wolffia–Lemna* vegetation (2014–2017); broad vertical lines = approximate limit of Frontenac Axis; broad yellow [light] line indicates Canada–United States border. Numbers identify populations referred to in the text: 1, Gananoque; 2, Maple Grove; 3, Sucker Brook; 4, Gananoque River main channel; 5, Marble Rock; 6, Gray's Creek; 7, Landon Bay; 8, Knight's Creek. Base image: Gananoque, 44.406450°N, 76.091095°W, Google Earth Pro 7.3.1.4507. Imagery date: 3 July 2018. Accessed: 14 March 2019.

site with long-time residents to obtain historical information on particular sites and populations.

*Azolla* taxonomy and identification is complex, and that of *A. cristata* is particularly challenging, largely because of the rarity of sporocarp and megaspore production (Svenson 1944; Lumpkin 1993). All known *Azolla* specimens from eastern Canada are sterile. Their identification relies on subtle microscopic characters, such as leaf trichome shape. Trichomes of the typically smaller-leaved *A. cristata* are bi- to tri-cellular compared with unicellular trichomes in *A. filiculoides* (Evrard and Van Hove 2004). The latter species (and *A. mexicana*) also frequently produce sporocarps (Svenson 1944; Lumpkin 1993). The specimens in question were examined in either a fresh or rehydrated condition through a light dissecting microscope (Wild M3B; Leica Microsystems, Wetzlar, Germany) at 40× magnification, with measurements made with the aid of an in-mount graticule (ocular micrometer).

*Azolla cristata* nomenclatural remains unsettled because of problems in interpretation of type specimens. Recent reviews of that problem conclude that *A. cristata* is the older, most appropriate name for this species (Evrard and Van Hove 2004; Pereira *et al.* 2011) and we follow that interpretation.

To assess the cold tolerance of *A. cristata*, plants from the Knight's Creek, L & G County population were collected in November 2016 and maintained in

cultivation over winter. One sample of approximately 20 floating fronds was kept in a container of creek water in a refrigerator at 4°C, and later became frozen in ice for approximately two weeks. A second, similar sample was maintained at approximately 17°C in the low natural light of a windowsill. Both were periodically examined through the winter season and into spring, with changes in size and appearance documented photographically.

## Results

*New eastern Ontario discoveries of Azolla cristata*

In September 2014, *A. cristata* was discovered along the Gananoque River in L & G County in patches that were almost continuous for 10.8 km upstream from the St. Lawrence River (Figures 1, 2, and 3). Subsequently, persistent populations of *A. cristata* have been found along tributaries of the St. Lawrence River in five separate watersheds within an area of approximately 56 km$^2$ in southern L & G County (Figure 4). Locations include the main course of the Gananoque River and its tributary Sucker Brook. The other subwatersheds encompass Gray's Creek, Knight's Creek (Figure 5), and Landon's Bay, all of which empty directly into the St. Lawrence River.

New records of *A. cristata* found from 2014 to 2017 were all within the Frontenac Axis (Table 1; Figure 4), a rugged upland landscape of erosion-resistant Precambrian bedrock characterized by an abundance of water bodies (Keddy 1995). In September 2014, we discovered large populations of *A. cristata* along the Gananoque River in patches extending from its confluence with the St. Lawrence River upstream for 10.8 km. The plants were conspicuous, forming large, dense, free-floating mats (Figure 3) suspended within a 5–10 mm thick growth of watermeal (*W. borealis* and *W. columbiana*), Small Duckweed (*Lemna minor* L.), Star Duckweed (*Lemna trisulca* L.), and Great Duckweed (*Spirodela polyrhiza* (L.) Schleiden). The brick-red colour of the *Azolla* patches was so conspicuous that the species was first noted from a vehicle moving at freeway speed on the Highway 401 bridge over the Gananoque River. In the Gananoque area, we observed the strong affinity of *Azolla* plants for *Wolffia* mats. Although some of the *Wolffia* mats examined did not support *Azolla*, all L & G County *A. cristata* populations were found amongst *Wolffia*.

In years of high abundance, *Azolla* was observed to grow in continuous expanses and was found in all the *Wolffia–Lemna* mats occupying side bays and shallow, quiet shore areas with reduced current along the lower Gananoque River. The *Wolffia–Lemna* mats remained continuously dense from the St. Lawrence River at Gananoque upstream for 10.8 km to Marble Rock. In some years, *A. cristata* plants and mat fragments piled up with millions of *Wolffia* plants into 10+ cm deep "drifts" on river obstructions and along the last several hundred metres of the Gananoque River shore in the

**FIGURE 5**. Portion of Knight's Creek Eastern Mosquito Fern (*Azolla cristata*) population (dark mat) in dense watermeal (*Wolffia* spp.) growth, Ivy Lea, Leeds and Grenville County, Ontario. Photo: D.F. Brunton, 10 November 2016.

town of Gananoque. The adjacent river shore at each site was distinguished by the great abundance of Tuckahoe (*Peltandra virginica* (L.) Schott & Endlicher), an otherwise provincially rare species (Oldham and Brinker 2009) with southern affinities (Toner *et al*. 1995).

Between September and November 2016, we also conducted searches for *Azolla* at 15 possible sites in southern L & G County where particularly large *Wolffia* mats were evident on GoogleEarth satellite imagery (Figure 4). Although these *Wolffia* mats appeared to be virtually identical in form, situation, and floristic association to the Gananoque River populations, we did not find any *Azolla* at these locations.

*Overwintering of Azolla cristata*

In our winter dormancy experimentation, refrigerated material from Knight's Creek failed to produce any new growth by late March (following a two-week freezing period). However, virtually all fronds from the second (room temperature) sample showed abundant new growth at the tips (Figure 6). Although the central axis of most of these plants was decaying, many bud tips were producing new growth, including several fragments that had already separated from the parent plant. The growth on most fronds, including fragments, continued vigorously into mid-April, at which time over half of most fronds constituted fresh green growth. It appears that *A. cristata* fronds, at least at room temperature, are capable of perennating from bud tips when those fronds persist in a dormant state throughout the winter months.

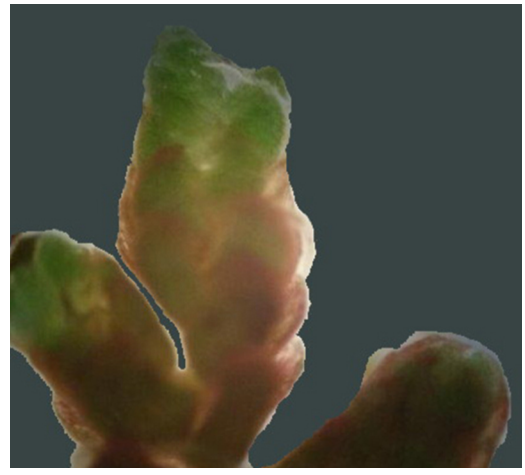

**FIGURE 6.** Pale-green-coloured, compressed, turion-like leaf-bundles (possibly winter buds) at tips of decaying Eastern Mosquito Fern (*Azolla cristata*) branches. Cultivated plants collected from Knight's Creek, Ivy Lea, Leeds and Grenville County, Ontario. Photo: H. Bickerton, 30 March 2017.

## Discussion

*Historical status of Azolla cristata in Canada*

*Azolla cristata* has been recorded growing outside cultivation in Canada in BC, southern ON, and southern QC. The BC records are all recent discoveries in artificial and/or recently disturbed wetland habitats in the urbanized southwestern part of the province (lower mainland and adjacent Vancouver Island). With no previous history of occurrence in western North America (Lumpkin 1993), and its occurrence only in disturbed sites heavily used by humans, the BC populations are reasonably considered to represent anthropogenic occurrences (Klinkenberg 2017). At least some historical southern ON records, however, were considered likely to represent natural range expansions (Macoun 1890; Cody and Schueler 1988).

*Azolla cristata* was first collected in Canada at Burlington Beach (western Lake Ontario), ON in 1862 (Macoun 1890; Cody and Britton 1989). It was not reported again in Canada until 1981 when a large population was found at the mouth of Knight's Creek in L & G County near Gananoque. Robert Griffin (pers. comm. 27 September 2016) reported observations of large *A. cristata* populations along the Gananoque River between Gananoque Lake and Marble Rock settlement "every few years" since the late 1960s. Griffin independently identified the species at that location years previously but was unaware of its significance until advised during the present study. Although abundant in 1981 (Cody and Schueler 1988), and despite periodic site inspections through 2000 (D.F.B. pers. obs.), *A. cristata* was not observed again at Knight's Creek until 2016 (H.J.B. pers. obs.).

Darbyshire (2002) discovered *A. cristata* at several locations in Ottawa, ON, and Gatineau, QC, in both the Rideau Canal and Rideau River in both 1997 and 1998. In 1998, *A. cristata* was observed only along the Ottawa River. This occurrence extended semi-continuously for ~10 km of the Rideau River in ON and 5 km of the Ottawa River in ON and QC. It could not be found in follow-up site visits in 1999 but was reported again from that area in 2003 when a large population was found in a different area of the Rideau River (Darbyshire and Thomson 2004). Although waterfowl dispersal was regarded as a possible vector, the urban location of these occurrences suggested to those investigators that the 2003 occurrence most likely resulted from the dumping of home aquaria (Darbyshire and Thomson 2004).

Discoveries of short-lived *Azolla* occurrences (believed to be *A. cristata*) were made elsewhere in southern ON after 2000. These were found either in artificial or disturbed wetlands and/or following wetland vegetation planting of nursery stock plants, e.g., in the Royal Botanic Garden, Hamilton (C. Rothfels pers. comm. 17 March 2004), Oshawa Second Marsh, Durham Regional Municipality (D. Leadbeater and J. Kamstra pers. comm. September 2017), and Niagara Regional Municipality (A. Garofalo pers. comm. November 2016). Most represented small populations but some (e.g., Oshawa

Second Marsh) involved thousands of plants covering several hectares. None of these populations are believed to have persisted more than two years.

*Origins and dispersal*

*Azolla cristata* is abundant and perhaps increasing in abundance in wetlands in the Oswego, NY area, ~100 km directly south of the L & G County sites (A. Nelson pers. comm. 23 December 2014; E. Hellquist pers. comm. 1 May 2018). Indeed, it was known to be common, even abundant, in eastern Lake Ontario shore marshes as long ago as the mid-19th century (Paine 1865). *Azolla* is known from wetlands frequented by migratory waterfowl along the NY shore of the Great Lakes from St. Lawrence County (Eldblom and Johnson 2010) to the Niagara Frontier region (Soper 1949; Eckel 2005; Weldy *et al.* 2018).

Waterfowl are widely identified as the probable vector for both short and long distance movements of many aquatic plant species (Garcia-Alverez *et al.* 2015; Coughlan *et al.* 2017). We frequently observed waterfowl, including Wood Ducks (*Aix sponsa*) and Canada Geese (*Branta canadensis*), loafing or preening in *A. cristata* patches along the Gananoque River, the former also apparently feeding among beds of *Azolla* and *Wolffia*. In October 2016, we observed plants adhering to the breast feathers of free-ranging Mute Swans (*Cygnus olor*) that were swimming through dense *Azolla* at the mouth of the Gananoque River. *Lemna* spp., a major constituent of the *Wolffia–Lemna* mats favoured by *A. cristata*, are known as a preferential, high-nutrient food source for waterfowl in general and swans in particular (Lumsden *et al*. 2017).

Costea *et al.* (2016) suggest that transport of plant propagules by waterfowl (internally) represents an underappreciated long-distance movement mechanism for various species in North America and indeed, Lovas-Kiss *et al.* (2018) document the long-distance transport of viable *Azolla* relative Floating Fern (*Salvinia natans* (L.) C. Allioni) macrospores in Europe. Similarly, Cranfill (1980) suggested that *A. cristata* populations in Kentucky may result from repeated introductions by migrating waterfowl. The suggestion by Cody and Schueler (1998) that such a process could explain the long periods of time between *Azolla* observation at Knight's Creek, L & G County, is supported by the distribution and habitat patterns noted here for both *Azolla* and waterfowl. Accordingly, dispersal by waterfowl from adjacent northern NY also seems the most plausible explanation for the comparable mass occurrences of *A. cristata* in L & G County and elsewhere in eastern ON and western QC.

The local distribution of *A. cristata* within individual waterways may also benefit from the physical transport of propagules by external agents. A large (0.6-ha) floating section of marsh turf consisting of Cattail (*Typha latifolia* L.) lifted off from the adjacent Wiltse Creek marsh in 1981 and blocked the section of the Gananoque River where *A. cristata* mats has been periodically observed since the 1960s. Smaller examples of such sediment-gouging marsh vegetation "rafts" occur sparingly but regularly along the river (R. Griffin pers. comm. 27 September 2016). Although no *Azolla* were observed during an 18 September 1981 investigation of the marsh blockage (Brunton 1981), such rafting could be responsible for the periodic downstream transport of *Azolla* plants.

It seems unlikely that the dumping of aquarium waste could explain recurring, independent populations of *A. cristata* across five subwatersheds in this lightly or uninhabited Canadian Shield landscape of L & G County. Indeed, *A. cristata* appears to be infrequently or rarely cultivated as a water garden or aquarium species in Canada, even in heavily urbanized areas. An online survey of 365 nurseries and aquaria active in the Greater Toronto Area (GTA) between 2011 and 2013 found that only 17 (4.6%) offered this species (L. Erdle pers. comm. 2017). Azan *et al.* (2015) reported that of 331 857 individual plant sales in one year by 20 stores in the GTA, only 931 (or 0.003%) consisted of *A. cristata* (as *A. caroliniana*).

*Extent and persistence of populations*

Some of the newly discovered L & G County *Azolla* populations were found to be immense, covering many hectares (Figure 3), in one case extending for kilometres. In 2016, we conservatively estimated a density of 13.5 *Azolla* plants/m$^2$ in a typical *Wolffia–Lemna* mat at the Maple Grove settlement ($n$ = 20 randomly chosen, 1-m$^2$ plots). Thus, this 2.6-km stretch of the Gananoque River that includes about 36 ha of apparently suitable habitat (identified from satellite images), supports about 485 000 plants. Extrapolating to the entire 10.8-km section of the Gananoque River along which *Azolla* was found implies an *Azolla* population of about two million plants. Even this large number, however, reflects only a portion of the total population that year because it excludes smaller sites off the Gananoque River. Despite that impressive estimate, in 2014 our field observations indicate that *Azolla* populations were even larger near the town of Gananoque—likely 200–300% more dense.

*Azolla cristata* has been considered short lived in the north of its range (Crow and Hellquist 2000). Populations in upstate NY appear to follow that pattern, persisting for several years in a given location, then disappearing for at least a period of years (A. Nelson pers. comm. 23 December 2014). Our finding that *A. cristata* has persisted at individual sites in L & G County for several years and probably even decades (R. Griffin pers. comm. 27 September 2016) is therefore notable. The Knight's Creek population, for example (Figure 5), has been known from its present location since at least 1981 (Cody and Schueler 1988; F.W. Schueler pers. comm. 6 November 2016). Despite periodic inspection in the intervening years (D.F.B. pers. obs.), *Azolla* was not observed again until 2016 (H.J.B. pers. obs.).

*Overwintering capacity*

The existence of these recurring *A. cristata* populations strongly suggests persistence over winter, either as dormant plants from the previous year or through the survival of propagules. This is consistent with observations near Lake Erie where a population believed to be *A. cristata* persisted over at least two growing seasons (2006 and 2007) in Black Creek (Fort Erie, ON; A. Garofolo pers. comm. 19 December 2016) and along the Rideau River in Ottawa in the late 1990s (Darbyshire 2002). Eric Hellquist (pers. comm. 28 May 2018) reports that *Azolla* plants in central NY were evident in May 2018 at a site where the species is reliably found; this is too early in the growing season for these plants to represent growth from the current year.

*Azolla cristata* was presumed unable to endure Canadian winter conditions, based on its apparent lack of persistence in ON (Darbyshire 2002). Because the −11°C average lowest winter temperature of Gananoque (Weather Spark 2018a) is only 3°C below the −8°C average lowest winter temperature of Oswego, NY (Weather Spark 2018b) where *A. cristata* is common (E. Hellquist pers. comm. 28 May 2018), "Canadian winter conditions" may not actually present a significant constraint to *Azolla* population sustainability in L & G County. The lower section of the Gananoque River where *Azolla* has been abundant in recent years was unfrozen on 2 March 2019 (D.F.B pers. obs.), also implying that aquatic temperature conditions are relatively moderate here.

Wong Fong Sang *et al.* (1987) found that *A. filiculoides* plants, frozen in a wild state between −10°C and −1°C for at least two weeks and then transferred to a 25°C growth chamber, started to grow again. Fronds of *A. filiculoides* are reportedly able to withstand hard frosts (−5°C) and prolonged ice cover (Lumpkin and Plucknett 1980). Janes (1998) found that although mature *A. filiculoides* plants in England died following a short (18 h) exposure to −4°C temperatures, they were capable of surviving encasement in ice for at least a week and only those plants that protruded above the ice were killed at sub-zero temperatures. Because *Azolla* can survive indefinitely at 4°C, Janes (1998) suggested that plants are capable of survival in fresh water below the ice where the temperature does not reach 0°C.

*Azolla cristata* is thought to be among the most cold-tolerant members of its genus (Lumpkin 1993). Consistent with that, in this study mats of apparently healthy *A. cristata* were evident at Knight's Creek on 9 November 2016 in 6°C water. Robust populations also were noted at Kinsman Park in Gananoque even later into that year on 19 November 2016 (K.L. McIntosh pers. comm. 19 November 2016).

We found no reference to turion-like structures in *A. cristata* in the botanical literature, although based on the growth observed in our cultivated sample (Figure 6; also see Results), these appear to exist. Eric Hellquist (pers. comm. 28 May 2018) also observed what appears to be perennating bud tips in *Azolla* populations in central NY in early May.

*Conclusions*

There is substantial evidence that *A. cristata* is naturally occurring in the Frontenac Axis of L & G County, ON. Large populations have persisted for 20+ or even 50+ year periods in lightly settled, rural locations there far removed from urban and suburban centres. Our observations, along with a reinterpretation of the earlier eastern ON and western QC data of Darbyshire (2002) and Darbyshire and Thomson (2004), imply that human-facilitated introductions are unlikely here. Interpretation of the likely origins of populations in the western Lake Ontario area is less clear because of their frequent occurrence in disturbed areas with high population densities.

The long period between observations of *Azolla* at some L & G County sites may not represent true absences, but may reflect periods when poorer growing conditions result in smaller, inconspicuous populations. The tiny population along the Gananoque River in 2015 between two "bumper" years, for example, could be a reflection of the documented ephemeral nature of *A. cristata* (Svenson 1944; Cranfill 1980; Lumpkin 1993). Small, inconspicuous populations may be normal in ON and elsewhere, with extensive populations such as those noted along the Gananoque, Ottawa, and Rideau Rivers, appearing only in years of especially favourable growth.

The occurrence of apparently self-sustaining *A. cristata* populations in eastern ON has phytogeographic and conservation implications. These occurrences are located within suggested plant migration routes of other uncommon plants with southern affinities. The Frontenac Axis area has long been recognized as a centre for such diversity, including provincially rare plant taxa of conservation concern, such as Pitch Pine (*Pinus rigida* P. Miller), Deerberry (*Vaccinium stamineum* L.), Appalachian Polypody (*Polypodium appalachianum* Haufler & Windham), Rue-anemone (*Thalictrum thalictroides* (L.) A.J. Eames & B. Boivin), and *Azolla* associate *P. virginica* (Dore *et al.* 1959; Cody 1982; Keddy 1995; Oldham and Brinker 2009).

Warming weather conditions in recent decades may be encouraging the persistence of *Azolla* populations in ON, QC, and BC. Warmer winters with longer ice-free periods and slightly warmer water temperatures would be expected to suppress barriers to the establishment and persistence of particular populations. The increased number of potential animal vectors in recent decades (especially migratory Wood Ducks and Canada Geese; Hughes and Abraham 2007; Zimmerling 2007) also increases potential opportunities for *Azolla* to be repeatedly transported into southeastern Canada.

## Author Contributions

Both authors contributed to the conceptualization of this article, investigation, methodology, formal analysis

of the data, writing of the original draft, review and editing. Both authors approved the final version of the manuscript.

## Acknowledgements

Our thanks to Ontario field-naturalists Michael J. Oldham, Albert Garofalo, Sarah Mainguy, Fred W. Schueler, James Kamstra, Dale Leadbeater, Karen L. McIntosh, and Carl. J. Rothfels for information on particular sites and populations in Ontario (ON), to field-botanist F. Lomer for information on *Azolla* distributional history in British Columbia, and to Andrew Nelson and Eric Hellquist (State University of New York, Oswego) and independent field-botanist David Werier for information on *Azolla* distribution in New York (NY). Lisa Erdle, Ontario Streams, Aurora ON, provided valuable information of the use of *Azolla* in the aquarium products trade. Important ecological observations on *Azolla* in NY were shared by Eric Hellquist, who also conducted an especially thorough and helpful review of the manuscript. An earlier draft of the manuscript benefited from reviews by Associate Editor Paul M. Catling and from Michael J. Oldham, Ontario Ministry of Natural Resources and Forestry, Peterborough, ON. We thank Parks Canada personnel Shalini Gupta and Josh Van Wieren at St. Lawrence Islands National Park, ON, for sharing documentation and observations on their Landon Bay discovery. Marble Rock Hamlet (Gananoque) resident Robert Griffin's keen observations of the landscape and natural features of his river were very helpful. We also thank the curatorial staff at the herbaria cited for their assistance in the examination of material under their care.

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Received 20 January 2018
Accepted 12 October 2018