U.S. Fish & Wildlife Service

Crested Mosquitofern (*Azolla cristata*)

Ecological Risk Screening Summary

U.S. Fish and Wildlife Service, May 2021
Revised, June 2021
Web Version, 7/23/2021

Organism Type: Plant
Overall Risk Assessment Category: High

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1 Native Range and Status in the United States

Native Range

From NIES (2021):

"Natural range [:] North America, Central America, South America."

From Yañez et al. (2020):

"It is widely distributed in the Americas, on the Southeast and Southwest of Canada and the United States, throughout Central America and South America up to Uruguay and Argentina (Arana 2016). In Argentina, it is distributed mainly from northern and Central regions […]."

According to Yañez et al. (2020), in southern South America, *A. cristata* is native to Brazil, Paraguay, Uruguay, and Argentina.

From Brunton and Bickerton (2018):

"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). […] 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)."

Weldy et al. (2021) report that *A. cristata* is native to the U.S. State of New York. Insufficient information was found to confirm the identification of *Azolla* spp. found in individual U.S. States, Commonwealths, or territories.

Status in the United States

Part of the native range of *Azolla cristata* is within the United States (see Native Range, above).

From Aber et al. (2010):

"In October 2009, low-height aerial photographs taken from a helium blimp revealed the presence of *Azolla* sp. over substantial portions of The Nature Conservancy (TNC) marsh complex at Cheyenne Bottoms in central Kansas. […] Although recorded previously in central Kansas, *Azolla* had not been seen in TNC marshes before, so its sudden appearance is remarkable."

"*Azolla cristata* is considered to be the most likely species (Van Hove, pers. comm.), although it is difficult to distinguish various species of the genus, and we have not made a positive identification."

Brunton and Bickerton (2018) mention repeated introduction of *A. cristata* to Kentucky, but no further information was available to determine if the species is established there.

This species is reportedly available for sale in the United States. However, the identification of these organisms in trade should be regarded with caution due to extensive taxonomic confusion (see Remarks).

From Tropical World Nursery (2021):

"Aquatic Floating plant Azolla (Fairy Moss [sic] one 16 oz deli cup
On sale $6.99
In Stock"

"Azolla cristata, aka Carolina Mosquit [sic] fern, Fairy Fern, Water Velve [sic] is a native plant to North America and is a true fresh water fern. Used as a fertilizer and for fish and poultry which [sic] love it. We supply this in a 16 ounce deli cup packed full."

Tropical World Nursery is based in Loxahatchee Groves, Florida.

Means of Introductions in the United States

From Aber et al. (2010):

"How *Azolla* entered TNC marshes [Cheyenne Bottoms, Barton County, Kansas] is unknown, but several means are possible. *Azolla* megasporocarps containing fertilized gametophytes or accompanied by microspores (containing massulae or sporophytes) could be brought in on the feet of waterfowl or shorebirds migrating northward in the spring. For example, some ponds around College Station, Texas were completely covered with *Azolla* during the summer of 2008."

"Another means of dispersal could be spores and plant fragments carried on boats or other equipment. This is considered unlikely in this case, because boats and other equipment are rarely used in TNC marshes. Still another possibility involves common use of *Azolla* as a freshwater aquarium plant; when aquarium water is released it may be transported into sewers and waterways (Whitley et al. 1999). The sewage treatment plant for the city of Hoisington is located less than one km directly west of TNC marshes."

"Perhaps most importantly for plant dispersal, Cheyenne Bottoms experienced flooding of historic proportion in 2007."

From Brunton and Bickerton (2018):

"Cranfill (1980) suggested that *A. cristata* populations in Kentucky may result from repeated introductions by migrating waterfowl."

Remarks

From Madeira et al (2016):

"[…] the identification of *Azolla* species is notoriously difficult and replete with historical, nomenclatural, and taxonomic issues and complications (Evrard and Van Hove, 2004). Reid et al. (2006) state that, 'The morphological similarity of *Azolla* species, together with their diminutive stature, have led to a long history of mistaken identifications, some of which have added to the taxonomic confusion.' The best identifications require the identification of reproductive features such as the glochidia from the microspore and the perine structure of the megaspore (Perkins et al., 1985). Unfortunately, reproductive structures are seldom available at the time when identifications are needed."

From Evrard and Van Hove (2004):

"We conclude therefore that there is no reason for considering more than two *Azolla* species in the American flora. One, *A. filiculoides*, has unicellular leaf trichomes, glochidia mainly unseptate or uniseptate, some with only a few, generally apical septae, and its perine is warty. The other species is characterized by bicellular leaf trichomes, glochidia mainly septate and a perine structure, quite variable, but not warty. Considering the priority rule this species must be named *A. cristata*. The observation of leaf trichomes under a light microscope is therefore the necessary and sufficient condition for identifying American sterile specimens."

"Most authors have confused *A. caroliniana* with *A. cristata*. The origin of this confusion is probably attributable to Mettenius (1847), whose description and illustrations of *A. caroliniana* are based on a specimen from Mexico, more than likely the type of *A. mexicana*, and not on the type of Michaux. This paper has been considered as the reference by the subsequent authors, including Mettenius (1867)."

"Most authors have also confused *A. microphylla* with *A. cristata*. The first descriptions and illustrations of Martius (1827) and of Mettenius (1847) are indeed probably based on the specimen of Pöppig, collected in Cuba, which belongs to *A. cristata*, and not on the Chamisso type. The subsequent authors have maintained this confusion."

Evrard and Van Hove (2004) synonymize *A. cristata* Kaulf. with *A. caroliniana* auct. non. Willd., *A. microphylla* auct. non Kaulf., *A. portoricensis* Spreng., and *A. mexicana* K. Presl in the most recent taxonomic redescription of American members of the genus *Azolla*. WFO (2021) recognizes *A. cristata* Kaulf., *A. caroliniana* Willd. and *A. microphylla* Kauf. as separate, valid species. Like Evrard and Van Hove (2004), WFO (2021) does not recognize *A. portoricensis* Spreng. as a valid species. WFO (2021) differs from Evrard and Van Hove (2004) in recognizing *A. mexicana* C. Presl. as valid and separate from *A. cristata* Kaulf. Because WFO (2021) is the taxonomic authority used for this risk screening process, this report followed WFO (2021) in treating *A. mexicana* C. Presl as separate from *A. cristata* Kaulf. Every effort has been made to include and consider information pertaining only to *A. cristata* Kaulf. Sources that synonymized *A. cristata* Kaulf. with *A. caroliniana* Willd. (such as Reid et al. 2006 and USDA NRCS 2021) were not used in conducting this risk screen but it is recognized that some amount of this information may indeed be applicable to *A. cristata* Kaulf.

A wide variety of common names have been applied to this species, including Eastern Mosquito Fern (Brunton and Bickerton 2018), Mexican Azolla (Hill and Coetzee 2017), Mosquito Fern (Weldy et al. 2021), and Water Fern (NIES 2021). All these names may also be applied to other *Azolla* species.

2 Biology and Ecology

Taxonomic Hierarchy and Taxonomic Standing

According to WFO (2021), *Azolla cristata* Kaulf. is the current valid scientific name of this species.

From ITIS (2021):

Kingdom Plantae
Subkingdom Viridiplantae
Infrakingdom Streptophyta
Superdivision Embryophyta
Division Tracheophyta
Subdivision Polypodiophytina
Class Polypodiopsida
Subclass Polypodiidae
Order Salviniales
Family Azollaceae
Genus *Azolla*
Species *Azolla cristata* Kaulf.

Size, Weight, and Age Range

From Yañez et al. (2020):

"Fronds 1-5 cm long or more […]"

From Weldy et al. (2021):

"Annual"

From Brunton and Bickerton (2018):

"*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 [Leeds and Grenville County, Ontario] for several years and probably even decades (R. Griffin pers. comm. 27 September 2016) is therefore notable."

Environment

From Yañez et al. (2020):

"This species inhabits lentic water bodies (e.g. ditches, ponds and temporary puddles) […]"

From Weldy et al. (2021):

"Still or slow moving water of lakes, ponds, and streams."

From Brunton and Bickerton (2018):

"*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)."

Climate

From Madeira et al. (2016):

"The distribution of […] *A. cristata* and *A. pinnata* subsp. *asiatica* are found in the lower lying, coastal warmer regions extending northward towards more tropical climatic regions [in Africa]."

From Brunton and Bickerton (2018):

"*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 [Leeds and Grenville County, Ontario]."

From Rizzo (2021):

"[…] from 0 to 300 m a.s.l. [above sea level]"

Distribution Outside the United States

Native

From NIES (2021):

"Natural range [:] North America, Central America, South America."

From Yañez et al. (2020):

"It is widely distributed in the Americas, on the Southeast and Southwest of Canada […] throughout Central America and South America up to Uruguay and Argentina (Arana 2016). In Argentina, it is distributed mainly from northern and Central regions […]."

According to Yañez et al. (2020), in southern South America, *A. cristata* is native to Brazil, Paraguay, Uruguay, and Argentina.

From Brunton and Bickerton (2018):

"Eastern Mosquito Fern (*Azolla cristata* G.-F. Kaulfuss (*A. caroliniana* auct., non C.L. Willdenow)); is found […] southward into South America (Svenson 1944; Wherry 1961; Lumpkin 1993; Crow and Hellquist 2000; Pereira *et al.* 2011). […] 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)."

Introduced

From Brunton and Bickerton (2018):

"Populations found along the Rideau and Ottawa Rivers in ON [Ontario, Canada] and Quebec (QU) were reported to represent non-native introductions (Darbyshire 2002; Darbyshire and Thomson 2004)."

"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."

"Recent occurrences in urban areas in southern BC [British Columbia] are reported as being introduced (Douglas *et al.* 2000; Klinkenberg 2017)."

No information was found to confirm establishment status of *A. cristata* in British Columbia. Douglas et al. (2000) report *A. caroliniana* Willd. as introduced and locally frequent in the Province, but do not list *A. cristata* as present in British Columbia.

From NIES (2021):

"Range in Japan [:] Miyagi, Ibaraki, Gumma, Saitama, Tokyo, Aichi, Gifu, Ahiga, Kyoto, Osaka, Wakayama, Hyogo, Okayama, Hiroshima, Kagawa, Ehime, Tokushima, Fukuoka, and Saga Pref[ecture]s."

From Ahad et al. (2012):

"The species of *Azolla* that grows in Kashmir [India] has been previously reported as *Azolla pinnata* R. Br. (Mir and Pandit, 2008), referring to the native Indian taxon *A. pinnata* subsp.

asiatica R.M.K. Saunders & K. Fowler. A detailed study of vegetative and reproductive characters of *Azolla* specimens collected from several water bodies in Kashmir Valley revealed that this species has been incorrectly identified and is, instead, *A. cristata* Kaulf."

From Masoodi and Khan (2012):

"We report here for the first time, the occurrence of *Azolla cristata* Kaulf. from India. *Azolla cristata* was collected from Wular lake, the largest freshwater lake in Kashmir, India. *Azolla cristata* Kaulf. is alien to the Indian flora and invasive in Kashmir."

From Langa et al. (2020):

"[…] *A. cristata* was found abundantly in all the rivers [sampled in southern Mozambique] except the Govuro and Inharrime Rivers."

From Hill et al. (2020):

"Distribution in subtropical regions of Limpopo, Mpumalanga and KwaZulu-Natal [South Africa] […] Recent establishment"

From Madeira et al. (2016):

"Twenty-one samples […] from north eastern South Africa, Mozambique, and Zimbabwe were identified as *A. cristata*, a western hemisphere species that has been introduced to the region."

"*A. cristata* was also sampled in Ghana and Uganda."

No further information regarding the status of *A. cristata* in Zimbabwe, Ghana, or Uganda was found.

From Mitić (2016):

"[In Croatia] Only eight freshwater alien plants were reported, out of them six are invasive (*Azolla cristata*, […])."

No further information regarding the status of *A. cristata* in Croatia was found.

From Domingues de Almeida (2018):

"[…] naturalized in Portugal at least since 1920 (Pinto da Silva 1940; Vasconcellos 1940; Pinto da Silva & Rainha 1948; Reed 1962; Almeida 1986; Lawalrée & Jermy 1993; Christenhusz & von Raab-Straub 2013)."

From NOBANIS (2021):

"*Azolla cristata* in Netherlands […]

Time of introduction: 1850 […]
Status: Not established
Frequency: Extinct […]
Comments: Last unvalidated observation in 1994 (waarneming.nl)"

From Rizzo (2021):

"The Pteridophytes [ferns and lycophytes] present in Sicily [Italy] are 58 (including species and subspecies), 6 Lycopodiopsida and 52 Polypodiopsida, including 4 allochthonous naturalized taxa (*Azolla cristata*, *Azolla filiculoides*, *Cyrtomium falcatum* and *Nephrolepis cordifolia*)."

Means of Introduction Outside the United States

From Brunton and Bickerton (2018):

*"*Although waterfowl dispersal was regarded as a possible vector, the urban location of these occurrences [in Ottawa, Ontario and Gatineau, Quebec] suggested to those investigators that the 2003 occurrence most likely resulted from the dumping of home aquaria (Darbyshire and Thomson 2004)."

From NIES (2021):

"Deliberate: For agricultural use (rice-duck farming)
Accidental: Spread within Japan by hitchhiking on water bird body."

From Madeira et al. (2016):

"The most likely explanation for the current distribution of alien *Azolla* species in southern Africa is that the initial invasion (*A. filiculoides*), reported in the Northern Cape region, slowly spread within the Orange River watershed, which empties westward towards the Atlantic Ocean, while secondary introduction(s), comprising *A. pinnata* subsp. *asiatica* and *A cristata* later occupied rivers emptying eastwards into the Indian Ocean. *A. cristata* populations constituted a separate introduction but the samples were originally mistakenly classified as *A. pinnata* subsp. *africana* (Hill, 1998) then later as *A. cristata* (Madeira et al., 2013) under the assumption that there had been only one introduction into southern Africa."

"Asuming-Brempong and Watanabe (1989) report the performance testing of *A. microphylla* [considered synonymous with *A. cristata* by this source] as a bio-fertilizer at a University of Ghana Agricultural Research Station in Kpong, Ghana, potentially the source of the introduction [to Ghana]. Additionally, Fiogbe et al. (2004) report the introduction of *A. microphylla* as a source of protein in low-cost feeds for tilapia at a research project at Porto-Novo in nearby Benin."

From NOBANIS (2021):

"Type of Introduction: Not known"

Short Description

From Yañez et al. (2020):

"Fronds 1-5 cm long or more, loosely imbricate; segments suborbicular, reddish."

From Ahad et al. (2012):

"[…] all plants examined were *Azolla cristata*, on the basis of the presence of bicelled trichomes, hook shaped, multiseptate glochidia, and a 3-float megaspore apparatus with a granular perine surface […]"

"The plants are free-floating, up to 4 cm long, and polygonal in shape. Sporophytes consist of a thin axial stem, bearing small leaves and delicate brown rootlets up to 6 cm long. Leaves are alternate, imbricate, and bilobed; the hyaline ventral leaf lobe and an aerial chlorophyllous dorsal lobe are covered with short bicellular trichomes and bear an extracellular cavity housing filamentous *Anabaena* […]. Rootlets are solitary, from stem branching points. Sporocarps are borne in pairs at the base of branches. Microspores are aggregated in stalked massulae, massulae with arrow shaped glochidia […]. Megasporocarps contain a solitary megaspore; megaspore apparatus with three floats, and granular perine […]."

Biology

From Brunton and Bickerton (2018):

"All known *Azolla* specimens from eastern Canada are sterile."

"The plants [in Ontario] were conspicuous, forming large, dense, free-floating mats […] 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 following information pertains to the genus *Azolla*, which includes *A. cristata*:

From Aber et al. (2010):

"It is a small aquatic fern typically described as a delicate, mosslike plant that floats or is stranded on the edge of quiet water bodies such as ponds, marshes and ditches (Steyermark 1963; McGregor et al. 1986). It often grows in large floating mats composed of masses of tiny ferns (Whitley et al. 1999)."

"The plants most usually reproduce asexually by fragmentation of the fronds as frequently as every two days (Watanabe 1982). *Azolla* also may reproduce sexually under special, poorly defined conditions. Each leaf has two lobes. The larger dorsal lobe is photosynthetic and hollow; the cavity is filled with the nitrogenfixing [sic] blue-green bacterium *Anabaena azollae*. The lower lobe is colorless, cup-shaped, and provides buoyancy (Wagner, 1997)."

"In winter, *Azolla* survives as either sporocarps, which fall to the bottom of water bodies, or as sporophytes that float."

Human Uses

From NIES (2021):

"For agricultural use (rice-duck farming)"

From Madeira et al. (2016):

"Asuming-Brempong and Watanabe (1989) report the performance testing of *A. microphylla* [considered synonymous with *A. cristata* by this source] as a bio-fertilizer at a University of Ghana Agricultural Research Station in Kpong, Ghana […] Additionally, Fiogbe et al. (2004) report the introduction of *A. microphylla* as a source of protein in low-cost feeds for tilapia at a research project at Porto-Novo in nearby Benin."

From Brunton and Bickerton (2018):

"[…] *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*)."

This species is reportedly available for sale in the United States. However, the identification of these organisms in trade should be regarded with caution due to extensive taxonomic confusion (see Remarks).

From Tropical World Nursery (2021):

"Aquatic Floating plant Azolla (Fairy Moss [sic] one 16 oz deli cup
On sale $6.99
In Stock"

"Azolla cristata, aka Carolina Mosquit [sic] fern, Fairy Fern, Water Velve [sic] is a native plant to North America and is a true fresh water fern. Used as a fertilizer and for fish and poultry which [sic] love it. We supply this in a 16 ounce deli cup packed full."

Tropical World Nursery is based in Loxahatchee Groves, Florida.

The following information pertains to the genus *Azolla*, which includes *A. cristata*:

From Aber et al. (2010):

"In China and southeastern Asia, it is grown as a natural fertilizer in rice paddies releasing up to 1100 kg N per hectare per year, nearly three times the rate compared to legume root nodules (Watanabe, 1982)."

Diseases

From Hill and Coetzee (2017):

"The biological control programme against *A. filiculoides* in South Africa using the Azolla specialist *S[tenopelmus] rufinasus* has been highly successful (McConnachie et al. 2003, 2004). However, field surveys showed that the agent utilised another Azolla species, thought to be the native *Azolla pinnata* subsp. *africana* (Desv.) Baker, which contradicted the host specificity trials (Hill 1998). However, molecular analysis showed that what we thought was the native species, *A. pinnata subsp. africana*, was a new invasive species, *A. cristata* Kaulfuss, a close relative of *A. filiculoides* (Madeira et al. 2016). Field surveys have shown that *S. rufinasus* is capable of establishing populations on *A. cristata* in the warmer, eastern part of the country […]"

Threat to Humans

No physical threats to humans were found. The following information pertains to threats to human well-being and livelihoods.

From Keller et al. (2018):

"Boat navigation has been affected by this species because it blocks channels (Mir and Pandit 2008), and the benefits from commercial fishing have declined because fishers now regularly need to hire additional labor to clear the water surface of *A. cristata* before they can cast their nets (AM, unpublished data from informal interviews with fishers […])."

"Continued spread of *A. cristata* and alligator weed in Wular Lake [India] will likely cause further decline in fish harvest. […] Additionally, water availability for municipal use, agriculture, and hydroelectricity generation will likely decline. These impacts will compound long-term reductions in environmental quality and ecosystem service supply, and will likely lead to increased poverty and vulnerability levels for local communities and a decrease in human well-being."

3 Impacts of Introductions

From NIES (2021):

"[In Japan:] Competition, hybridization, nutrient enrichment.
Affected organism: Native *Azolla* (*A. imbricate* [sic] and *A. japonica*) (competition, hybridization)
Freshwater communities (nutrient enrichment)"

From Keller et al. (2018):

"*A. cristata* was first recorded in Wular Lake [in India] between 2002 and 2004 when it was already forming thick mats, competing with other plants, and blocking navigation (Mir and Pandit 2008; note that it was originally misidentified as *A. pinnata*, later corrected by Masoodi and Khan [2012]; […])."

"[…] it has spread rapidly in Wular Lake and forms mats up to 10 cm thick ([…] Masoodi and Khan [2012]). […] Boat navigation has been affected by this species because it blocks channels (Mir and Pandit 2008), and the benefits from commercial fishing have declined because fishers now regularly need to hire additional labor to clear the water surface of *A. cristata* before they can cast their nets (AM, unpublished data from informal interviews with fishers […])."

4 History of Invasiveness

The history of invasiveness is High. *Azolla cristata* has been introduced outside of its native range in North America, Africa, Europe, and Asia, where it has become established in multiple locations. Established populations are known in Canada, South Africa, Mozambique, India, Italy, Japan, and Portugal. Negative impacts of introduction have been documented in India and Japan, including forming thick mats, competing with other plants, impeding navigation, and increasing difficulty and costs to commercial fishing.

5 Global Distribution

Figure 1. Known global distribution of *Azolla cristata*. Map from GBIF Secretariat (2021). Locations in the Netherlands, Ghana, Uganda, Zimbabwe, and the Ivory Coast will not be included in the climate match as they are not known to represent established populations.

Additional source locations will be added to the climate match in eastern Canada, India, Italy, and Japan to represent additional established nonnative populations in those countries (Ahad et al. 2012; Brunton and Bickerton 2018; NIES 2021; Rizzo 2021).

This species is native to North, Central, and South America but limited georeferenced locations were available within the native range of *A. cristata*. No georeferenced occurrences were available to represent the established population in Portugal.

6 Distribution Within the United States

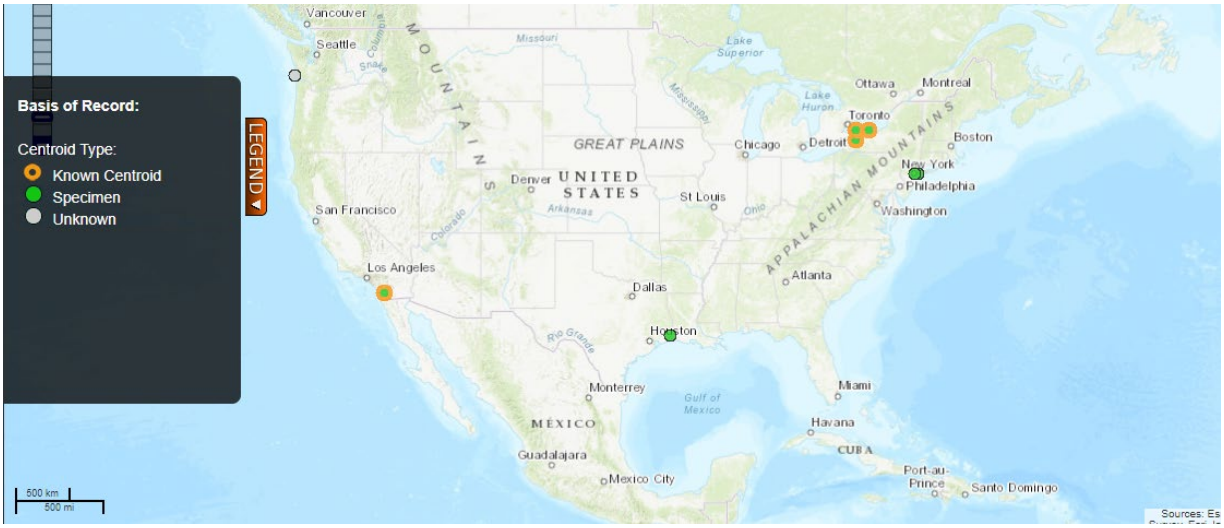

Figure 2. Known distribution of *Azolla cristata* in the United States. Map from BISON (2021).

7 Climate Matching

Summary of Climate Matching Analysis

The Climate 6 score (Sanders et al. 2018; 16 climate variables; Euclidean distance) for *Azolla cristata* in the contiguous United States was 0.492, indicating a high overall climate match. (Scores greater than or equal to 0.103 are classified as high.) Much of the contiguous United States had a high to medium climate match locally. The highest climate matches were found in the Great Lakes region, the interior Northeast, and on the Texas Gulf Coast; *A. cristata* climate matching source locations representing native populations were situated in each of these areas. Other areas with high match included the Ohio River basin, the Atlantic Coast south of Long Island, the remainder of the Northern Gulf Coast, and the Desert Southwest into California. The lowest climate matches were found in the Pacific Northwest along the Pacific Coast and just to the east of the Cascade Range. Other areas of low match included the northern Great Plains and the Sierra Nevada. The central Great Plains, the interior Southeast, the Seattle area, and coastal New England had medium match. A mix of high, medium, and low matches were scattered throughout the Rocky Mountains. Most States received high individual Climate 6 scores. Idaho, Montana, Nebraska, South Dakota, and Wyoming received medium individual Climate 6 scores; Minnesota, North Dakota, and Rhode Island received low individual Climate 6 scores.

Azolla cristata is native within the United States. Due to a historical lack of clarity and consensus on *Azolla* taxonomy, it is likely that the range of *A. cristata* is broader than shown in the source locations for the United States. Therefore, the climate match results may be an underestimate of true climate match.

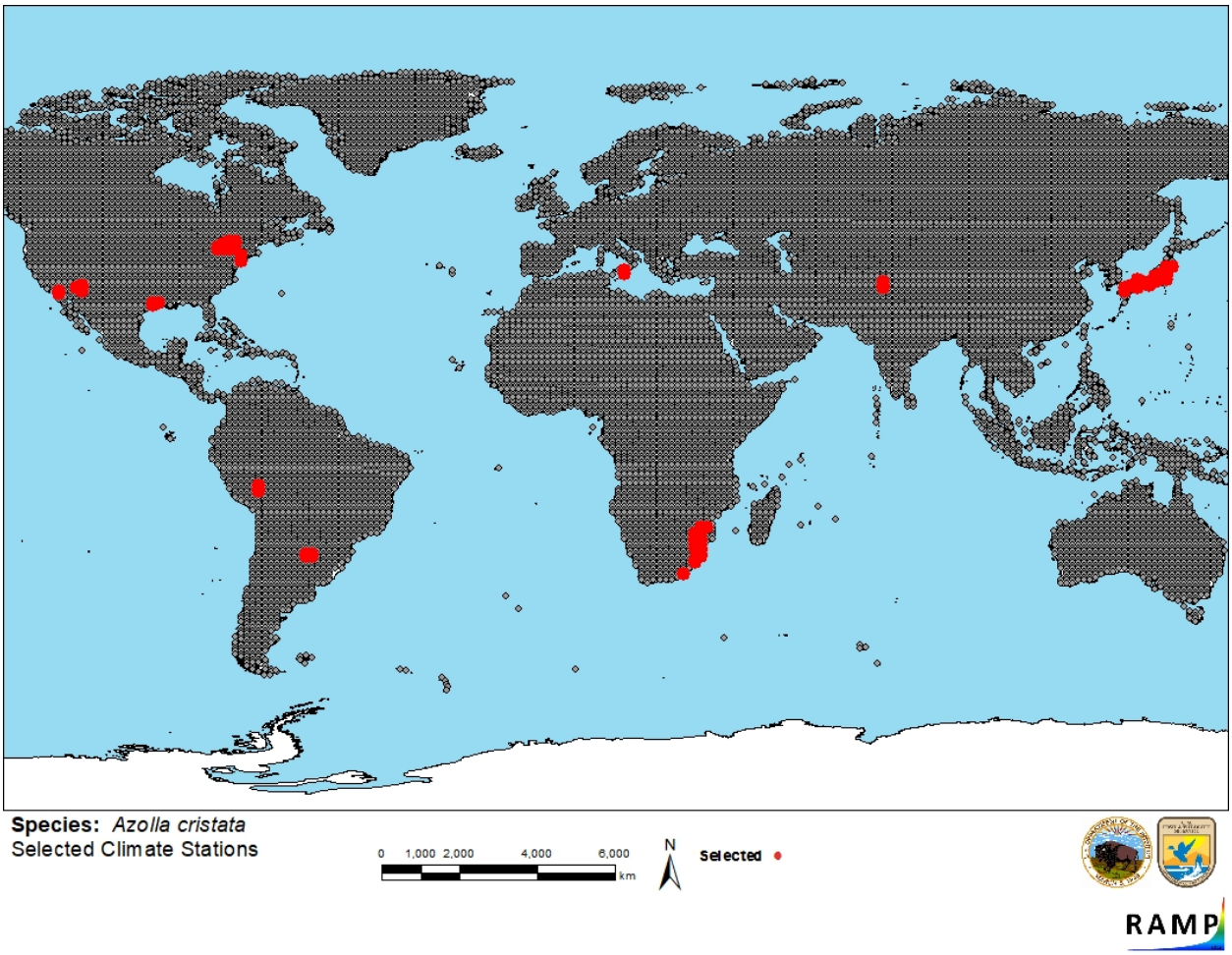

Figure 3. RAMP (Sanders et al. 2018) source map showing weather stations selected as source locations (red; United States, Canada, Peru, Argentina, Mozambique, Eswatini, South Africa, Italy, India, and Japan) and non-source locations (gray) for *Azolla cristata* climate matching. Source locations from GBIF Secretariat (2021) and BISON (2021). Additional source locations from Ahad et al. (2012), Brunton and Bickerton (2018), NIES (2021), and Rizzo (2021). Selected source locations are within 100 km of one or more species occurrences, and do not necessarily represent the locations of occurrences themselves.

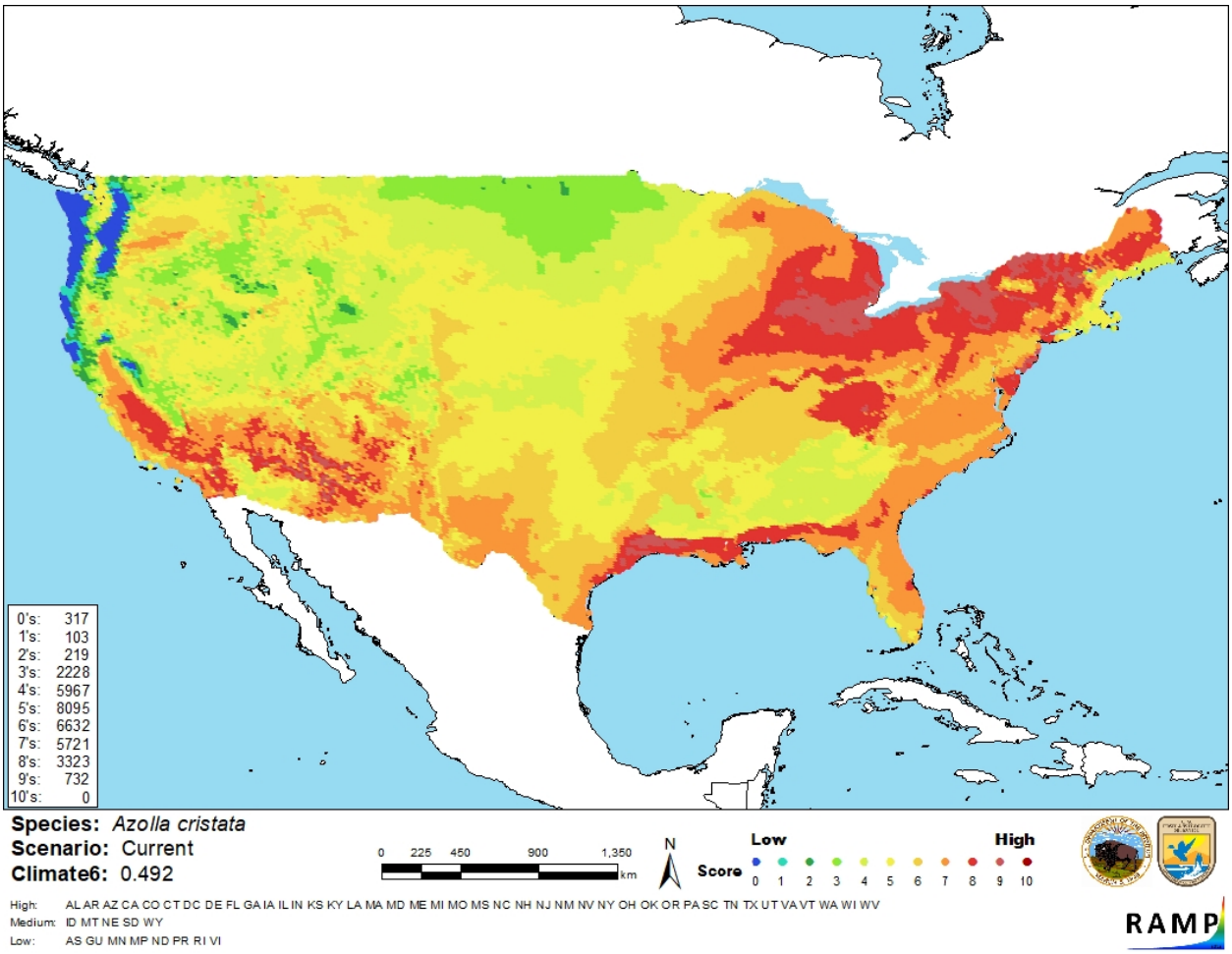

Figure 4. Map of RAMP (Sanders et al. 2018) climate matches for *Azolla cristata* in the contiguous United States based on source locations reported by GBIF Secretariat (2021), BISON (2021), Ahad et al. (2012), Brunton and Bickerton (2018), NIES (2021), and Rizzo (2021). Counts of climate match scores are tabulated on the left. 0/Blue = Lowest match, 10/Red = Highest match.

The High, Medium, and Low Climate match Categories are based on the following table:

Climate 6: (Count of target points with climate scores 6-10)/ (Count of all target points)	Overall Climate Match Category
0.000≤X≤0.005	Low
0.005<X<0.103	Medium
≥0.103	High

8 Certainty of Assessment

The certainty of this assessment is High. There is significant confusion over the identification and definition of species in the genus *Azolla*. This taxonomic uncertainty has resulted in confusion over the native range of *A. cristata* in the Americas as well as difficulty in identifying

nonnative populations to the species level. Incomplete information on the native range in the United States likely led to an underestimate of true climate match, but a high climate match was estimated even without complete distributional information. Negative impacts of introduction have been clearly documented for an established population of *A. cristata* in India for which the identity has been verified. Information on the biology and ecology of *A. cristata* was readily available, although the uncertainty around species identification applies to this information as well.

9 Risk Assessment

Summary of Risk to the Contiguous United States

The Crested Mosquitofern, *Azolla cristata,* is a small floating aquatic fern native to North, Central, and South America. This species is native in the United States, however the exact extent of the native range of this species is unclear due the taxonomic confusion associated with the genus *Azolla*. This fern is a nitrogen fixer and as such has been used in agriculture, particularly in rice production. *A. cristata* is rarely found in the aquarium and water garden trades. This species has been reported as introduced and established in Canada, India, Japan, South Africa, Mozambique, Portugal, and Italy. Multiple peer-reviewed reports document negative impacts of introduction including forming thick mats, competing with other plants, blocking navigation, and increasing difficulty and costs to commercial fishing. The history of invasiveness is High. The overall climate match to the contiguous United States is High. A majority of high match was found where *A. cristata* is likely native, in the East and the Southwest. Despite the uncertainty in the taxonomy of the *Azolla* genus, the certainty of this assessment is High because of clear documentation of negative impacts and because the high climate match likely underestimates true climate match. The overall risk assessment category for *Azolla cristata* is High.

Assessment Elements

- **History of Invasiveness (Sec. 4): High**
- **Overall Climate Match Category (Sec. 7): High**
- **Certainty of Assessment (Sec. 8): High**
- **Remarks, Important additional information:** The taxonomy of the *Azolla* genus is unresolved.
- **Overall Risk Assessment Category: High**

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