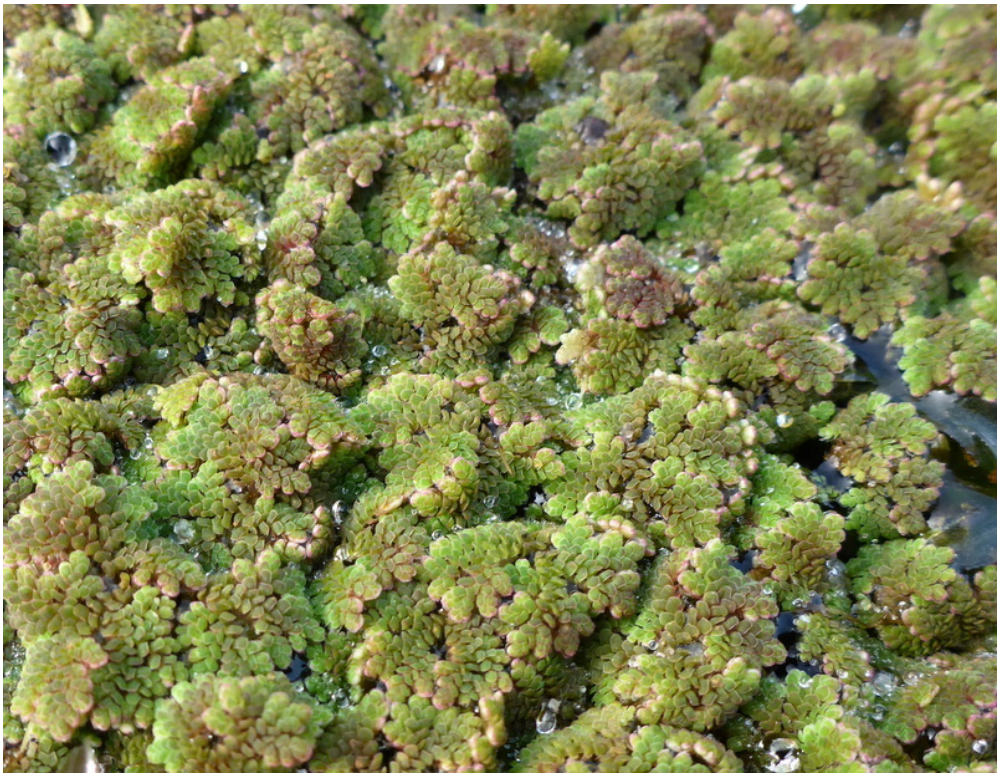
Carolina Mosquitofern (*Azolla caroliniana*)

Ecological Risk Screening Summary

U.S. Fish & Wildlife Service, April 2021
Revised, July 2021
Web Version, 8/5/2021

Organism Type: Plant
Overall Risk Assessment Category: High



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1 Native Range and Status in the United States

Native Range

According GRIN-Global (2021), *Azolla caroliniana* is native to the following countries: Canada (Ontario), the United States (Indiana, Massachusetts, New York, Ohio, Wisconsin, Alabama, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, South Carolina, Virginia and Texas), Mexico (Chiapas), Cuba, Jamaica, Puerto Rico, Costa Rica, Guatemala, Honduras,

Nicaragua, Panama, French Guiana, Guyana, Suriname, Venezuela (Zulia), Brazil, Columbia, Peru, Argentina and Uruguay.

From Madeira et al. (2013):

"Florida's native *Azolla* has historically been considered to be *Azolla caroliniana* Willd. (Godfrey et al., 1961; Wunderlin and Hansen, 2003).

From Lumkpkin and Plucknett (1980):

"Prior to their dispersal by man, the species were endemic to the following areas: *A. caroliniana*, eastern North America and the Caribbean.

From Shi and Hall (1988):

"*A. caroliniana* is indigenous to the eastern United States, Caribbean, the West Indies and Mexico […]"

From Darbyshire (2002):

"[…] it has been found only twice in Ontario (Cody and Schueler 1988). The first collection in Canada is of plants taken at Burlington Beach (near Hamilton) in 1862. The plant does not seem to have persisted there, and has not been seen since the original collection. An extensive population found at Knights Creek (near Gananoque) in 1981 also did not persist the following year (Cody and Schueler 1988)."

Status in the United States

A large portion of the native range of *Azolla caroliniana* is within the United States (see section above).

According to NatureServe (2021) *Azolla caroliniana* is "possibly extirpated" from Arkansas, "critically imperiled" in Washington D.C. and Illinois, "vulnerable" in Indiana, New Jersey and North Carolina, and "imperiled" in Missouri and New York.

From Svenson (1944):

"[…] occasionally introduced in the eastern United States, Hawaii, […]."

From Imada and Kennedy (2020):

"*Azolla filiculoides* and *A. caroliniana* are among three North American species that have become […] introduced horticulturally into Hawai'i […]. In Hawai'i it can often be found covering the water surface in taro paddies. […] *A. caroliniana* was apparently relatively recently introduced, represented in the herbarium by only two Hawaiian vouchers collected in 1985 (O'ahu) and 1994 (Moloka'i), both identified in 1994 by Alan R. Smith (UCBerkeley)."

From Cohn and Renlund (1953):

"This species is apparently a recent introduction to New Jersey. […] Taylor (1915) lists it as being found "in the Morris Canal near Bloomfield, New Jersey" and Small (1935) mentions it as being present in Essex County as the "remains of plantings connected with mosquito-control activities." […] Although its natural range is given as Massachusetts and New York to Louisiana and westward, Muenscher (1944) does not indicate its presence in New Jersey. It may be assumed then that this colony represents the first natural appearance of the fern in the state."

From Darbyshire (2002):

"Small (1935) considered the occurrences of *A. caroliniana* around southeastern New York State and northern New Jersey (and by implication further north) to be colonies naturalized from human introductions. Fernald (1950) also considered northern occurrences as being likely spread from cultivation."

According to EDDMapS (2021), *A. caroliniana* has been introduced to Hawaii and lists the infestation status as Positive.

Azolla caroliniana appears to be readily available in the plant trade and can be found online through various aquaria supply vendors such as Pond Megastore (2021), Pond Plants Online (2021), Dragonfly Aquatics (2021), etc. It is also available by large, nationwide chain stores such as Walmart (2021) and is sold by the pound.

Azolla caroliniana is on Illinois's Aquatic Life Approved Species List (Illinois DNR 2015).

Means of Introductions in the United States

From Darbyshire (2002):

"Small (1935) considered the occurrences of *A. caroliniana* around southeastern New York State and northern New Jersey (and by implication further north) to be colonies naturalized from human introductions. Fernald (1950) also considered northern occurrences as being likely spread from cultivation."

No information on the means of introduction in Hawaii was found.

Remarks

The taxonomic authority used for plants by this screening process considers *Azolla caroliniana* a valid species (WFO 2021). WFO (2021) states *A. filiculoides* and *A. cristata* are considered separate, valid species. Multiple other peer-reviewed resources indicate these species are synonymous with *A. caroliniana*. Every effort has been made to only consider information pertaining to *A. caroliniana* and not another valid *Azolla* species in this screening.

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. In other words the taxonomy proposed by Mettenius (1867) and so remarkably documented by Strasburger (1873) has to be rehabilitated, with nevertheless *A. caroliniana* Willd. sensu Mettenius replaced by *A. cristata* Kaulf."

From Metzgar et al. (2007):

"Likewise, the taxonomic status and rank of three sympatric New World taxa (*A. caroliniana, A. mexicana*, and *A. microphylla*) have also generated considerable debate. Numerous authors maintain them as distinct species (Svenson 1944; Moore 1969; Saunders and Fowler 1993). Others have contributed evidence or reviews suggesting that they be regarded as a single species (Zimmerman et al. 1989, 1991; Evrard and Van Hove 2004) or that the extent of the taxonomic conundrum is such that some of the names may have been misapplied and will require a detailed study of type material (Dunham 1986; Dunham and Fowler 1987). Reid et al. (2006) produced the first molecular phylogeny of *Azolla* and hypothesized that *A. caroliniana* was distinct whereas *A. microphylla* and *A. mexicana* should be considered the same species."

"Based on the results of their three-locus study, Reid et al. (2006) recommended recognizing *A. caroliniana* as a separate species but suggested that *A. microphylla* and *A. mexicana* be treated as a single species. The results of our six-locus data support the conclusions of Reid et al. (2006) and do not confirm the morphological conclusion of Evrard and Van Hove (2004) that the entire CAR-MICMEX complex should be recognized as a single species."

From Pereira et al. (2011):

"The clustering analysis with the different types of data (morphological, RAPD and combined) supported the two-section ranking, the *A. pinnata* and *A. nilotica* in section Rhizosperma and five distinct species on section *Azolla Azolla* (*A. mexicana, A. microphylla, A. caroliniana, A. filiculoides* and *A. rubra*)."

From Madeira et al. (2013):

"The status of *A. caroliniana* is perhaps the most debatable within the genus because Willdenow (1810) described the holotype *A. caroliniana* ("habitat in aquis Carolinae") from a sterile specimen. This has led to various interpretations including that the original specimen was actually *Azolla filiculoides* Lamarck (Dunham and Fowler, 1987; Evrard and Van Hove, 2004). Regardless of what the holotype represents, an "*A. caroliniana*" species with a unique megaspore perine structure exists (Perkins et al., 1985; Pereira et al., 2001)."

"The discovery of a previously uncharacterized Ecuadorian *Azolla*, which appears to be a paternal ancestor of *A. caroliniana*, indicates that *A. caroliniana* is a hybrid species."

Difficulty in correctly identifying *Azolla caroliniana* from its congeners and the many taxonomic changes proposed to the genus make difficult to fully understand the distribution of the species.

From Roy et al. (2014):

"[…] cryptic species make early detection of new species difficult. For example the alien aquatic fern, *Azolla filiculoides*, is well established in Britain, but the morphological characters distinguishing it from *Azolla caroliniana* are unclear so it is not known whether both species are actually present."

From Pereira et al. (2001):

"With regard to the existence of two species in Portugal, we offer two explanations: either *A. caroliniana* never existed in our country and the herbarium specimens where incorrectly identified or, if it existed, it probably overlapped with *A. filiculoides*."

2 Biology and Ecology

Taxonomic Hierarchy and Taxonomic Standing

According to WFO (2021), *Azolla caroliniana* Willd. is the current valid common name of this species.

From GBIF Secretariat (2021):

Kingdom: Plantae
Phylum: Tracheophyta
Class: Polypodiopsida
Order: Salviniales
Family: Salviniaceae
Genus: *Azolla*
Species: *Azolla caroliniana* Willd.

Size, Weight, and Age Range

From Pandey (2012):

"*Azolla caroliniana* (1–4 cm) […]"

From Shi and Hall (1988):

"The diameter of the sporophyte is usually about 1 cm for *A. pinnata (A. imbricata), A. mexicana, A. micro- phylla,* and *A. caroliniana*."

From Wagner (1997):

"The maximum biomass attained by *Azolla* spp., as reported by various researchers, is 3190 kg dry weight ha-1 for *A. caroliniana* and ranges from 640 to 2170 kg ha-1 […]"

Environment

From Wagner (1997):

"The optimum temperature for *Azolla* spp. is between 18° and 28° (Tuan & Thuyet, 1979), although some species can survive a very wide temperature range of about -5° to 35°C (Lumpkin, 1987a). According to Watanabe (1982), the optimum temperature for *A. pinnata, A. mexicana,* and *A. caroliniana* is about 30°C. Growth rates are reduced above 35°C and no species can survive at prolonged temperatures above 45°C (Lumpkin, 1987a). Hechler and Dawson (1995) found that, in *A. caroliniana*, nitrogenase activity could be detected at temperatures between 5° and 40°C, was relatively high at between 15° and 35°C and was maximum at 25°C."

From Palmer (1912):

"It has found the still waters of South Walsham and Ranworth Broads particularly suited to its needs. It is most partial to the reed swamps of *Typha angustifolia*, so characteristic of the borders of our fen-lakes and with this protection it is seen in large crowded expanses."

"It seems probable that the spread of the species to the other rivers of the Norfolk system, the Yare and the Waveney, will be prevented by the brackish nature of the water below Acle Bridge. I understand that a hard winter would probably kill the plant off but its abundant sporocarps would carry it over the succeeding spring."

From Pandey (2012):

"*A. caroliniana* is an aquatic fern occurring in ditches and stagnant water, although it can grow on the wet mud surfaces […]."

Climate

From Pandey (2012):

"*A. caroliniana* is distributed widely throughout temperate, sub-tropical and tropical regions of the world."

Distribution Outside the United States

Native

According the GRIN-Global (2021), *Azolla caroliniana* is native to the following countries: Canada (Ontario), […] Mexico (Chiapas), Cuba, Jamaica, […], Costa Rica, Guatemala, Honduras, Nicaragua, Panama, French Guiana, Guyana, Suriname, Venezuela (Zulia), Brazil, Columbia, Peru, Argentina and Uruguay.

From Shi and Hall (1988):

"*A. caroliniana* is indigenous to the […] Caribbean, the West Indies and Mexico […]"

From Lumkpkin and Plucknett (1980):

"Prior to their dispersal by man, the species were endemic to the following areas: *A. caroliniana*, eastern North America and the Caribbean.

From Darbyshire (2002):

"[…] it has been found only twice in Ontario (Cody and Schueler 1988). The first collection in Canada is of plants taken at Burlington Beach (near Hamilton) in 1862. The plant does not seem to have persisted there, and has not been seen since the original collection. An extensive population found at Knights Creek (near Gananoque) in 1981 also did not persist the following year (Cody and Schueler 1988)."

Introduced

From Dana et al. (2001):

According to Dana et al. (2001), *Azolla caroliniana* is listed as a "Naturalised [*sic*] Species Showing a Potential Threat to Spanish Ecosystems."

Thiébaut (2007) lists *A. caroliniana* as invasive in France.

From Shi and Hall (1988):

"[…] introduced into eastern Spain, France, Italy, and China."

From Imada and Kennedy (2020):

"*Azolla filiculoides* and *A. caroliniana* are among three North American species that have become naturalized in Europe and South Africa, […] and agriculturally into Asia (Lumpkin 1993).

From Protopopova et al. (2006):

"*A. caroliniana* Willd. colonize aquatic ecosystems [in the Steppe zone of Ukraine]."

From Prokopuk (2016):

"*Azolla caroliniana* Willd. belonging to invasive species-transformers of the aquatic flora of Ukraine was found in water bodies of Kiev used for recreation."

From Darbyshire (2002):

"In September of 1997 a population of *A. caroliniana* was found in the westerly pond of Brown's Inlet off the Rideau Canal in Ottawa, Ontario […] Other vegetation studies in this pond during the previous two years had failed to detect the fern (personal observations)."

"Continued observations during the next two years did not detect *A. caroliniana* at Brown's Inlet. It is presumed that these winter conditions [including full draw down] proved too harsh for the vegetative material to over-winter."

"The occurrence of the plant in Brown's Inlet […] was most likely a deliberate introduction."

"In late summer of 1998, colonies of *A. caroliniana* were seen in substantial mats along the shores of the Rideau River."

"Systemic and repeated searches in August and September of 1999 throughout the areas of the previous year's infestation failed to detect a single plant of *A. caroliniana*. Less detailed searches in 2000 were also negative. The large population seems to be the result of a single introduction at a point close to collection site number 9 […]. Figure 2 [in source material] indicates that phosphorous concentrations would not have been a limiting factor in 1999, so the complete disappearance is most likely the result of winter-kill."

Means of Introduction Outside the United States

According to Imada and Kennedy (2020), *A. caroliniana* was introduced to Asia for agricultural purposes.

From Madeira et al. (2019):

"*Azolla. caroliniana* was introduced [to the Yangtze River Watershed, China] because of broad tolerance for drought, heat, and cold (Watanabe Journal Pre-proof and Liu, 1992). As *A. caroliniana* fixes large amounts of nitrogen in the spring it was especially useful in early rice triple cropping systems, as well as for early-medium and medium rice varieties (Li 1984)."

From Prokopuk (2016):

"*Azolla caroliniana* Willd. belonging to invasive species-transformers of the aquatic flora of Ukraine was found in water bodies of Kiev used for recreation."

From Darbyshire (2002):

"The introduction could have easily occurred here from the dumping of aquarium contents."

Short Description

From WFO (2021):

"Plants rarely fruiting, minute, mostly 5–10 mm wide, dichotomously branched almost throughout; upper lf-lobes 0.5–0.6 mm,much smaller than the lower, not much imbricate; massulae ca 0.3 mm, the glochidia without cross-walls; […]"

From eFloras (2008):

"Plants dark green or with margins of bright crimson or whole plants dark red, free-floating or forming multilayer mat to 4 cm thick under good conditions; plants infrequently fertile. Stems prostrate, 0.5--1 cm. Largest hairs on upper leaf lobe near stem with 2 or more cells; broad pedicel cell often 1/2 or more height of hair, apical cell curved, with tip nearly parallel to leaf surface. Megaspores without raised angular bumps or pits, densely and uniformly covered with tangled filaments."

From Svenson (1944):

"Glochidia not septate, or rarely with 1 or 2 septae at apex; plants elongate (frequently 2-6 cm. long), with closely appressed, imbricate, papillose, oblong to ovate leaves (1 mm. long); micro-sporangia 35-100 in an indusium; massulae 4-6; megasporangia with raised, irregularly hexagonal markings."

From Evrard and Van Hove (2004):

"All the papers having described leaf trichomes on the upper leaf lobe have presented them as […] bicellular (sometimes accompanied by tricellular) in *A. caroliniana,* […]"

From Cohn and Renlund (1953):

"The plants are green from their first appearance in July to early September, at which time a color change becomes apparent. By mid-September they are a deep rusty-red, except for those plants that are shaded, these retaining their green color. The leaf tip of the green plant is bright red. During the color change this red hue is at first more intense in the tip of the lower lobe. Later, the entire plant becomes rusty-red. Different authors ascribe this change to varying environmental factors such as light, nutriment, and others."

Biology

From Pandey (2012):

"It is dispersed by spores or stem fragments. *A. caroliniana* (aquatic pteridophyte) form a symbiotic relationship with the cyanobacteria *Anabaena azollae* (nitrogen-fixing blue-green algae), which fixes atmospheric nitrogen and grows in a cavity in the dorsal lobe of the fern's frond."

From Imada and Kennedy (2020):

“In Hawai‘i it can often be found covering the water surface in taro paddies.”

From Darbyshire (2002):

“*Azolla caroliniana* is a plant of slow-moving or stagnant water in ponds, lakes, marshes, swamps, streams, rivers, ditches, etc. (Lumpkin 1993). It is the most cold-resistant species in the genus (Lumpkin 1993) […]”

“Species of *Azolla* are heterosporic, but reproduction in *A. caroliniana* seems to be entirely vegetative. Megaspores are unknown, although microspores are sometimes detected and are produced under condition of crowding (Lellinger 1985),”

“A symbiotic relationship has formed between species of *Azolla* and the cyanobacterium (blue-green alga) *Anabaena azollae* Strasb. (and possibly other bacteria) […]”

“[…] it was abundant, often completely swathing the shore for considerable distances. Mats became sparser at the upstream and downstream extremes of the distribution and where the river shores were exposed to strong currents or waves. Mats were mostly along shores and becoming thick and continuous in quite bays and around protecting emergent vegetation such as Cattails (*Typha latifolia* L.) or Reed Canary Grass (*Phalaris arundinacea* L.).”

Human Uses

From Imada and Kennedy (2020):

“These tiny water ferns are well known for their association with nitrogen-fixing blue-green algae, leading to their economic use as a green fertilizer. They have also been exported horticulturally as water plants, leading to their spread as invasive weeds of slow-moving waterways.”

From Dhir (2019):

“Aquatic plant species, namely, *Ipomoea aquatica, Azolla caroliniana, Lemna minor, Phragmites australis, Typha latifolia, Salix atrocinerea*, and *Scirpus validus* have shown capacity for removing PPCPs [pharmaceuticals and personal care products] such as ibuprofen, triclosan, diclofenac, carbamazepine, sulfadiazine, acetaminophen, tonalide, oxybenzone, galaxolide, and naproxen from wastewater.”

From Wagner (1997):

“*Azolla-Anabaena* has many uses. It can be utilized as a biofertilizer on rice and many other crops, an animal feed, a human food, a medicine, and a water purifier. It may also be used for the production of hydrogen fuel, the production of biogas, the control of weeds, the control of mosquitoes, and the reduction of ammonia volatilization that accompanies the application of chemical nitrogen fertilizer.”

From Pandey (2012):

"The accumulation of Pb, Mn, Zn, Cu and Ni in naturally growing *A. caroliniana* on FA [fly ash] pond was much higher in comparison to the previously reported plant species like *Typha latifolia* (Cattail) and *Fimbristylis dichotoma* (Rush) from the marshy and wet areas of FA ponds (Maiti and Jaiswal, 2008). Thus, this comparison shows that *A. caroliniana* is more [sic] efficient candidate for removal of heavy metals from FA pond)

From Brunton and Bickerton (2018):

"Azan et al. (2015) reported that of 331 857 individual plant sales in one year by 20 stores in the GTA [Greater Toronto Area], only 931 (or 0.003%) consisted of *A. cristata* (as *A. caroliniana*)."

Azolla caroliniana appears to be readily available in the plant trade and can be found online through various aquaria supply vendors such as Pond Megastore (2021), Pond Plants Online (2021), Dragonfly Aquatics (2021), etc. It is also available by large, nationwide chain stores such as Walmart (2021) and is sold by the pound.

Diseases

No information on diseases available.

Threat to Humans

No information on threat to humans available.

3 Impacts of Introductions

From Prokopuk (2016):

"In water bodies of Kiev, *A. caroliniana* is a potent cenosis-forming species and dominant. Under conditions of warm summer-autumn period of 2014 in water bodies of Kiev, its life strategy corresponded to that of "species-transformers". On the one hand, this species produces resources (in particular, nitrogen), whereas on the other hand – it intensively consumes other resources (light, oxygen, and nutrients), which results in changes in the cenotic structure of communities. […] In addition, intensive development of *A. caroliniana* resulted in changes in the cenoses. In autumn, communities of the association Lemno-Spirodeletum polyrhizae widely distributed in summertime gave way to the cenoses of the association Lemno-Azolletum carolinianae and monodominant communities of *A. caroliniana*.

Mass development of *A. caroliniana* in the water body of Kiev is an example of invasion of alien species conditioned by intensive anthropogenic load on aquatic ecosystems. Thus, *A. caroliniana* is a dangerous component of the flora of macrophytes of Ukraine."

From Darbyshire (2002):

"In some situations *A. caroliniana* has been a serious weed disrupting aquatic ecology and interfering with human activities (Thieret 1980)."

"[…] it was abundant, often completely swathing the shore for considerable distances. Mats became sparser at the upstream and downstream extremes of the distribution and where the river shores were exposed to strong currents or waves. Mats were mostly along shores and becoming thick and continuous in quite bays and around protecting emergent vegetation such as Cattails (*Typha latifolia* L.) or Reed Canary Grass (*Phalaris arundinacea* L.)."

From Protopopova et al. (2006):

"[…] *A. caroliniana* [in Ukraine] promotes bogging and by that not only interferes with processes of development and life activity of free-floating species, but also adversely affects nearly all aquatic organisms (Dubyna & Protopopova, 1980; Shelyag-Sosonko & Dubyna, 1984; Dubyna et al., 2002)."

"In the lower reaches of the Danube, in Danube Biosphere Reserve, the spread of alien water fern species *Azolla filiculoides* and *A. caroliniana* affects conditions of populations of native protected and relict species *Salvinia natans* and *Trapa natans* L., which are listed in the Red Data Book of Ukraine ([Shelyag-Sosonko et al.] 1996)."

4 History of Invasiveness

The History of Invasiveness category for *Azolla caroliniana* is High. *A. caroliniana* has become widespread outside of its native range. Much of this is due to intentional introduction for agricultural, horticultural and ornamental purposes in Africa, Asia, Europe and Hawaii. Negative impacts of introduction have been documented such as forming thick mats which clog waterways and outcompeting native species for light, oxygen, and nutrients, some of which are locally threatened. Additionally, *Azolla caroliniana* is found in the aquarium trade but quantities and duration of trade were not found.

5 Global Distribution

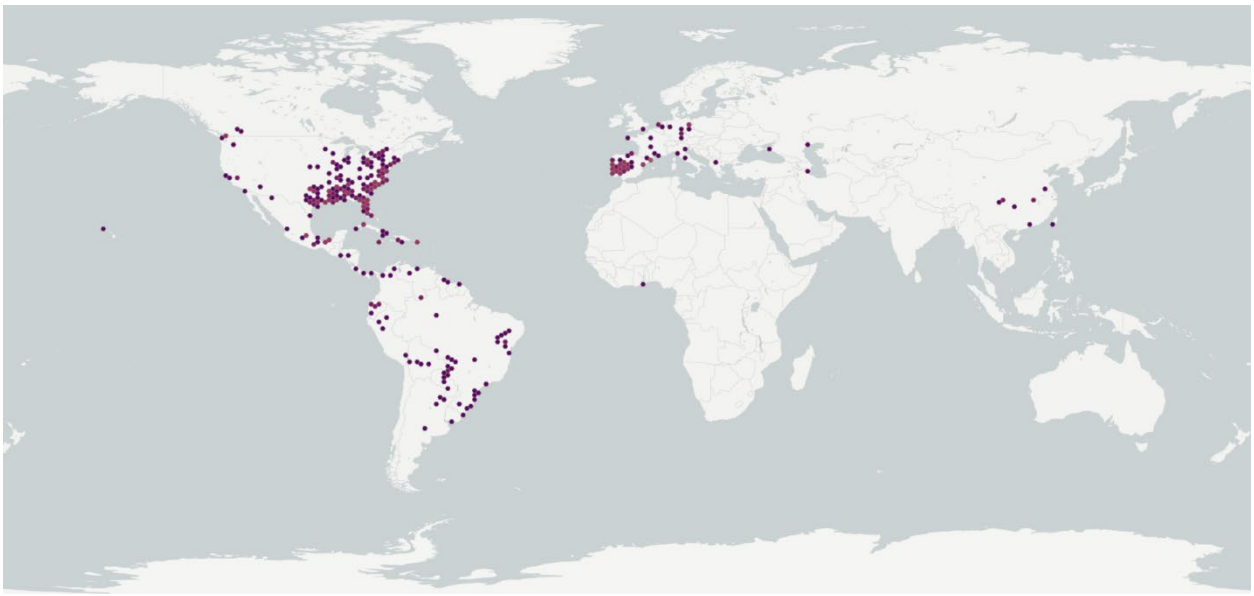

Figure 1. Known global distribution of *Azolla caroliniana*. Observations are reported from the Americas, Europe and portions of eastern Asia. Map from GBIF Secretariat (2021).

Locations in the following areas will not be included in the climate match as they do not represent wild, established populations: Western United States, Ghana, Germany, the Netherlands, Russia, Albania, Azerbaijan, and Taiwan.

Additional locations in Ukraine, provided by Prokopuk (2016), will be used to select source points for the climate match.

6 Distribution Within the United States

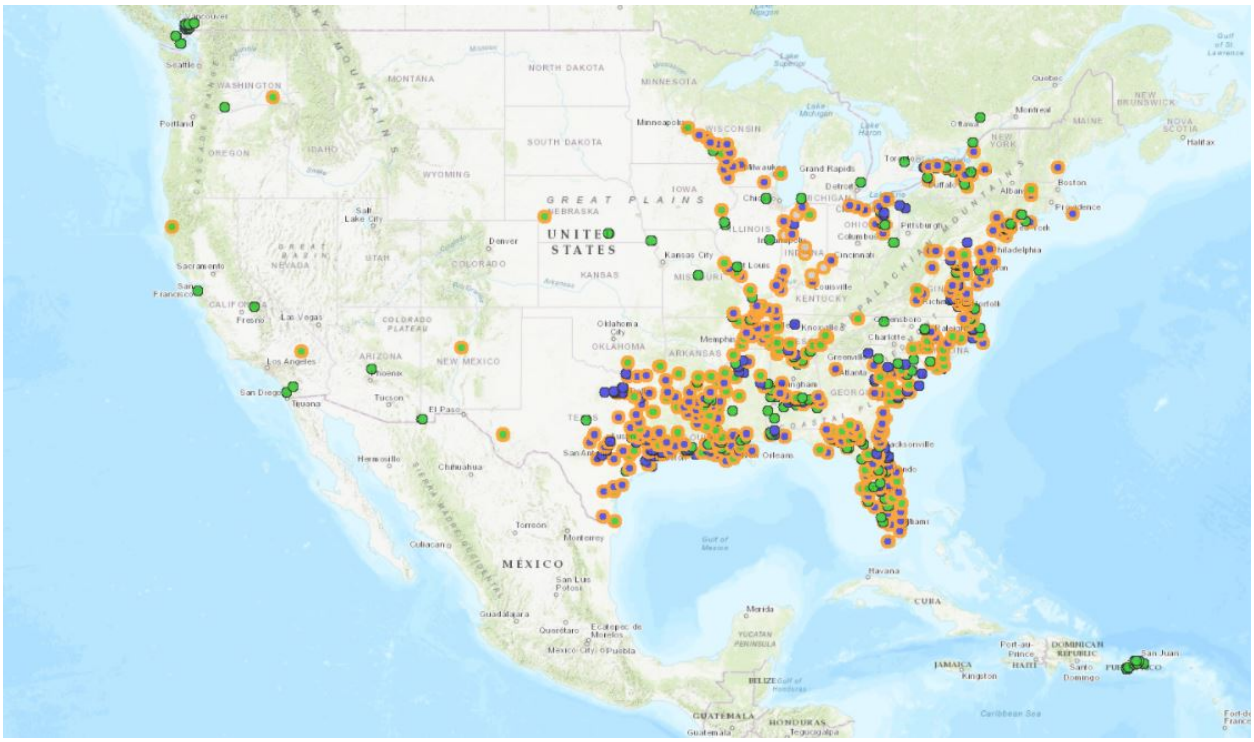

Figure 2. Known distribution of *Azolla caroliniana* in the United States, including Puerto Rico. Map from Bison (2021). Blue dots indicate observation based records, green dots indicate specimens based records, orange rings around the dots indicate centroid records instead of exact coordinates.

Locations in Washington, California, Arizona, and Nebraska will not be included in the climate match as no established populations have been documented in those locations.

Additional locations will be added in the climate match to represent the naturalized populations in Hawaii provided by Imada and Kennedy (2020), and EDDMapS (2021).

7 Climate Matching

Summary of Climate Matching Analysis

The majority of the contiguous United States had a high climate match. The area of highest match is found within the native range of this species in the eastern United States. Additional areas of high match are found along the Pacific Coast and northern Midwest. Small patches of low and medium-low match are found in the West. Medium match is primarily found in the interior West and coastal Northeast. The overall Climate 6 score (Sanders et al. 2018; 16 climate variables; Euclidean distance) for the contiguous United States was 0.791, high. (Scores greater than or equal to 0.103 are classified as high.) All States had high individual Climate 6 scores.

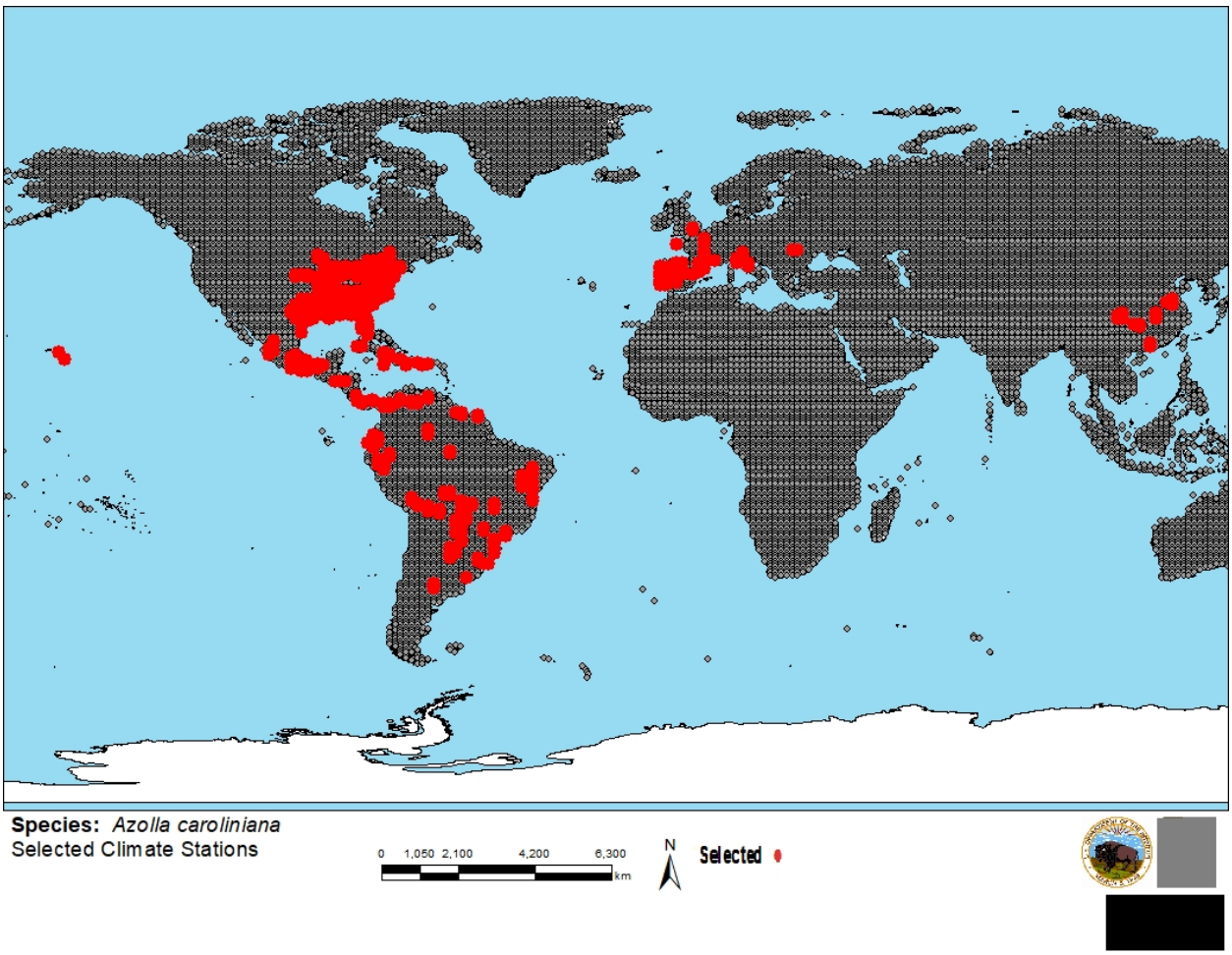

Figure 3. RAMP (Sanders et al. 2018) global source map showing weather stations selected as source locations (red; Canada, United States, Mexico, Cuba, Haiti, Dominican Republic, Jamaica, Puerto Rico, Honduras, El Salvador, Costa Rica, Panama, Colombia, Venezuela, Guyana, French Guiana, Ecuador, Peru, Brazil, Bolivia, Paraguay, Argentina, Uruguay, United Kingdom, Portugal, Spain, France, Italy, Ukraine, China) and non-source locations (gray) for *Azolla caroliniana* climate matching. Source locations from GBIF Secretariat (2021), Prokopuk (2016), Imada and Kennedy (2020), and EDDMapS (2021). Selected source locations are within 100 km of one or more species occurrences, and do not necessarily represent the locations of occurrences themselves.

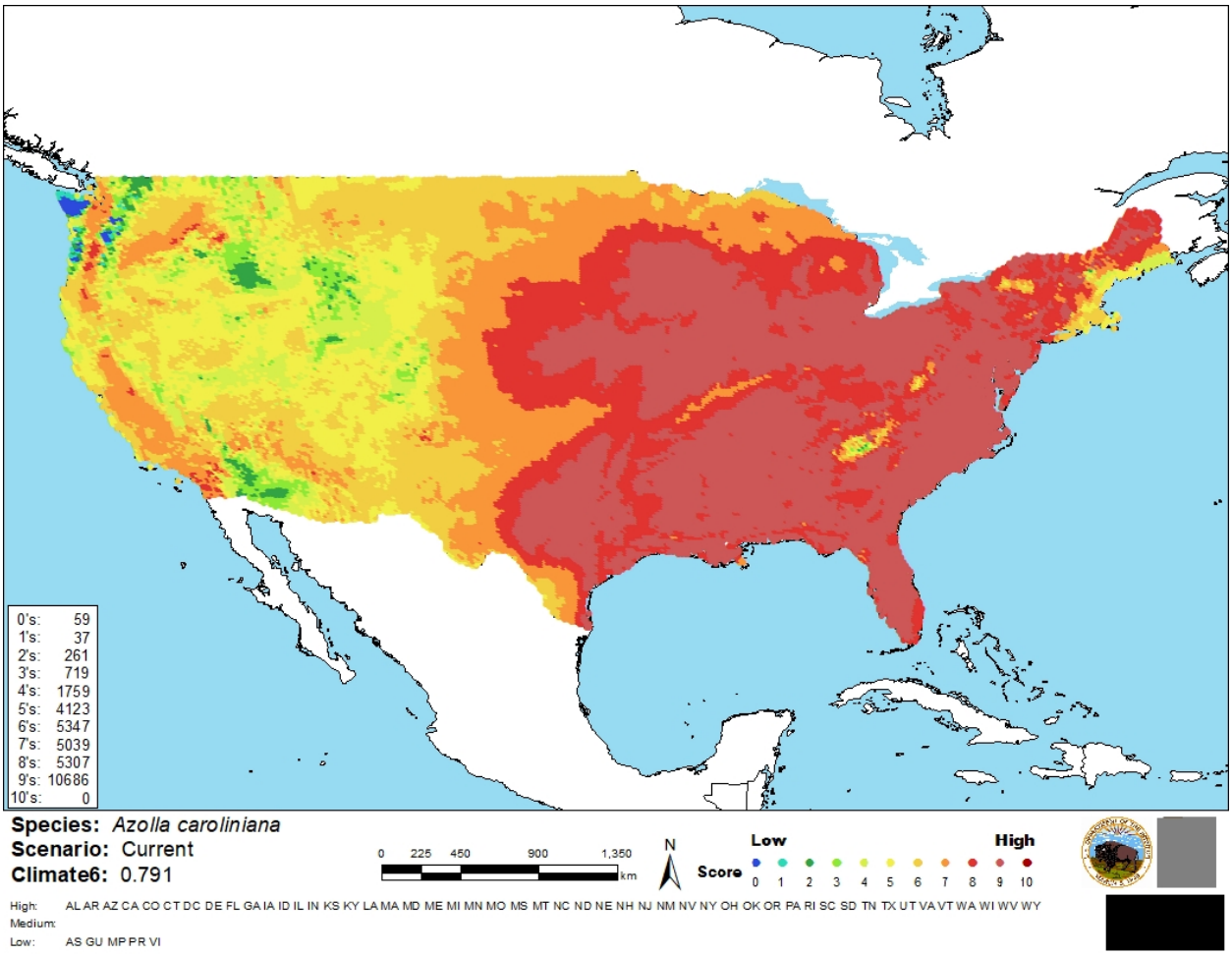

Figure 4. Map of RAMP (Sanders et al. 2018) climate matches for *Azolla caroliniana* in the contiguous United States based on source locations reported by GBIF Secretariat (2021), Prokopuk (2016), Imada and Kennedy (2020), and EDDMapS (2021). Counts of climate match scores are tabulated on the left. 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 Medium. Information is available on the native range, biology and ecology. Uncertainty in the understanding of the species' native range provides confusion in identifying areas of introduction of *A. caroliniana*. While *A. caroliniana* has been reported as

introduced outside of its native range, and information on impacts is available, much of the primary sources for the impact information were not available in English. Due to continually changing taxonomy of the *Azolla* genus and difficulty in distinguishing *A. caroliniana* from some congeners it may be difficult to fully understand the extent of its invasiveness and impacts.

9 Risk Assessment

Summary of Risk to the Contiguous United States

Azolla caroliniana, the Carolina Mosquitofern, is an aquatic plant native to much of the eastern United States, Central and South America, as well as the Caribbean. This species has been introduced to many countries to be used in agriculture as a green fertilizer, for horticultural and ornamental purposes, and pest control. *A. caroliniana* has become established outside of its native range in Hawaii, Spain, France, Italy, Ukraine, and China. Some information on impacts of introduction were found including competition with native plants, some of which are locally threatened, interference with recreational activities, and it can change water quality and hydrology. Information on documented negative impacts of introduction was available, therefore the history of invasiveness is High. The climate match for the contiguous United States was High, with high match found throughout the native range in the eastern States, with additional areas of high match found in the West and Midwest. The certainty of this assessment is Medium, due to the taxonomic confusion of the *Azolla* genus and primary sources for the impacts of introduction not being available in English. The overall risk assessment category for *Azolla caroliniana* is High.

Assessment Elements

- **History of Invasiveness (Sec. 4): High**
- **Overall Climate Match Category (Sec. 7): High**
- **Certainty of Assessment (Sec. 8): Medium**
- **Remarks/Important additional information:** No additional remarks.
- **Overall Risk Assessment Category: High**

10 Literature Cited

Note: The following references were accessed for this ERSS. References cited within quoted text but not accessed are included below in Section 11.

Brunton DF, Bickerton HJ. 2018. New records for eastern mosquito fern (*Azolla cristata*, Salviniaceae) in Canada. Canadian Field-Naturalist 132(4):350–359.

Cohn J, Renlund RN. 1953. Notes on *Azolla caroliniana*. American Fern Journal 43:7–11.

Dana ED, Sanz-Elorza M, Sobrino E. 2021. Plant Invaders in Spain [Check-list] "The unwanted citizens." Available: https://w3.ual.es/personal/edana/alienplants/checklist.pdf (March 2021).

Darbyshire SJ. 2002. Ephemeral occurrence of the mosquito fern, *Azolla caroliniana,* at Ottawa, Ontario. Canadian Field-Naturalist 116(3):441–445.

Dhir B. 2019. Removal of pharmaceuticals and personal care products by aquatic plants. Pages 321–340 in Prasad MN, Vithanage M, Kapley A. Pharmaceuticals and personal care products: waste management and treatment technology. Butterworth-Heinemann.

Dragonfly Aquatics. 2021. *Azolla* (*Azolla caroliniana*) floating pond plant. Available: https://www.dragonflyaquatics.com/shop/sale-items/azolla-azolla-caroliniana-floating-pond-plant/ (March 2021).

EDDMapS. 2021. Early Detection and Distribution Mapping System. Tifton: University of Georgia, Center for Invasive Species and Ecosystem Health. Available: https://www.eddmaps.org/distribution/uscounty.cfm?sub=23047&map=density (July 2021).

eFloras. 2008. *Azolla caroliniana*. Flora of North America. Available: http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200005217 (July 2021).

Evrard C, Van Hove C. 2004. Taxonomy of the American *Azolla* species: a critical review. Systematics and Geography of Plants 74(2):301–318.

GBIF Secretariat. 2021. GBIF backbone taxonomy: *Azolla caroliniana* Willd. Copenhagen: Global Biodiversity Information Facility. Available: https://www.gbif.org/species/2650105 (March 2021).

GRIN-Global. 2021. Taxon: *Azolla caroliniana* Willd. U.S. Department of Agriculture. Available: https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomydetail?id=102986 (March 2021).

Illinois [DNR] Department of Natural Resources. 2015. Aquatic life approved species list. Springfield: Illinois Department of Natural Resources. Available: https://www.ifishillinois.org/programs/aquaculture/aquatic_approved_species.pdf (November 2020).

Imada CT, Kennedy BH. 2020. New Hawaiian plant records from Herbarium Pacificum for 2019. Bishop Museum Occasional Papers 129:67–92.

Lumpkin TA, Plucknett DL. 1980. *Azolla*: Botany, physiology and use as a green manure. Economic Botany 34(2):111–153.

Madeira PT, Dray FA, Tipping PW. 2019. Molecular identification of Azolla in the Yangtze River watershed, China. Aquatic Botany 159.

Madeira PT, Center TD, Coetzee JA, Pemberton RW, Purcell MF, Hill MP. 2013. Identity and origins of introduced and native *Azolla* species in Florida. Aquatic Botany 111:9–15.

Metzgar JS, Schneider H, Pryer KM. 2007. Phylogeny and divergence time estimates for the fern genus *Azolla* (Salviniaceae). International Journal of Plant Sciences 168(7):1045–1053.

NatureServe. 2021. NatureServe Explorer: an online encyclopedia of life, version 7.1. Arlington, Virginia: NatureServe. Available: https://explorer.natureserve.org/ (March 2021).

Palmer WE. 1913. *Azolla* in Norfolk. Nature 92:233.

Pandey VC. 2012. Phytoremediation of heavy metals from fly ash pond by *Azolla caroliniana*. Ecotoxicology and Environmental Safety 82:8–12.

Pereira AL, Martins M, Oliveira MM, Carrapico F. 2011. Morphological and genetic diversity of the family Azollaceae inferred form vegetative characters and RAPD markers. Plant Systematics and Ecology 297:213–226.

Pereira AL, Teixeira G, Sevinate-Pinto I, Antunes T, Carrapico F. 2001. Taxonomic re-evaluation of the *Azolla* genus in Portugal. Plant Biosystems 135(3):285–294.

Pond Megastore. 2021. *Azolla caroliniana,* fairy moss. Available: https://pondmegastore.com/products/azolla-caroliniana (March 2021).

Pond Plants Online. 2021. *Azolla | Azolla caroliniana*. Available: https://www.pondplantsonline.com/products/azolla-azolla-caroliniana?variant=8042220165 (March 2021)

Prokopuk MS. 2016. New record of *Azolla caroliniana* in water bodies of Kiev. Hydrobiological Journal 52(2):56–58.

Protopopova VV, Shevera MV, Mosyakin SL. 2006. Deliberate and unintentional introduction of invasive weeds: A case study of the alien flora of Ukraine. Euphytica 148:17–33.

Roy HE, Peyton J, Aldridge D, Bantock T, Blackburn TM, Britton R, Clark P, Cook E. Dehnen-Schmutz K, Dines T, Dobson M, Edwards F, Harrower C, Harvey M, Minchin D, Noble DG, Parrott D, Pocock MJO, Preston CD, Roy S, Salisbury A, Schonrogge K, Sewell J, Shaw RH, Stebbing P, Stewart AJA, Walker KJ. 2014. Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. Global Change Biology 20:3859–3871.

Sanders S, Castiglione C, Hoff M. 2018. Risk Assessment Mapping Program: RAMP. Version 3.1. U.S. Fish and Wildlife Service.

Shi DJ, Hall DO. 1988. The *Azolla-Anabaena* association: Historical perspective, symbiosis and energy metabolism. Botanical Review 54(4):353–386.

Svenson HK. 1944. The New World species of *Azolla*. American Fern Journal 34(3):69–84.

Thiebaut G. 2007. Invasion success of non-indigenous aquatic and semi-aquatic plants in their native and introduced ranges. A comparison between their invasiveness in North America and France. Biological Invasions 9:1–12.

USDA, NRCS. 2021. *Azolla caroliniana* Willd. Carolina mosquitofern. The PLANTS database. Greensboro, North Carolina: National Plant Data Team. Available: https://plants.usda.gov/core/profile?symbol=AZCA (March 2021).

Wagner GM. 1997. *Azolla*: A review of its biology and utilization. The Botanical Review 63:1–26.

Walmart. 2021. *Azolla caroliniana* portion in 4 oz container - floating live plants for aquariums or ponds. Available: https://www.walmart.com/ip/Azolla-caroliniana-portion-in-4-oz-container-Floating-Live-Plants-for-or-Aquariums-or-Ponds/111426397 (March 2021).

[WFO] World Flora Online, 2021. *Azolla caroliniana* Willd. World Flora Online – a project of the World Flora Online Consortium. Available: http://www.worldfloraonline.org/taxon/wfo-0001109619 (March 2021).

11 Literature Cited in Quoted Material

Note: The following references are cited within quoted text within this ERSS, but were not accessed for its preparation. They are included here to provide the reader with more information.

Azan S, Bardecki M, Laursen AE. 2015. Invasive aquatic plants and the aquarium and ornamental pond industries: a risk assessment for Southern Ontario (Canada).Weed Research 55: 249–259.

Cody WJ, Schueler FW. 1988. A second record of the mosquito fern, *Azolla caroliniana*, in Ontario. Canadian Field-Naturalist 102:545–546.

Darbyshire SJ, Thomson S. 2004. Mosquito fern in Ottawa. Trail & Landscape 38:21–23.

Dubyna DV, Yu R. Shelyag-Sosonko OI, Zhmud et al. 2002. Danube Biosphere Reserve: the plant cover. Kyiv, Ukraine: Phytosociocentre Press. (In Ukrainian.) [Source material did not give full list of authors.]

Dubyna DV, Protopopova VV. 1980. New for the USSR water ferns from the family Azollaceae. Ukrayins'k Bot Zhurn 37(5):20–32. (In Ukrainian.)

Dunham DG. 1986. Taxonomic re-evaluation and species recognition of *Azolla* Lam., with particular reference to section *Azolla*. Doctoral disserataion. Portsmouth, England: Portsmouth Polytechnic.

Dunham DG, Fowler K. 1987. Taxonomy and species recognition in *Azolla* Lam. Pages 7–16 in *Azolla* utilization. Manila, Philippines: International Rice Research Institute.

Fernald ML. 1950. Gray's manual of botany. A handbook of the flowering plants and ferns of the central and northeastern United States and adjacent Canada. 8th edition. New York: American Book Company.

Godfrey RK, Reinert GW, Hook RD. 1961. Observations on microsporocarpic material of *Azolla caroliniana*. American Fern Journal 51:89–92.

Hechler WD, Dawson JO. 1995. Factors affecting nitrogen fixation in *Azolla caroliniana*. Transactions of the Illinois State Academy of Science 88:97–107.

Lellinger DB. 1985. A field manual of the ferns & fern-allies of the United States and Canada. Washington D.C.: Smithsonian Institution Press.

Li S. 1984. *Azolla* in the paddy fields of eastern China. Pages 169–178 in Organic Matter and Rice. Los Banos, Phillipines: International Rice Research Institute.

Li ZX, Zu SX, Mao MF, Lumpkin TA. 1982. Study on the utilization of eight *Azolla* species in agriculture. Scientia Agriculturae Sinica 1:19–28. (In Chinese).

Lumpkin TA. 1993. Azollaceae Wettstein. *Azolla* family. Flora of North America north of Mexico. New York: Oxford University Press.

Lumpkin TA. 1987a. Collection, maintenance, and cultivation of *Azolla*. Pages 55–94 in Elan GH, editor. Symbiotic nitrogen fixation technology. New York: Marcel Dekker.

Maiti SK, Jaiswal S. 2008. Bioaccumulation and translocation of metals in the natural vegetation growing on fly ash lagoons: a field study from Santaldih thermal power plant, West Bengal India. Environmental Monitoring and Assessment 136:355–370.

Mettenius G. 1867. Filicinae-Rhizocarpae-Salviniaceae. Pages 51–55 in Kotschy T, Peyritsch J. Plantae Tinneanae. [Source material did not give full citation for this reference.]

Moore AW. 1969. *Azolla*: biology and agronomic significance. Botanical Review 35:17–34.

Muenscher WC. 1944. Aquatic plants of the United States. Ithaca, New York. [Source material did not give full citation for this reference.]

Perkins SK, Peters GA, Lumpkin TA, Calvert HE. 1985. Scanning electron microscopy of perine architecture as a taxonomic tool in the genus *Azolla* Lamarck. Scanning Electron Microscopy 4:1719–1734.

Reid JD, Plunkett GM, Peters GA. 2006. Phylogenetic relationships in the heterosporous fern genus *Azolla* (Azollaceae) based on DNA sequence data from three noncoding regions. International Journal of Plant Sciences 167(3):529–538.

Shelyag-Sosonko, Yu.R, editors, et al. 1996. Red Data Book of Ukraine. Kyiv: Ukrainian Encyclopedia Press. (In Ukrainian.) [Source material did not give full list of authors for this reference.]

Shelyag-Sosonko YR, Dubyna DV. 1984. Dunayskie Plavni State Reserve. Kyiv, Ukraine: Naukova Dumka Press. (In Russian.)

Small JK. 1935. Ferns of the vicinity of New York. Being description of the fern-plants growing naturally withing a hundred miles of Manhattan Island. Lancaster, Pennsylvania: The Science Press.

Strasburger E. 1873. Ueber *Azolla*. Jena, Hermann Dabis. [Source material did not give full list of authors for this reference.]

Saunders RMK, Fowler K. 1993. The supraspecific taxonomy and evolution of the fern genus *Azolla* (Azollaceae). Plant Systematic and Evolution 184:175–193.

Taylor N. 1915. Flora of the vicinity of New York. Memoirs of the New York Botanical Garden 5.

Thieret JW. 1980. Louisiana ferns and fern allies. Lafayette, Louisiana: Lafayette Natural History Museum.

Tuan DT, Thuyet RQ. 1979. Use of *Azolla* in rice production in Vietnam. Pages 395-405 in International Rice Research Institute, nitrogen and rice. Los Banos, Philippines: International Rice Research Institute.

Watanabe I. 1982. *Azolla-Anabaena* symbiosis--Its physiology and use in tropical agriculture. Pages 169–185 in Dommergues YR, Diem GH, editors. Microbiology of tropical soils and plant productivity. The Hague, the Netherlands: Martinus Nijhoff/W. Junk.

Watanabe I, Liu CC. 1992. Improving nitrogen-fixing systems and integrating them into sustainable rice farming. Plant Soil 141:57–67.

Wunderlin RP, Hansen BF. 2003. Guide to the vascular plants of Florida. 2nd edition. Gainesville: University Press of Florida.

Zimmerman JW, Lumplin TA, Watanabe I. 1989. Classification of *Azolla* spp., section *Azolla*. Euphytica 43:223–232.

Zimmerman WJ, Watanabe I, Ventura T, Payawal P, Lumpkin TA. 1991. Aspects of the genetic and botanical status of Neotropical *Azolla* species. New Phytologist 119:561–566.