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Dominique Jeton Groffman

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Leveraging Herbarium Records for a Niche Analysis of Ripariosida hermaphrodita (L.) Weakley & B. D. Poind. (Virginia mallow)

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TITLE: Leveraging Herbarium Records for a Niche Analysis of *Ripariosida hermaphrodita* (L.) Weakley & B. D. Poind. (Virginia mallow)

AUTHOR: Dominique Jeton Groffman
The Eli Kirk Price Endowed Flora of Pennsylvania Intern

DATE: June 5, 2020

ABSTRACT:

Weedy and rare plant species that thrive in disturbed habitats, but with restricted ranges, present a unique field of ecological research. Native to the Great Lakes drainage and the Ohio, Kanawha, Susquehanna, and Potomac river basins, Virginia mallow, *Ripariosida hermaphrodita* (L.) Weakley & D.B. Poind. (Malvaceae), is one such pioneer species that, despite its weedy habit, is rare throughout its range from Indiana west to Pennsylvania and from Tennessee north to Ontario, Canada. This rhizomatous perennial can reach around four meters in one growing season and is cultivated as a biofuel crop in Eastern Europe. Virginia mallow yields perfect, white, terminal cymose panicle inflorescences for several months in late summer, maturing into capsule fruits. Recent molecular phylogenetic work has motivated the revision of Virginia mallow from *Sida hermaphrodita* (L.) Rusby to the newly monotypic *Ripariosida*. This work attempts to clarify the known extent of Virginia mallow through an ArcGIS niche analysis using georeferenced herbarium specimens collected over the last century and a half. 248 wild collected and unduplicated records were assigned levels of geographic uncertainty from none to most: 51 specimens could not be georeferenced (Level 0), 83 specimens were georeferenced with a general locality statement (Level 1), 106 specimens were georeferenced with a precise locality statement (Level 2) and 7 specimens have GPS coordinates taken at the time of collection (Level 3). Analyses

were completed on subsets of specimens (Level 3, Level 2-3 and Level 1-3) to understand the effects of geographic uncertainty on results. Large-scale, latitudinal climate variables were accompanied by finer-scale variables including soil and topography, land use, and proximity to nearest water and transportation to define the range of Virginia mallow. Occurring most frequently in flood plains, stream terraces, slopes, till plains, (rail)road cuts and urban land, Virginia mallow favors disturbance, both natural and mechanical, and is tolerant of both drought and periodic inundation. The most common soil type found for Virginia mallow was loam, of various kinds, followed by 'urban land.' *Ripariosida hermaphrodita* also clearly prefers high sun exposure, occurring on predominantly south/southeastern exposures and absent from northern exposures. Its tendency to occupy disturbed and at least intermittently wet areas was underscored by a mean distance to nearest road/railroad of 83 m and to nearest water of 165 m for Level 1-3 specimens. Our findings provide the foundation for further research into the population genetics and demography on the of this rare yet weedy, native perennial through comprehensive field work.

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I. Introduction

Plant species that thrive in high disturbance regimes occupy a uniquely sensitive and conspicuous niche in today's increasingly industrialized and developed world. Such plants may be qualified as "weeds", proliferating on the margins on developed land, such as roadsides, powerline rights-of-way, and abandoned parking lots. Despite their high visibility and tenacity, these "weeds" may be vulnerable as developed areas are often unprotected and subject to ongoing land-use change. One such species is the native perennial *Ripariosida hermaphrodita* (L.) Weakley & D.B. Poind., commonly known as Virginia mallow or Virginia fanpetals (Figure 1). Virginia mallow is notable in that, while weedy – fast-growing, preferring full sun, and occupying disturbed soil – this native mallow is rare throughout its range in the Great Lakes drainage and Ohio, Kanawha, Susquehanna and Potomac river basins. The cause of its rarity has been under debate for nearly half of a century, targeting soil specificity, low germination potential, the threat of invasive species and shade intolerance (Thomas 1979, Spooner et al. 1985, Bickerton 2011). However, there is consensus that habitat destruction may be a primary limiting factor to the survival of *Ripariosida hermaphrodita* given its natural riparian habitat (Environment Canada 2015). Due to the fertile and flat soils of waterways and their role in transportation, continued habitat loss through practices including stream channelization and modern agriculture threaten the survival of many riparian species, including Virginia mallow (Leuven and Poudevigne 2002). However, *Ripariosdia hermaphrodita* has occupied disturbed regions other than the increasingly rare, inundated streambeds it once thrived in. For at least a century and a half, a Thomas C. Porter specimen from 1863 was along a canal at Safe Harbor on the Susquehanna River, Virginia mallow has been collected along ruderal land including the margins of cultivated fields, roadside embankments and manmade waterways. Given the seemingly contradictory rare yet pioneer existence of Virginia mallow, this native perennial presents a heuristic opportunity to leverage the tools of Aeronautical Reconaissance Coverage Geographic Information System (ArcGIS) (Esri) to complete a niche analysis to better understand the limits of its distribution and range.

Figure 1. *Ripariosida hermaphrodita* (Virginia mallow) inflorescence and terminal leaves.

Known habitat of Ripariosida hermaphrodita *(L.) Weakley & D. B. Poind.*

The native range of the rare and weedy Virginia mallow, *Ripariosida hermaphrodita* has been debated and nuanced for nearly half of a century (Iltis 1963, Thomas 1979, Spooner et al. 1985, Environment Canada 2015). Further complicating the disagreement surrounding the native locality of Virginia mallow, misidentification of *Ripariosida hermaphrodita* with the previously congeneric *Napaea dioica* L. was not uncommon (Iltis 1963). Analysis of herbaria specimens and field work in the northeast United States and southern Ontario, Canada where *Ripariosida hermaphrodita* is found, alongside experimental research in Russia, Poland and Ukraine where Virginia mallow is an attractive biofuel source (Matyka and Kús 2017, Chudzik et al. 2010, Nahm and Morhart 2018, Kujawski et al. 1997) aid our understanding of its known occurrence history and the limiting factors of growth. While the data supporting this research does not greatly differ from prior studies of Virginia mallow distribution, founded on digitized herbaria specimens from the past century and a half, the methods of mapping and niche analysis take advantage of contemporary datasets and programs, namely ArcGIS and the Maximum Entropy species distribution modelling software Maxent V. 3.4.1 (Phillips et al. 2017)

1. Soil and topography

In the seminal 1979 study from L. K. Thomas Jr. on the distribution and ecology of *Ripariosida hermaphrodita*, field observations and soil samples from four sites were analyzed. Thomas found all soils to be loose and sandy, two samples loamy sand, and all with low organic matter content. He noted the great range in soluble salt available to the Virginia mallow rooted in these soils, postulating this may be a "factor in their survival as well as their rarity if they can tolerate high nutrient concentrations when many other species cannot" (57). Further, Thomas notes the aeration in the soil samples studied provided for by their rocky particle size and proposes decline in *Ripariosida hermaphrodita* populations may be due to soil compaction, such as on Theodore Roosevelt Island in Washington, D.C. where Virginia mallow is presumed extirpated (NatureServe Explorer 2.0). In a later study with emphasis on the populations along the Ohio and Kanawha Rivers, Spooner et al. (1985) note the predominance of Virginia mallow along disturbed and fill soils of railroad rights-of-way and roadsides. Spooner et al. also cite two soil series associated with a majority of known natural populations of *Ripariosida hermaphrodita*, Sloan and Wheeling soils.

The Sloan soil series is distinguished by its fine-loamy and poorly drained soil that are formed in alluvium and occur on flood plains and streamside depressions formed from glaciation with slopes of 0-2% (Soil Survey Staff, USDA Soil Series Descriptions). Wheeling soils are also fine-loamy, yet well-drained and formed in alluvium occurring on stream terraces and in valleys. With these findings in mind, Spooner et al. claim “the physical-chemical properties of the soils are not a factor limiting the geographical distribution” of Virginia mallow.

More recent evidence from Matyka and Kús (2017) attempts to make sense of these conflicting findings in a long-term experimental approach to clarify the relationship between soil quality and yield of Virginia mallow. Microplots of *Ripariosida hermaphrodita* were grown in six, distinct soils supported by the local bedrock over a seven-year period during which climactic variables were also recorded. The researchers found the highest yields, calculated as number of shoots, shoot diameter, and plant height, obtained in brown soil generated from loess (loosely compacted, windblown sediment common to the Midwestern United States) (C), and in black soil building from medium loam on heavy loam (F). The lowest yields were found in brown soil building from light sandy loam on poor sandy loam (B), in brown soil building from light loam on heavy loam (E), as well as the same black soil that bore high-yielding Virginia mallow (F). Matyka and Kús declare *Ripariosida hermaphrodita* performs poorly in poor soils (B) and in good quality yet heavy soils (E, F), although their results demonstrate Virginia mallow may also grow well in certain good quality, heavy soil (F). When accounting for climactic variability, Matyka and Kús recorded the lowest yields in the years with the highest relative temperatures and lowest relative rainfall. While the researchers do not provide yearly records, only averages over the eight-year period, achieving both low and high yields in the same good quality, heavy soil (F) may have been due to unfavorable climactic conditions. This last finding underscores how factors limiting the growth of Virginia mallow are interdependent and often difficult to distill from one another when performing a niche analysis.

2. Climate

No literature available on the distribution of *Ripariosida hermaphrodita* stipulates the climactic variables limiting its potential range. Rather, topography, hydrology, and the surrounding vegetation community are cited as more clear indicators for the niche occupied by Virginia mallow (Environment Canada 2015). Our niche analysis includes nineteen Bioclimactic variables from Worldclim (Fick and Hijmans 2017), such as temperature annual range and precipitation of the wettest and driest months. While these variables may indicate a latitudinal range in which Virginia mallow might occur, we do not anticipate these variables alone will elucidate why *Ripariosida hermaphrodita* occurs where it does at a much finer scale.

3. Hydrology and disturbance

As hinted in the binomial for Virginia mallow, this herbaceous perennial historically thrived along riverine terraces and floodplains. Seeds of *Ripariosida hermaphrodita* are readily scarified by flowing sediment, increasing the chance of successful germination (Spooner et al. 1985). An anatomical investigation of the ovule development of Virginia mallow found one third of the ovules failed to develop beyond the juvenile stage (Chudzik et al. 2010). It may be that Virginia mallow, which flowers for several months of the late summer, has a high ratio of flowers that fail to mature to fruit, and riverine habitats increasing seed germination potential through scarification are necessary for the success of this species.

During his review of the populations of *Ripariosida hermaphrodita* occurring in the Potomac and Susquehanna watersheds, Thomas (1979) found all populations, with two exceptions, closely associated with a flowing stream. The author further acknowledges Virginia mallow's observed preference for disturbed habitats as a pioneer species. Spooner et al. (1985) also cites the riverine terrace and floodplain habitat of Virginia mallow populations “at the edges of woods near streams and rivers” (218). However, the authors’ review of the Kanawha and Ohio rivers emphasizes most known populations instead occur in “sunny, moist, disturbed situations along roadsides and railroad rights-of-way” (218). Apparently, both mechanical and natural disturbance provide favorable habitat for *Ripariosida hermaphrodita.* For our niche analysis, we calculated the proximity of each georeferenced point to recognized bodies of water and to roads, railroads and trails to provide quantitative support for the anecdotal evidence that such correlations exists.

4. Shade tolerance

Part and parcel to the disturbance regime often associated with stands of Virginia mallow is the availability of high sunlight. All populations reviewed by Thomas (1979) in the Susquehanna and Potomac watersheds were partially shaded or without shade entirely. The critical habitat of Virginia mallow identified by the Canadian government in southern Ontario is distinguished by full sun to partial shade (Environment Canada 2015). Although this herbaceous perennial exhibits behavior of a pioneer species, preferring low shade environments with disturbed, loose soils, *Ripariosida hermaphrodita* is also notably uncommon wherever it occurs (Spooner et al. 1985, COSEWIC 2010, Environment Canada 2015). This may be due to out-competition by associated taxa that are more shade tolerant following the early stages of succession. For instance, rights-of-ways and roadsides that are regularly mowed encourage *Ripariosida hermaphrodita* survival, while populations in abandoned areas left to succession may disappear over time (Chris Frye, personal communication).

Conservation status

Globally, *Ripariosida hermaphrodita* is ranked as Vulnerable (G3), while Vulnerable (N3) in the United States and Critically Imperiled (N1) in Canada (NatureServe Explorer 2.0). Among the states and territories where *Ripariosida hermaphrodita* occurs, it is Presumed Extirpated (SX) in Washington, D.C., Possibly Extirpated (SH) in Tennessee, Critically Imperiled (S1) in Indiana, Maryland, Virginia and Ontario, Canada, Imperiled (S2) in Pennsylvania, Imperiled/Vulnerable (S2S3) in Kentucky and Vulnerable (S3) in Ohio and West Virginia. Michigan and Massachusetts have not yet been ranked by The Nature Conservancy's NatureServe platform.

Efforts to protect critical habit of Virginia mallow are limited to southern Ontario, where Virginia mallow occurs at only two known sites; in the County of Haldimand and within the Regional Municipality of Niagara, together comprising 120 square acres (Bickerton 2011). According to this 2011 Recovery Strategy, the population in Haldimand County is within a managed conservation area, while the population in Niagara occurs along a gas pipeline corridor. The Canadian government has released periodic reviews on the status of *Ripariosida hermaphrodita* since 2010 given its national status as Critically Imperiled (N1). Per the Species At Risk Act, in which Virginia mallow is listed, the Canadian government has described and identified the critical habitat of Virginia mallow in Ontario (Environment Canada 2015). Their findings corroborate the work completed elsewhere in the Great Lakes drainage and the Ohio, Kanawha, Susquehanna and Potomac watersheds (Spooner et al. 1985, Thomas 1979). Following the Ecological Land Classification rubric, the biophysical attributes of the critical habitat of Virginia mallow belong to the Forb Mineral Meadow Marsh, the Cultural Thicket and the Cultural Meadow (Lee et al. 1998). The latter two are ecosites of anthropogenic origins, and all three classifications are associated with riparian areas, bottomlands and floodplains. Environment

Canada (2015) found soil organic content to be medium to high, with variable texture and pH. These habitats are also distinguished by full sun to partial shade. Within this report, the accompanying vegetative taxa of each of the habitats is also listed.

For many riparian species, including Virginia mallow, habitat destruction may be a dominant limiting factor of their distribution (Leuven and Poudevigne 2002). According to Spooner et al. (1985), "no populations of *S. hermaphrodita* are protected from destruction in any part of its geographic range" (222). To investigate this claim, conservation status since 1985 of known Virginia mallow habitat is clarified using the Protected Areas Database from the U.S.G.S. (2018).

Taxonomic Revision

Of interest here, *Ripariosida hermaphrodita* has undergone recent taxonomic revision within the greatly contested, non-monophyletic genus *Sida* L. of the family Malvaceae (Weakley et al. 2017). Previously recognized as *Sida hermaphrodita* (L.) Rusby, recent phylogenetic evidence suggests Virginia mallow was a particular outlier in the genus *Sida*, variable in molecular, geographical and morphological characteristics (Tate et al. 2005, Bayer and Kubitzki 2003, Fuertes et al. 2003, Fryxell 1998). Based on nrDNA internal transcribed spacer (ITS) data, Virginia mallow was placed as sister to *Sida hookeriana* Miquel, a woody shrub native to southwestern Australia, forming an inconclusive, heterogenous clade (Fuertes et al. 2003). According to the most recent molecular phylogeny of the Malvaceae using amplified ITS regions of 121 species from 68 genera, a Bayesian analysis demonstrates *Sida hermaphrodita* to be placed as sister to the *Plagianthus* alliance, primarily woody shrubs and trees endemic to New Zealand and Australia, again as an outlier species (Tate et al. 2005). These findings are consistent with previous results based on geography, chromosome number and reproductive morphology (Bayer and Kubitzki 2003).

Virginia mallow, unlike *Sida hookeriana* and members of *Plagianthus*, is an herbaceous perennial native to northeastern North America. In light of the disparity in geography and habit, alongside recent molecular evidence, there is support for the Weakley et al. (2017) revision of *Sida hermaphrodita* as *Ripariosida hermaprhodita*, the sole member of the monotypic *Ripariosida*, so named because of the historic riverine habitat of Virginia mallow. In light of this taxonomic revision, Virginia mallow further deserves our attention to better understand its distribution and the evolutionary relatedness to other members of Malvaceae.

II. Methods

Herbaria data gathering and cleaning

Digitized specimens of *Ripariosida hermaphrodita* in herbaria across the United States and Canada were located through the Regional Networks of North American Herbaria (SEINet) and the Consortium of Northeastern Herbaria (CNH) database hosted on Symbiota, as well as the New York Botanical Garden's database "sweetgum" and the Smithsonian National Herbarium database. Additional searches in the University of Michigan Herbarium, the Willard Sherman Turrell Herbarium of Miami University, and The Ohio State University Herbarium databases were completed. Specimen records gathered from the above-mentioned searches were then compared with "Preserved Specimen" search results of the Global Biodiversity Information Facility (GBIF) and iDigBio. In all cases, "*Sida hermaphrodita*" was used as the search string, as the revised binomial *Ripariosida hermaphrodita* is not yet widely accepted by herbaria.

Following the compiling of results from available herbaria databases, the records were confirmed for correct identification, particularly vigilant for misidentification with previously congeneric *Napaea dioica* L. Otherwise, Virginia mallow is a fairly distinct species, easily identified by its five-lobed, palmate leaves and perfect, white, axillary inflorescences. Duplicate records were then consolidated, and explicitly cultivated specimens were indicated and removed from the georeferencing process.

Georeferencing

During the georeferencing process, specimens were assigned a rank of geographic uncertainty from none to most (Level 0 – Level 3). Level 0 indicates the specimen cannot be georeferenced because no locality finer than county is provided. These specimens were not incorporated in the niche analysis. Level 1 indicates the specimen may be georeferenced from a general locality statement, but not to its exact location (i.e. a town polygon). Level 2 indicates the specimen may be georeferenced from a precise locality statement with a high degree of certainty and specificity. Level 3 indicates the specimen is accompanied by GPS coordinates taken at the time of collection and confirmed by our georeferencing process. Ranks were assigned to each non-duplicate and wild collected specimen while georeferenced using the GEOLocate standard client web application (Rios and Bart 2010). Practices for georeferencing according to the Mid-Atlantic Megalopolis Georeferencing Guidelines (Mancini et al. 2019), the BioGeomancer Consortium Guide to Best Practices for Georeferencing (Chapman and Wieczorek 2006), and the HerpNET Georeferencing Quick Reference Guide (Wieczorek et al. 2012) were adhered to. While using GEOLocate, localities provided by specimen labels were entered into the "Locality string" box, followed by state or territory and county. Errors in transcription yielding typing mistakes or misspellings were corrected at this stage. For localities not recognized by GEOLocate, manual georeferencing was completed, often making use of the Measure tool. Habitat strings indicating a more precise location (ie: "along creek") were incorporated at this stage to further correct the resulting GPS coordinates. All Level 1-3 specimens were georeferenced using the WGS 84 datum, providing a latitude and longitude (degree), radius of coordinate uncertainty (m), and an error polygon if helpful. Both Jeton Groffman and Skema georeferenced all specimens in GEOLocate to ensure quality control. Notes made during the georeferencing process justifying manual adjustments and interpretations of the locality strings are included in the original table.

ArcGIS niche analysis

Using ArcMap (ESRI, v.10.3.1), the specimens georeferenced to Level 1-3 were uploaded as a .csv file. The data was exported as *x,y* coordinates of latitude and longitude using the WGS 84 datum. Following export as a shapefile, we were able to demarcate populations of *Ripariosida hermaphrodita* using 10 miles as the maximum distance between points within the same population. This process was somewhat arbitrary among the densely populated sections of the Ohio and Kanawha Rivers. Following this initial step, we began the niche analysis with our variables of interest. For each variable, analyses were completed on subsets of the specimens to distill the effect of geographic certainty: Level 3 specimens, Level 2-3 specimens and Level 1-3 specimens.

1. Climate

Climatic data was sourced from the nineteen bioclimatic variables published by Worldclim and averaged from the years 1970-2000 at the finest resolution available of 30s, or 1km (Worldclim v. 2, Fick and Hijmans 2017). Each .tif file was uploaded and the symbology adjusted from “stretched” to “classified”, prompting the creation of a histogram of values. The number of classes was increased to its maximum value (32) for visualization purposes. The tool “Extract values from points” from the ArcGIS Spatial Analyst toolkit was used to generate values that intersected with the coordinate pair from GEOLocate. These values were then exported through the "Table to Excel" tool into nineteen distinct Excel files.

2. Soil and topography

Soil series and aspect data for the United States was sourced from the Soil Survey Geographic Dataset (SSURGO) of the Natural Resources Conservation Service of the U.S. Department of Agriculture (Soil Survey Staff). Twenty-nine relevant polygons of geographic soil map units were downloaded using the SSURGO downloader web client. Once these project files were reformatted for use in ArcMap, georeferenced *Ripariosida hermaphrodita* points were Spatially Joined using the Analysis toolkit in ArcGIS to the localized intersecting polygons. The results were exported through the "Table to Excel" function. Not all attributes were populated for each of the local polygons. Only attributes of interest to this study are included in the results. For the Ontario, Canada population, soil data was acquired from the Soil Survey Complex published by the Ontario Ministry of Agriculture, Food and Rural Affairs and Agriculture and Agri-Food Canada (2020). Since we needed to extract data for only one point, attributes were downloaded manually from the attribute table of the intersecting polygon.

3. Hydrology

The National Hydrography Dataset provided the data for our proximity analysis to the nearest body of water (U.S. Geological Survey, 2019). The Area shapefile was less comprehensive than the Flowline file which includes ephemeral sources of water such as storm drains and provides sufficient detail for the study. Near analyses were run with both datasets, however, using the ArcGIS Analysis toolkit. The input feature was the point coordinates projected into D North American 1983 datum ("LatLong_Final_D_North_American_1983") and the near feature was the

National Hydrography Dataset file (e.g. "NHDFlowline"). Geodesic proximity was calculated in meters to the single, nearest feature for each georeferenced *Ripariosida hermaphrodita* point.

4. Proximity to transportation

To analyze proximity to transportation networks, the U.S.G.S. Transportation Dataset from the National Map was incorporated (2017). Railroad, road segment and trail shapefiles were incorporated in the Near analysis using the ArcGIS Analysis toolkit for all relevant states. Geodesic proximity was calculated in meters to the single nearest feature with input features as the projected point coordinates ("LatLong_Final_D_North_American_1983") and near features as the road, railroad and trail segments for each state. For the southern Ontario point, data was sourced from the Ontario Road Network published by the Ministry of Natural Resources and Forestry (2020). Distance (m) was calculated manually given the presence of a single point in Ontario using the Measure tool.

5. Land use and land cover

Land use data for 2001-2014 was acquired from the National Land Cover Database (NLCD), released by the Multi-Resolution Land Characteristics consortium at a 30m resolution for the continental United States (Yang et al. 2018). The eight adjacent pixels, as well as the intersecting pixel, are included for the analysis, excluding open water. This process was performed manually for the relevant records (n = 25). For temporal accuracy, land use data prior to 2001 was acquired from the Enhanced Historical Land-Use and Land-Cover Data Sets of the U.S.G.S. (GIRAS) (Price et al. 2006) for the years 1970-1989. The Northeast and Southeast Conterminous U.S. datasets were downloaded at varying resolutions making use of the Anderson Level II land classification system (Anderson et al. 1976). Intersecting land use types for each relevant point were again recorded manually by class number corresponding to a given land use type, a dictionary for which can be found here. For the decades included in our dataset prior to 1970, the ISLSCP II Historical Land Cover and Land Use, 1700-1990 dataset was included, published by the Oak Ridge National Laboratory (Goldewijk et al. 2007). However, the half-degree resolution for this dataset does not lend confidence to our findings for this period (1863-1969). For this dataset, as with GIRAS, intersecting land cover type was recorded manually. Both of these historic datasets made use of color indices providing a class number corresponding to a land use type. The dictionary for the ISLSCP II dataset can be found here. The remaining decade, 1990-2000, cannot be accurately represented at this time with the available land use data and includes the southern Ontario, Canada population. For more comprehensive findings, habitat as noted on the herbaria labels was included in our analysis alongside derived land use and land cover data. For the Ontario, Canada population, land cover data was extracted from the Southern Ontario Land Resource Information System (2020) V. 3 published by the Ministry of Natural Resources and Forestry and based on the Ecological Land Classification system (Lee et al. 1998). However, this data is only relevant for the years 2000-2015.

6. Protected areas

Conservation statuses of the locations of known *Ripariosida hermaphrodita* specimens were extracted from the Protected Areas Database 2.0 (PAD-US) (U.S. Geological Survey, 2018). The North Atlantic-Appalachian, South Atlantic Gulf, and Great Lakes regional datasets were uploaded as polygons. For the southern Ontario point, known conservation status of the Taquanyah Conservation Area in Haldimand County was incorporated. Variables of interest for each managed area include manager type, manager name, local designation, state/territory, public access, protection status, date founded and acreage. Additional research, when missing, on acreage, date founded and other metrics were located.

Maxent species distribution modeling

The nineteen bioclimatic variables at 30s resolution downloaded from Worldclim were included in a Maximum Entropy species distribution model known as Maxent (V. 3.4.1) published by the American Museum of Natural History (Phillips et al. 2017). Logarithmic analysis of the bioclimatic variables alongside the georeferenced *Ripariosida hermaphrodita* specimens generated a model of realized and potential niches. All environmental variables were continuous and default parameters for Regularization were kept. Response curves and jackknife outputs were selected. Analyses with and without the three Massachusetts specimens were included given their high likelihood of cultivation and uncertainty as to whether or not these populations are naturalized, as they have not been documented since the turn of the nineteenth century. For the model including the Boston, Massachusetts population, 139 presence records were used for testing, while 137 presence records were used for testing in the model without the Boston, Massachusetts population.

Field reconnaissance

Twelve records in the southeast Ohio River region were visited on April 18th and May 2nd, 2020. Detailed observations were made of the habitat, locality, population size, and descriptions of the specimens. Herbarium-quality collections accompanied by GPS coordinates were made by Jeton Groffman to be donated to the herbarium of Morris Arboretum of the University of Pennsylvania (MOAR). Accompanying photographic and video media will be made available on the Mid Atlantic Herbaria Consortium portal hosted by Symbiota.

III. Results

Georeferencing results

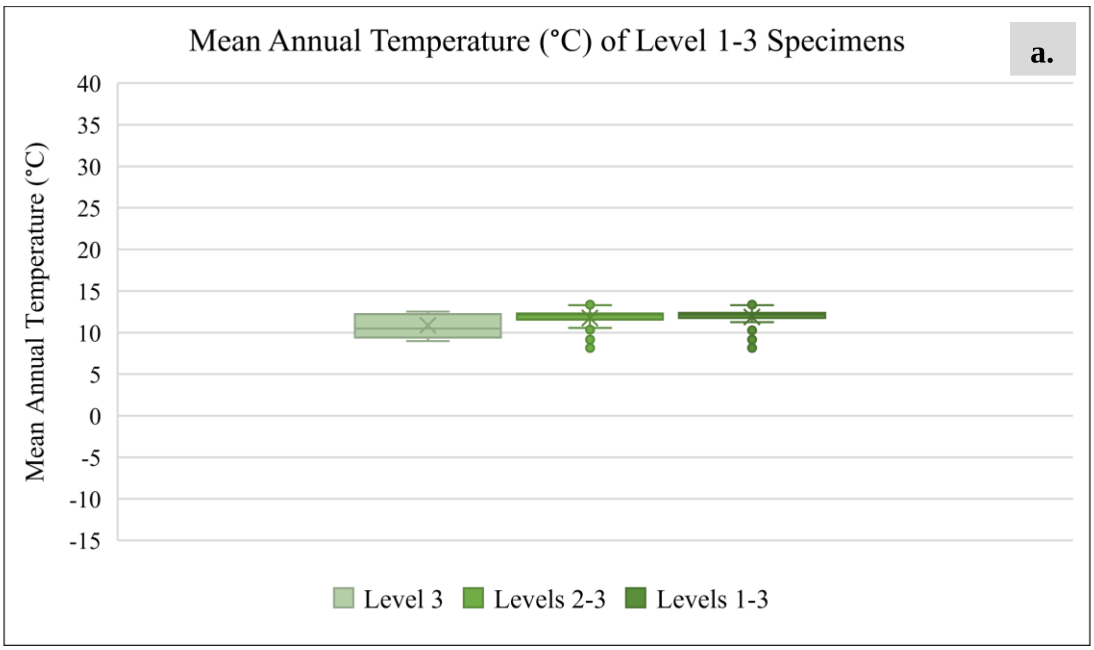
Sixteen cultivated specimens were indicated and removed from the georeferencing process. Seven Level 3 specimens were accompanied by a latitude and longitude made by the collector. 106 specimens were accompanied by a precise locality string to grant the rank of Level 2, while an additional 83 specimens were accompanied by a general locality string to grant the rank of Level 1. In total, 196 specimens were georeferenced to various degrees of certainty and uploaded to ArcMap. Following export as a shapefile, 51 populations were demarcated among the occurrences. This allowed us to visualize groupings of occurrences along distinct watersheds.

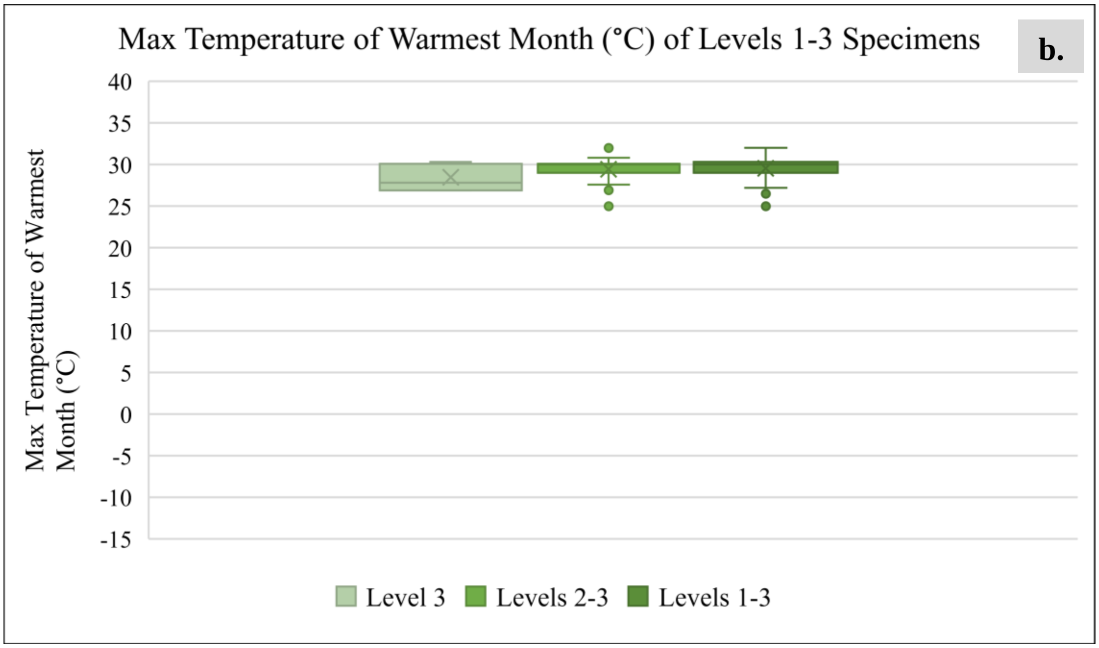
Niche analysis

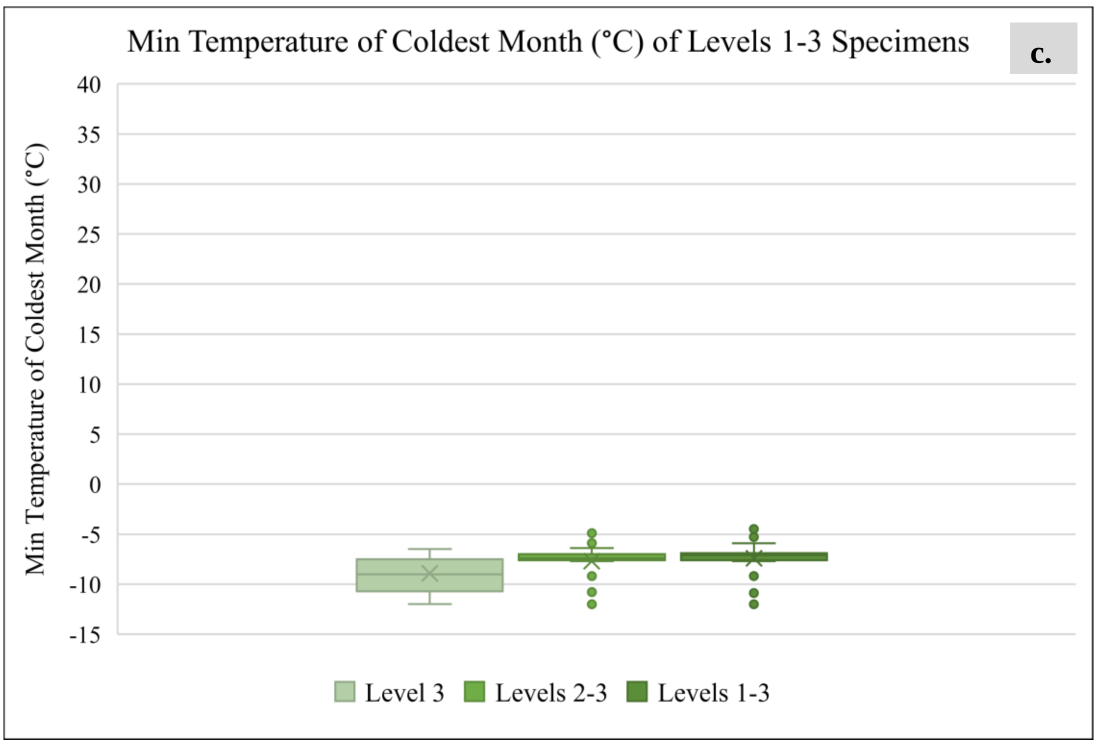
1. Large-scale trends

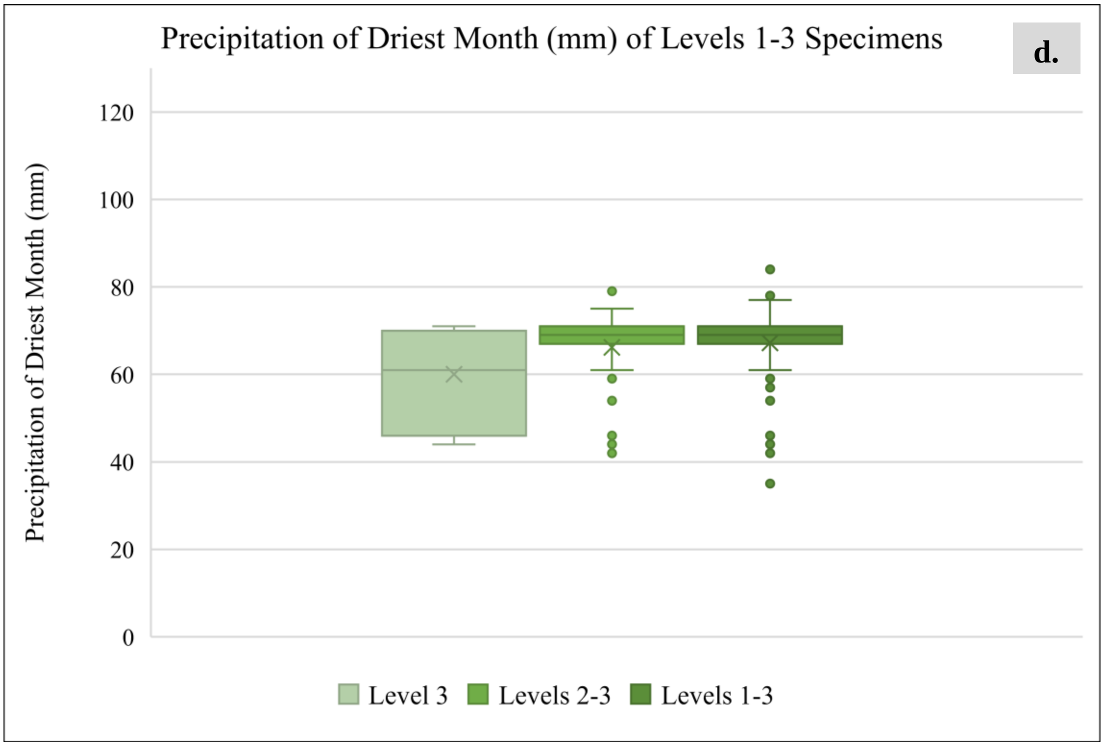
Our many variables differed considerably in resolution as well as scale. Climatic data provided large-scale, latitudinal limitations of the niche of Virginia mallow. For several of the nineteen Worldclim bioclimatic variables, the range of data points was very tight when calculating the Inner Quartile Range (IQR) as well as the Upper – Lower Fence representing all data points with the exception of outliers ($n > 1.5$ x Q3; $n < 1.5$ x Q1). The Boston, Massachusetts population was included for the following box-and-whisker plot analyses. Mean annual temperature, calculated as the sum of the mean of the average monthly temperatures over the course of a year (O'Donnell and Ignizio 2012) has a Upper – Lower Fence value of only ~1.8 °C for Level 1-3 specimens with a median of 12.2 °C (Figure 1a). The Upper – Lower Fence of the Minimum temperature of coldest month for Level 1-3 specimens has a range of only ~2.1 °C around a median of -7.1 °C, while Maximum temperature of warmest month for Level 1-3 specimens ranges by

~3.7 °C around a median of 30 °C (Figure 1b-c). In addition to temperature meas a. certain precipitation variables indicate broad limitations to the niche of Virginia mallow. For instance, Precipitation of driest month for Level 1-3 specimens was tightly correlated around a median of 69 mm, ranging only by ~12 mm, excluding outliers (Figure 1d). Precipitation seasonality, found by calculating the standard deviation monthly precipitation divided by the mean monthly precipitation value, indicates the amount of variation in monthly precipitation over the year (O'Donnell and Ignizio 2012). This variable also had a narrow Upper – Lower Fence of only ~5.0 mm for Level 1-3 specimens (Figure 2e).




Max Temperature of Warmest Month (°C) of Levels 1-3 Specimens
b.
Max Temperature of Warmest Month (°C)
40
35
30
25
20
15
10
5
0
-5
-10
-15
Level 3
Levels 2-3
Levels 1-3


Min Temperature of Coldest Month (°C) of Levels 1-3 Specimens
c.
Min Temperature of Coldest Month (°C)
40
35
30
25
20
15
10
5
0
-5
-10
-15
Level 3
Levels 2-3
Levels 1-3



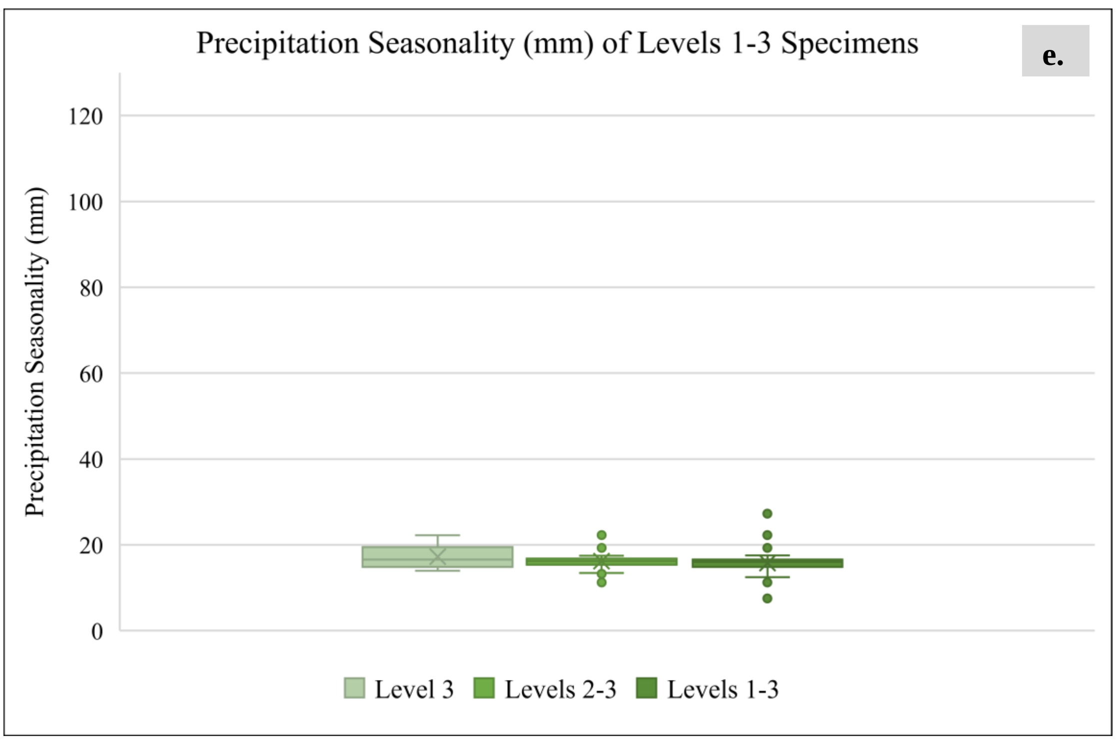


Figure 2a-e. Box-and-whisker plots of bioclimatic variables from Worldclim with geographic certainty subsets (Level 3, Level 2-3 and Level 1-3). Median values are indicated with an "X". Outliers (n > 1.5 x Q3; n < 1.5 x Q1) are indicated with dots above and below the upper and lower quartiles.

Five of the nineteen variables had statistically significant (*p-value* < 0.05) differences between the geographic certainty subsets (Level 3, Level 2-3 and Level 1-3): Temperature seasonality (p-value = 0.032), Minimum temperature of coldest month (p-value = 0.029), Mean temperature of coldest month (p-value = 0.027), Precipitation of driest quarter (p-value = 0.045) and Precipitation of coldest quarter (p-value = 0.034). These values include the Boston, Massachusetts population. When the Boston, Massachusetts population is removed from the analyses, the differences between geographic certainty subsets for these five variables remain statistically significant. In this second analysis, the p-value of eleven of the nineteen variables decreases from the first analysis, while the p-value of the remaining eight variables increases. Differences between the geographic certainty subsets become newly statistically significant for Annual mean temperature alone (p-value = 0.041).

When completing a linear Pearson correlation analysis on the nineteen bioclimatic variables including the Boston, Massachusetts population in the Microsoft Excel Data Analysis ToolPak the highest correlations are found between Annual mean temperature and Maximum temperature of warmest month (0.969), Minimum temperature of coldest month (0.951), Mean temperature of warmest quarter (0.896), and Mean temperature of coldest quarter (0.962). Similarly, Annual precipitation is strongly correlated with the variables Precipitation of driest month (0.926), Precipitation of driest quarter (0.962) and Precipitation of wettest quarter (0.921). When the Boston, Massachusetts population is removed from the Pearson correlation analysis, several variables become highly correlated, including precipitation seasonality. Precipitation seasonality, is highly correlated with Minimum temperature of coldest month (-0.809) and Mean temperature of coldest quarter (-0.713). Further, Precipitation of driest month was found to newly correlate with Isothermality (-0.716) defined as the amount of diurnal temperature oscillation relative to seasonal temperature oscillation (O'Donnell and Ignizio 2012).

To visualize how well climate indicates the range of *Ripariosida hermaphrodita*, we ran several analyses of the nineteen bioclimatic variables with a logarithmic output in Maxent, a maximum entropy species distribution modelling software. Without the Boston, Massachusetts population, the predicted suitable habitat was more tightly correlated to the known data points throughout its range (Figure 3a-b). While the model's predicted habitat corresponds to known populations along the primary river basins, including the Ohio, Kanawha and Potomac rivers, certain anomalous regions including the eastern shore of Maryland and western Massachusetts indicate where the model may have shortcomings. With or without the Boston, Massachusetts population, Maximum temperature of warmest month (40.8%; 41.7%), then Precipitation of driest quarter (19.8%; 22.5%), followed by Precipitation seasonality (13.7%; 16.1%) had the largest contribution to the models. According to the model with the Boston, Massachusetts population, two variables did not contribute at all (0%), Annual mean temperature and Precipitation of driest month.

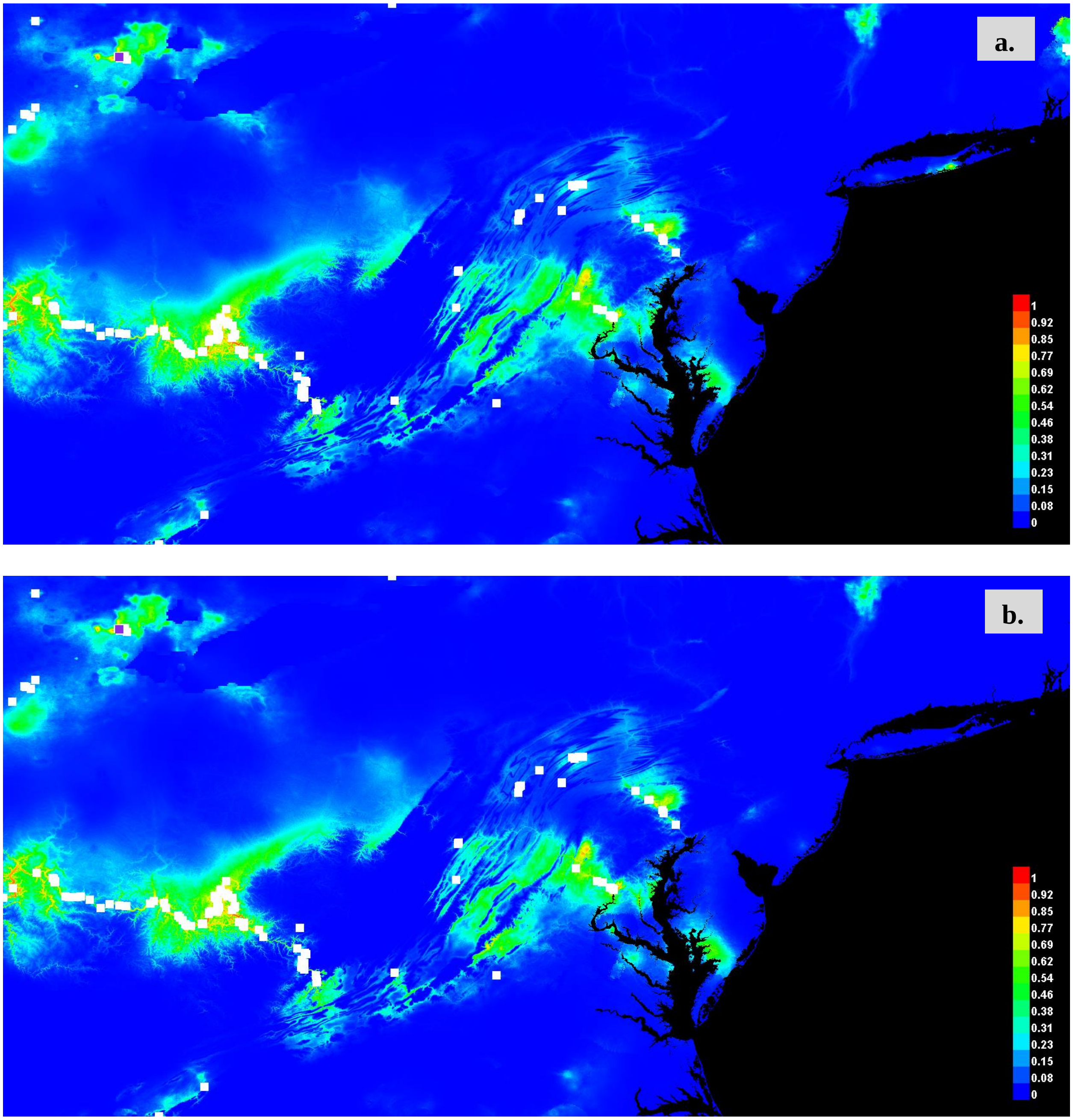


Figure 3a-b. Maxent (maximum entropy) species distribution model of *Ripariosida hermaphrodita* as predicted by nineteen bioclimatic variables at 30s resolution from Worldclim. **a)** With Boston, Massachusetts population and **b)** without Boston, Massachusetts population.

2. Fine-scale trends

In addition to climate, soil and topography were incorporated in the niche analysis to further understand the distribution of Virginia mallow at scale finer than latitudinal. From the relevant twenty-nine soil map units downloaded from the Soil Survey Geographic Database (SSURGO) numerous attributes were provided, however only those of interest are mentioned below. Geomorphic description, component type, drainage class, particle size and representative aspect provided the most comprehensive and useful understanding of the underlying soil and geology of known Virginia mallow sites. Geomorphic descriptions for the occurrences included in this study across geographic certainty subsets are dominated by flood plains, followed by stream terraces, then by hillslopes, and finally mountain slopes. Of the occurrences with known soil data, these four geomorphic descriptions represent 87% of the data points for Level 1-3 specimens (n = 163) and 89.6% of the data points for Level 2-3 specimens (n = 97). Level 3 specimens (n = 7) are accompanied by GPS coordinates made at the time of collection and are therefore the most geographically reliable. Of these records, three occurrences are in flood plains, one occurrence is within a stream terrace, one is within a till plain, one is on urban land and one occurrence is on a hillslope.

To further understand what factors of soil and topography align occurrences or distinguish populations from one another, findings of component type and associated characteristics including particle size and drainage class are discussed below. Fifty-seven unique components are represented by Level 1-3 specimens, forty-six unique components are represented by Level 2-3 specimens and six unique components are represented by Level 3 specimens (Figure 4). Of these components, soil series Huntington (n = 16), Elkinsville (n = 14), Cateache (n = 12), Wheeling (n = 9) and Nolin (n = 9) are the most common, along with the miscellaneous components Urban

Land (*n* = 16) and Udorthents (*n* = 14), or disturbed soil where the top layer has been removed and replaced with gravel and sand (Turenne, 2018). Despite the great number of components represented by the data, certain characteristics remain consistent throughout the soil types. According to the U.S.D.A. Natural Resources Conservation Service's Official Soil Series Descriptions portal, these five most common named soil series are all well drained and very deep, occurring either on stream terraces and slopes or formed in alluvium of river valleys and flood plains. The Huntington, Elkinsville and Nolin series consist of fine-silty soils, while the Cateache and Wheeling series consist of fine-loamy soils. These findings are further supported by additional results of the SSURGO dataset. For Level 1-3 specimens with available soil data (*n* = 195) 68.7% of the records occur on some type of loam, primarily silt loam, loam, fine loamy, and loamy-skeletal soil. A significant portion of the records (21%) occur on urban land. This more ambiguous particle size descriptor is likely used to identify soil that has been imported or otherwise mechanically altered such that the characteristics of the native soil are unknown. For both Level 1-3 specimens (*n* = 139) and Level 2-3 specimens (*n* = 91) about three-quarters of the records occur in dominantly well drained soil. The remaining records occur in poorly drained soils and only three records occur in excessively drained soil.

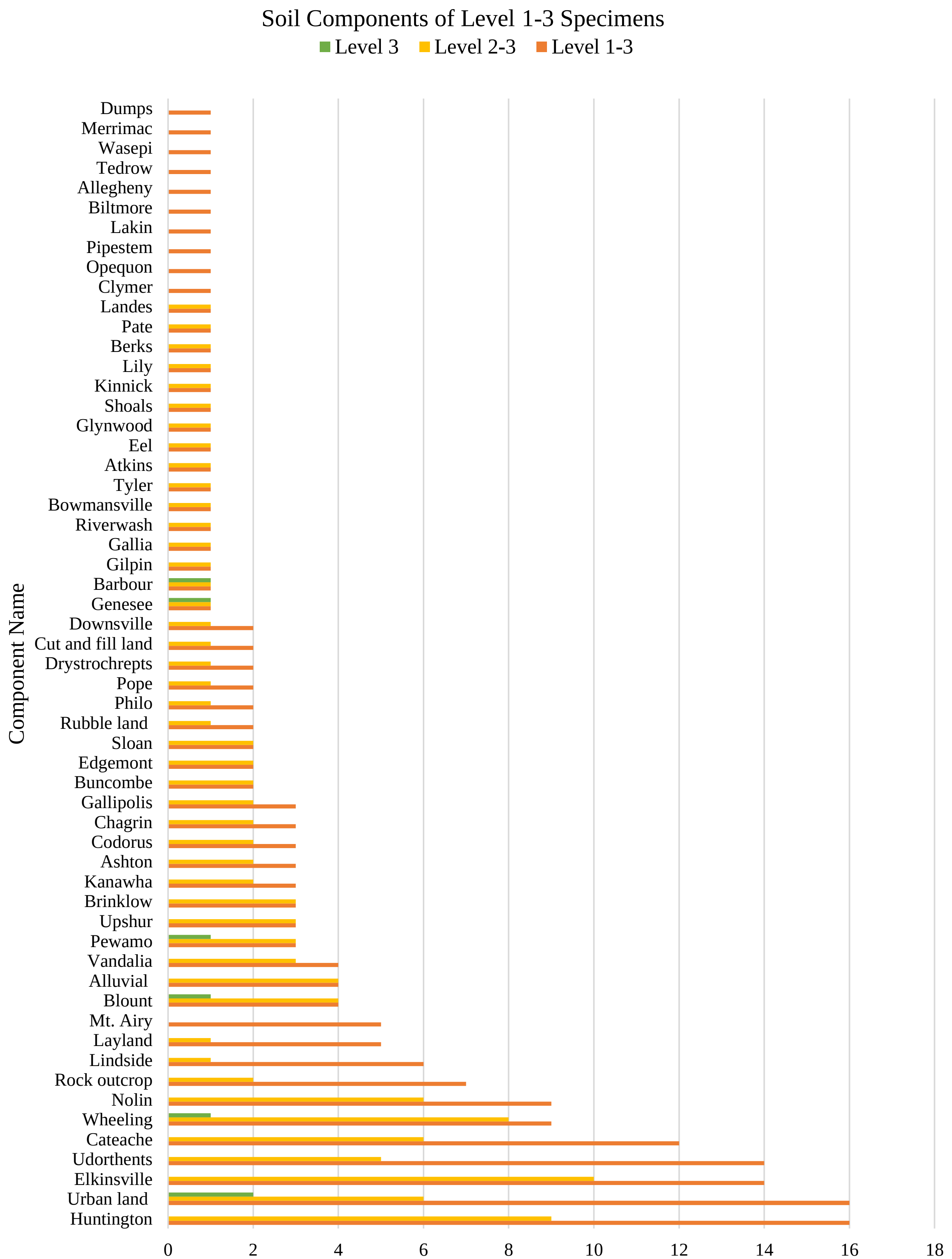


Figure 4. Unique soil components of georeferenced Virginia mallow records by geographic certainty subset: Level 3 (green), Level 2-3 (orange) and Level 1-3 (red). Data sourced from spatially joined map unit polygons of the Soil Survey Geographic Database (SSURGO).

One of the most valuable pieces of soil and topographical information obtained from the SSURGO Spatial Join analysis is the representative aspect of the georeferenced records. Aspect, the compass direction of a slope face, contributes to variation in microclimate in a given region (Matthew et al.). Aspect is measured in degrees (°) and may be demarcated into coordinate directions (Figure 5).

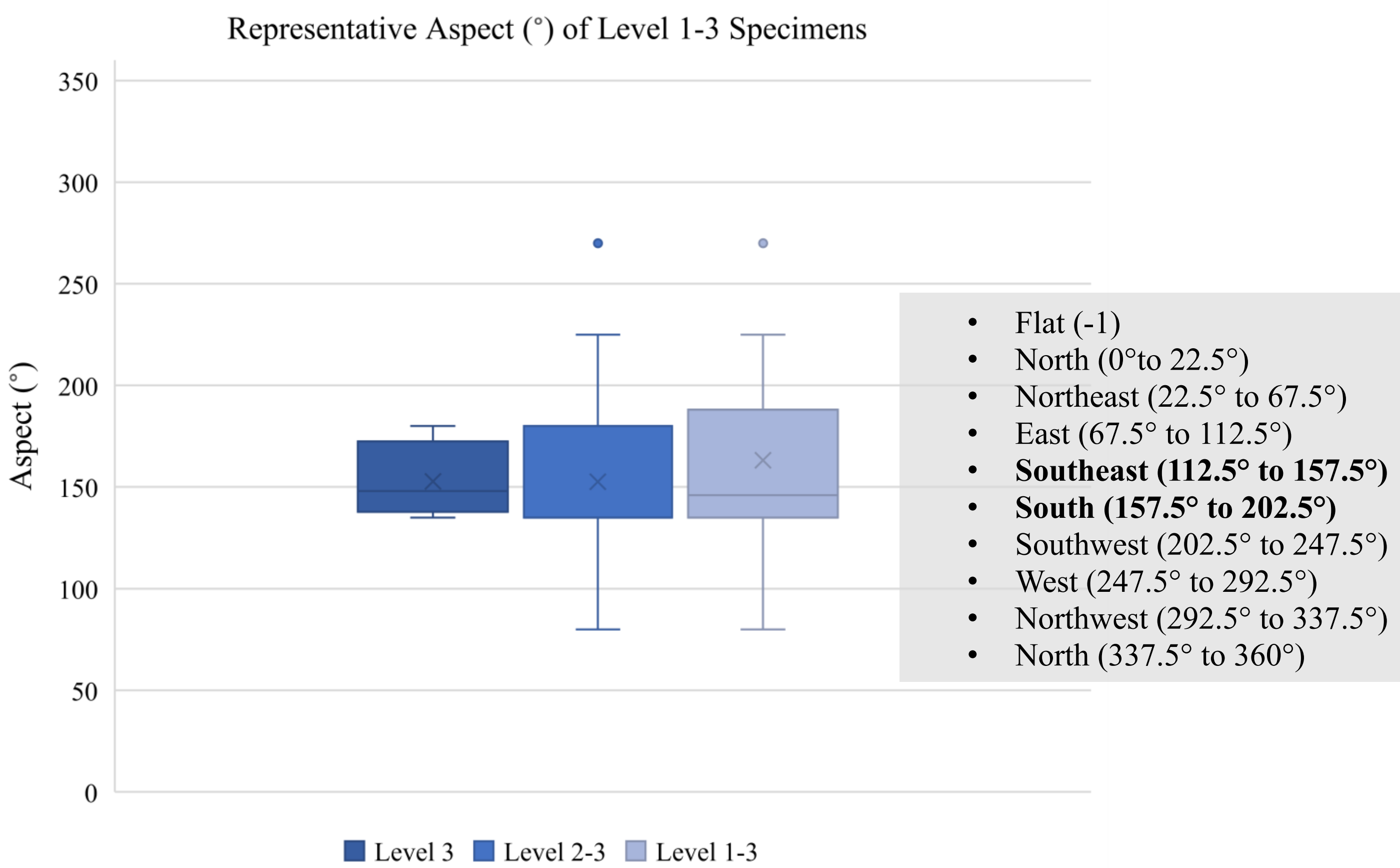
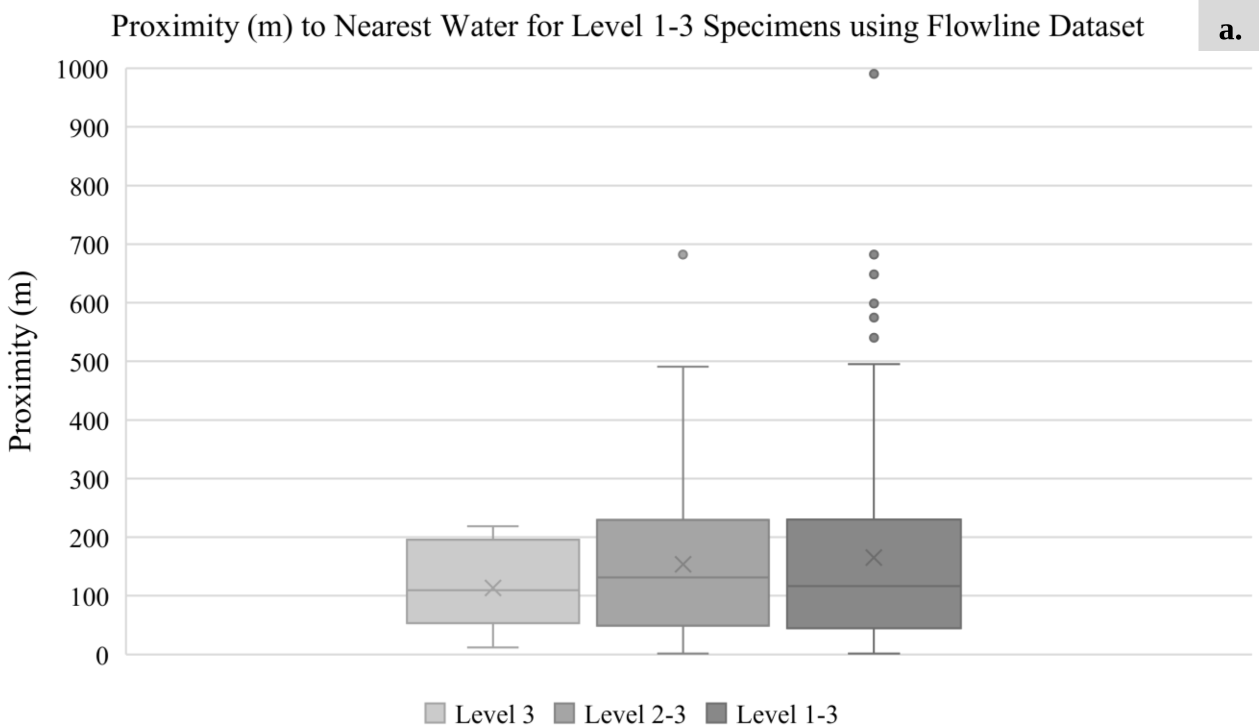


Figure 5. Representative aspect (°), coordinate direction of slope face, for georeferenced populations of Virginia mallow analyzed by geographic certainty subsets. Data sourced from spatially joined map unit polygons of the Soil Survey Geographic Database (SSURGO).

For Level 3 specimens with available aspect data (*n* = 4), the mean representative aspect was 152.75°, followed by Level 2-3 specimens (*n* = 69) with a mean of 152.65° and Level 1-3 specimens (*n* = 123) with a mean of 163.10° (Figure 4). All values indicate a predominance of either southeast or south-facing slopes with known Virginia mallow populations. As depicted in the figure, no records included in this research occur along north-facing slopes. Rather, aspect ranges from 135° to 180° (southeast-facing to south-facing) for Level 3 specimens, and from 80° to 270° (east-facing to west-facing) for Level 2-3 and Level 1-3 specimen subsets.

Alongside soil and topographical data, proximity to nearest water and transportation are incorporated to further define the niche of *Ripariosida hermaphrodita*. Reported as a riverine species, this claim is investigated using the ArcGIS Near analysis tool and data from the U.S.G.S. National Hydrographic Dataset to measure proximity (m) to the nearest source of water (Figure 6). Two analyses were completed, with the Flowline dataset including ephemeral and intermittent sources of water and using the Area dataset limited to perennial sources of water. With the Flowline dataset, for Level 3 specimens (n = 7) the mean distance was 113 m with an Upper – Lower Fence range of 349.7m (Figure 6a). For Level 2-3 specimens (n = 113) the mean distance increased to 153.5 m with an Upper – Lower Fence range of 646 m. Finally, for Level 1-3 specimens (n = 196) the mean distance increased slightly to 165.3 m with an Upper – Lower Fence range of 949 m. Several outliers for the Level 2-3 and Level 1-3 subsets include possible adventive populations, such as two records from the Back Bay Fens of Boston and one record from West Cambridge, Massachusetts. Additional outlier records from Wayne County, Michigan and from nearby Lansing, Michigan indicate populations of disputed nativity. However, another outlier record from Clermont County, Ohio is situated well within the undisputed region of the Ohio River drainage just east of Cincinnati. The mean proximity (m) to the nearest water source was much greater when using the Area dataset, limited to perennial water sources (Figure 6b). For Level 3 specimens, the mean distance was 797.8 m, then 378.6 m for Level 2-3 and 399.9 m for Level 1-3. The range (6881.95 m) is greatly expanded for Level 1-3 specimens with the Area dataset compared with the Flowline dataset (989.19 m) and far more records become outliers in this second analysis.



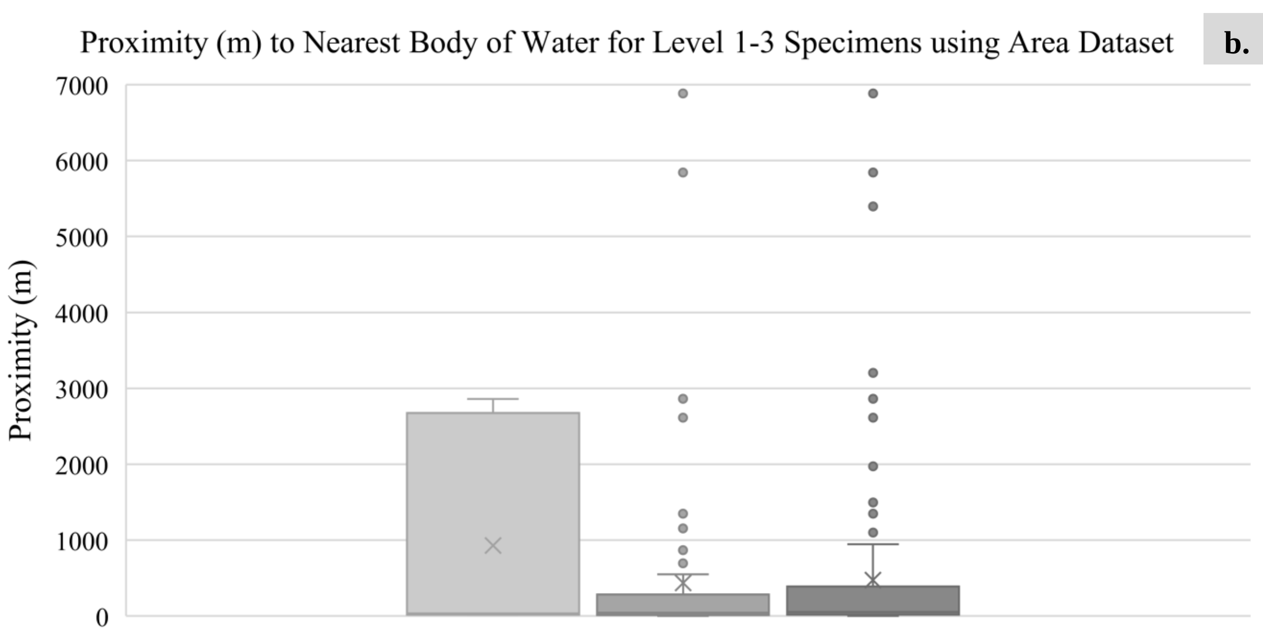


Figure 6a-b. Distance (m) of Virginia mallow occurrences to the nearest water source using the (**a**) Flowline dataset including intermittent and ephemeral water versus the (**b**) Area dataset limited to perennial water, both of the National Hydrographic Dataset from the U.S.G.S. Findings compared among subsets of geographic certainty: Level 3 (*n* = 7), Level 2-3 (*n* = 113) and Level 1-3 (*n* = 196) specimens.

Virginia mallow is known to prefer disturbance, both natural and mechanized, occurring in both riparian and ruderal sites. To provide support to this claim, in addition to proximity to the nearest water source, proximity to the nearest road, railroad or trail of each Virginia mallow occurrence is also recorded. Data is sourced from the U.S.G.S. Transportation Dataset of The National Map. For Level 3 specimens, the mean distance (m) is 121.3 m, while the Upper – Lower Fence range is 501.9 m (Figure 7). For Level 2-3 specimens, the mean and the range decrease to 65.9 m and 119.9 m, respectively. When all georeferenced records are included (Level 1-3), the mean increases slightly to 83.5 m and the Upper – Lower Fence range increases as well to 162.9 m. The values of these geographic subsets do not differ significantly (p-value = 0.387), perhaps due to the high number of outlier records for both Level 2-3 and Level 1-3 subsets.

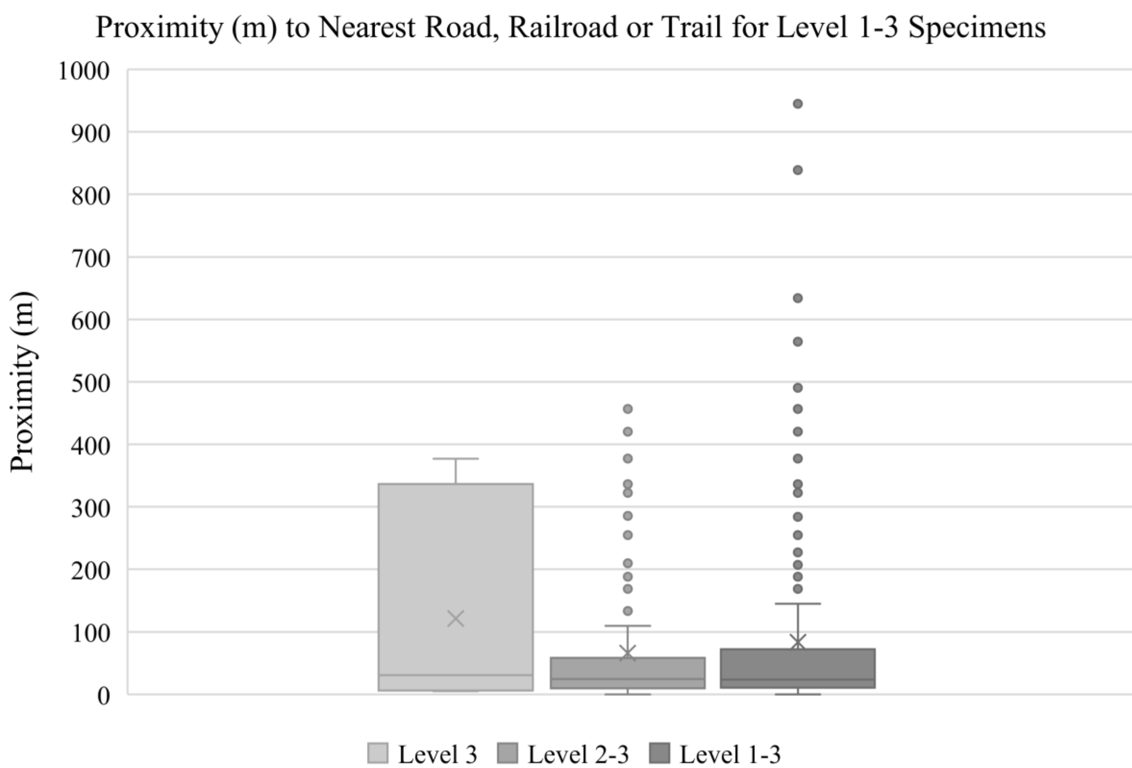
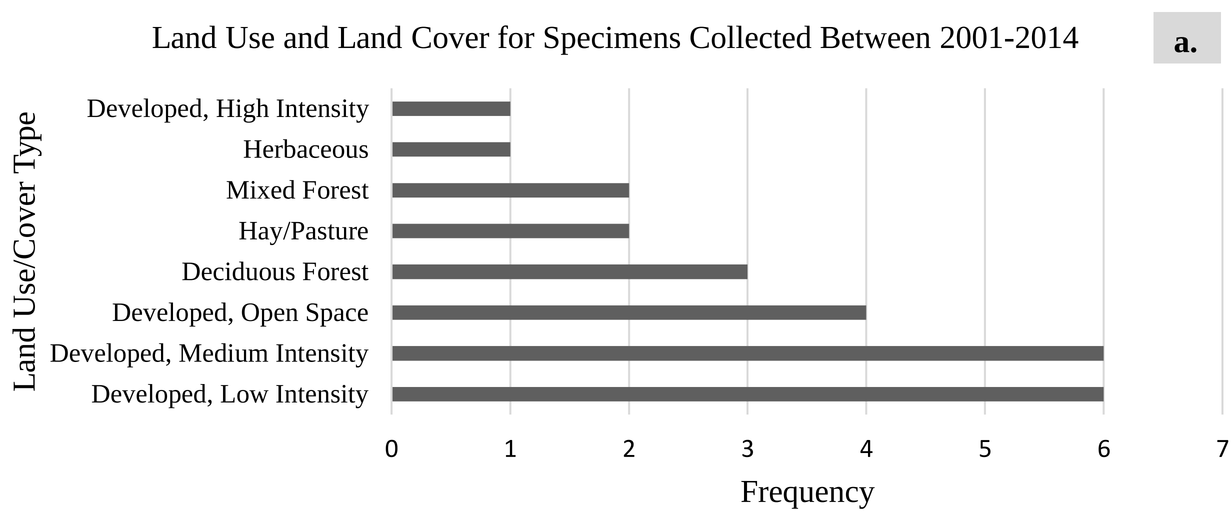
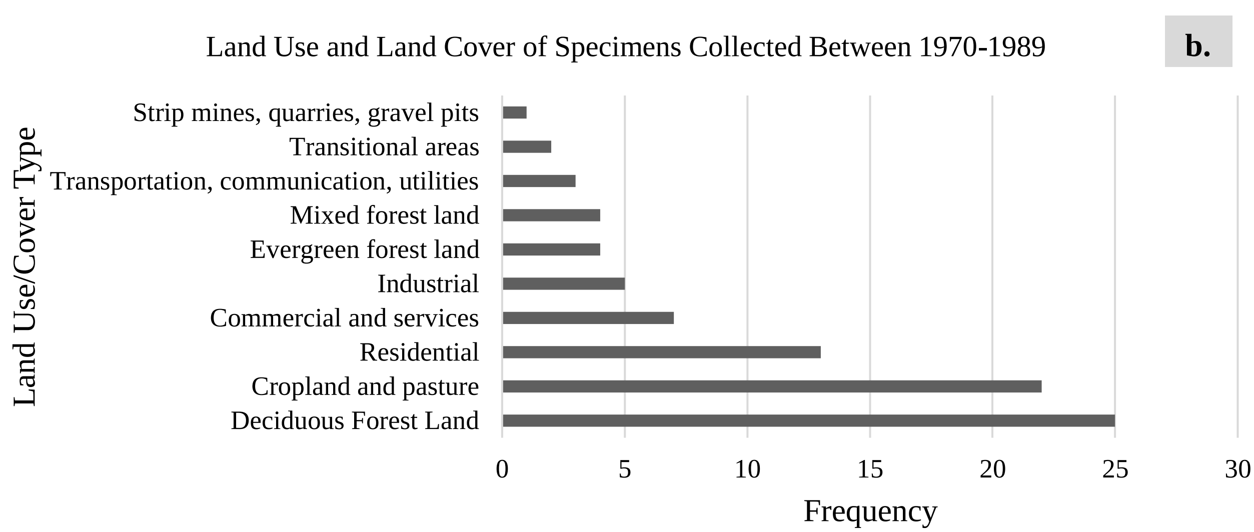


Figure 7. Distance (m) of Virginia mallow occurrences to the nearest road, railroad or trail using the U.S.G.S. Transportation Dataset of The National Map. Findings compared among subsets of geographic certainty: Level 3 (*n* = 7), Level 2-3 (*n* = 113) and Level 1-3 (*n* = 196) specimens.

The remaining fine-scale variable analyzed is land use and land cover. Across temporal markers, several patterns remain consistent (Figure 8). For the twenty-five collections made between 2001 and 2014, 76% occur on developed land (Figure 8a). Similarly, for the 86 records collected between 1970 and 1989, 61.6% occur on developed land (Figure 8b). Using low-resolution data for the decades between 1863 and 1969, 60.7% of the 53 relevant records occur on developed land (Figure 8c). Developed land is defined as any land use type that involves management, including cultivated, industrial and residential areas.





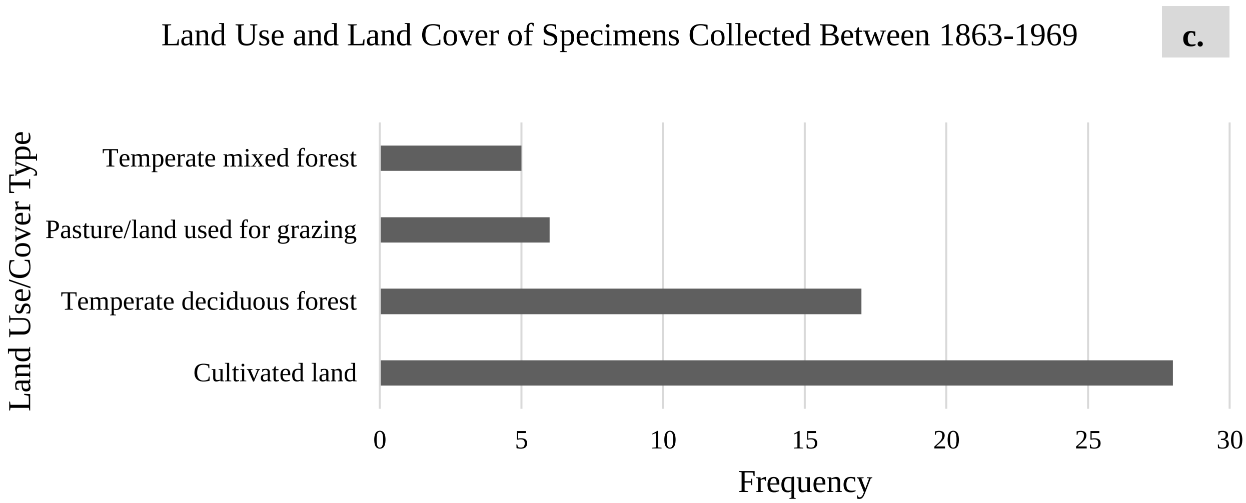


Figure 8a-c. Frequency of overlapping and adjacent land use/cover data for Level 1-3 Virginia mallow occurrences by temporally relevant databases. **a)** Records collected between 2001 and 2014, **b)** records collected between 1970-1989 and **c)** records collected between 1863 and 1969. Developed land is defined as land that has been modified by humans for cultivation, industry, resource extraction, or housing.

Field reconnaissance

In addition to completing an ArcGIS niche analysis, I was able to return to twelve known sites of collection of Virginia mallow included in this research. The extent of my field research was limited to the southeast Ohio River region from downtown Cincinnati eastward to Maysville, Kentucky, closely following the Ohio River and its creeks. The research took place during two outings on April 18th and May 2nd, 2020. Of the twelve records visited, *Ripariosida hermaphrodita* was found at four distinct locations representing five total records. Of those five sites, the change of population size over time can only be ascertained from three records representing four sites. When revisiting to site of the 1992 M. J. Becus collection in the southern tip of Woodland Mound Park in Hamilton County, Ohio, the population size has gone unchanged. Becus notes “1 plant with three flowering stems” in 1992. During my visit on April 18th, 2020, a single clump of only five stems were noted, although the number of unique plants present is uncertain as Virginia mallow is rhizomatous. During his 2014 collection at the nearby Woodland Mound Boat Ramp in Clermont County, Ohio, no population size was indicated by M. A. Vincent. I noted about 100 stems occupying 20 ft of roadside, but given the lack of temporal information, it is unknown whether this population has dwindled or not. While A. W. Cusick also neglected to note a population size at his 1985 collection along Bullskin Creek in Clermont County, M. J. Becus returned to the site in 2002 and noted "1000s of large flowering plants". Unfortunately, when visiting Bullskin Creek on May 2nd, 2020, I noted only around 150 stems on both the southwest and southeast banks of the creek. Conversely, the population at the overgrown Henry Franklin "Slim" Sallee Ball Field grew fifty-fold from the "approximately 10 plants growing" noted by J. S. McCormac in 1988. This was by far the largest population I observed during my field research, consisting of at least 800 stems and spanning around 250 ft by 20 ft in length and depth. Of the sites where *Ripariosida hermaphrodita* was found, site longevity ranged from 1988 to the present, with the most recent collection made in 2014. Earlier collections visited spanned from 1978 to 1986 with no Virginia mallow found. No collections made prior to 1978 were located within the field research area.

While limited in scope, this field research provides observational findings to support the results of the ArcGIS niche analysis. Fine-scale variables, including soil and topography, proximity to water and to transportation, and land use or land cover are particularly relevant. An additional variable of shade tolerance not incorporated in the niche analysis was clear from field work, as supported by the literature (Thomas 1979, Spooner et al. 1985, Bickerton 2011). In every revisited population of Virginia mallow, proximity to development or disturbance was present, whether along the side of a road or bridge (M. J. Becus 1998, A. W. Cusick 1985, M. J. Becus 2002) or along the margin of a cleared area (M. A. Vincent 2014, J. S. McCormac 1988). Proximity to sources of water was also indisputable as every revisited population was anywhere from ten feet (A. W. Cusick 1985 and M. J. Becus 2002, M. A. Vincent 2014) to a couple of hundred feet (M. J. Becus 1998, J. S. McCormac) from the nearest creek or the Ohio River. Full sun was an additional constant among revisited populations, while partial to full shade at revisited sites with no *Ripariosida hemaphrodita* located was similarly consistent.

IV. Discussion

Fundamental and realized niches of Virginia mallow

The native perennial *Ripariosida hermaphrodita* (Virginia mallow) is both rare and weedy, thus posing an interesting challenge to define its niche. Incorporating both large-scale and fine-scale variables in an ArcGIS niche analysis of georeferenced herbarium specimens alongside select field reconnaissance to a dozen of these sites allows us to narrow the fundamental and realized niches of this species. Firstly, we may address the fundamental, or possible niche of Virginia mallow (Blonder et al. 2014). Keeping in mind the Hutchinsonian theory of the *n*-dimensional hypervolume to describe the fundamental niche of a species (1957), each variable included in our niche analysis serves as an axis, and the suitable values of that axis describe possible Virginia mallow habitat. At the broadest scale, the nineteen bioclimatic variables included in our analysis provide latitudinal constraints on possible Virginia mallow niche (Figure 2). Variables with narrow ranges of values indicate particularly telling axes: mean annual temperature (median = 12.1 °C, range = 1.8 °C), maximum temperature of warmest month (median = 30 °C, range = 3.7 °C), minimum temperature of coldest month (median = -7.1 °C, range = 2.1 °C), precipitation of driest month (median = 69 mm, range = 12 mm) and precipitation seasonality (median = 16 mm, range = 5mm). Outside of these suitable climatic constraints, survivability of *Ripariosida hermaphrodita* may diminish. However, these bioclimatic variables do not define the fundamental niche at a local scale, as the region encompassing these temperature and precipitation values is greater than the known distribution of Virginia mallow. When completing a Maxent species distribution model with georeferenced herbarium records and nineteen bioclimatic variables, proposed habitat is not restricted to known sites but rather shares a similar pattern, which may indicate either gaps in our knowledge of Virginia mallow distribution via herbarium records, or limitations to the model (Figure 3a-b).

To ascertain the factors limiting *Ripariosida hermaphrodita*'s distribution at a finer scale, soil and topographical data reveals a higher degree of consistency across soil types than previously suggested, despite the more than fifty unique components represented by the data (Figure 4) (Spooner et al. 1985). For the geographic certainty subset Level 1-3, various subclasses of loam represent over two-thirds of the records, followed by 20% on urban land. This leaves around 10% of records that occur on soil with native particle sizes other than loam. This finding is further supported by three-quarters of the Level 1-3 records found in well-drained soils, with very few records found in either extremely well drained or poorly drained soils, bolstering the work of Matyka and Kus (2018). In the Northern Hemisphere, the overwhelmingly south and southeast-facing aspect of slopes with Virginia mallow populations indicates a preference for a microclimate that receives more direct solar radiation and is both warmer, drier and windier (Figure 5) (Smith et al. 2003). Thus, particle size, drainage and aspect form additional axes of the fundamental niche of Virginia mallow, further limiting its possible range at a local scale.

Beyond loamy and well-drained soil and south-facing slopes, known *Ripariosida hermaphrodita* populations are correlated with proximity to both water and transportation. For Level 1-3 specimens, this mean distance is 165.3 m to the nearest water source and only 83.5 m to the nearest transportation (Figures 6, 7). The lower value for proximity to transportation may indicate the fact that there are more roads than waterways, or that transportation maps are more complete than hydrographic maps. Additionally, this figure may explain the element of collector's bias in the records, as collecting along a road, railroad or trail offers convenience. Regardless, fundamental niche of Virginia mallow is limited by its distance from water and from transportation, with maximum proximities of 990.57 m to water and 945.08 m to transportation. It is unclear when both are present, as was observed in the field research component, whether reliance on one feature is greater than the other. This necessary proximity to water is bolstered by the geomorphic descriptions of soil units intersecting with Virginia mallow sites. Flood plains, followed by stream terraces, then by hillslopes, and finally mountain slopes dominate the geomorphology and tell us where *Ripariosida hermaphrodita* has been found, but not necessarily where it is absent. With this available data, however, the fundamental niche may be further adjusted from southeast and south-facing slopes of loamy, well-drained soil at most 1000 m away from both water and transportation, to include stream terraces and flood plains and slopes that intersect with these other axes. Land use and land cover data further supports the notion that Virginia mallow can thrive in both natural and manmade disturbance regimes. Developed land, encompassing land altered mechanically for agriculture, industry, resource extraction or residential use represents 76% of land use types for specimens collected in the last two decades, and around 61% of land use types for collections made prior to 2000 (Figure 8). These figures underscore the historic success of Virginia mallow in developed areas, indicating the fundamental niche of Virginia mallow must encompass ruderal zones. This finding may also be in part explained by collector's bias, as with proximity to transportation, given that populations in developed areas may be more visible and thus convenient to collect.

Study limitations

If the fundamental niche is defined by suitable values of the above-mentioned variables, the realized niche is the subset of the fundamental niche where Virginia mallow actually occurs. Our understanding of the realized niche of *Ripariosida hermaphrodita* is limited by how representative our data is. The 196 uncultivated and non-duplicate herbarium specimens providing the basis for this study yield a patchworked distribution. Sampling bias, or collector bias, both temporally and spatially, confuses absence of data with absence of Virginia mallow in a given area. Thus, we can confidently state where Virginia mallow does occur, or has occurred, and the associated variables, but we can less confidently state where Virginia mallow is not.

For those sites where Virginia mallow populations are known, geographic uncertainty for Level 1 and Level 2 specimens sheds doubt on certain fine scale variables, including soil and topography and land use or land cover. While a precise coordinate pair is given for these records in the georeferencing process, this is misleading. In fact, the coordinate pair represents the centroid of a circle with a given error radius. Thus, for variables such as soil and topography that have high local variability visualized as distinct polygons of soil type in ArcGIS, using a precise GPS coordinate for these imprecise specimens may be misleading. For large-scale variables including climate, however, the geographic uncertainty of these records is negligible. Furthermore, Level 3 specimens, accompanied with GPS coordinates made at the time of collection, provide the most reliable geographic information. However, only seven Level 3 records were included in the study, preventing replicability for this geographic certainty subset. Even still, insignificant differences between values of Level 3 specimens and Level 2 and 1 specimens may be explained by the distribution of Level 3 records throughout the major basins and drainages relevant to this study.

It is not clear that Ripariosida hermaphrodita is no longer present at all sites where no Virginia mallow was found during field reconnaissance. However, for certain populations where Virginia mallow was not rediscovered, development over the past four decades is clearly a threat to the continued survival of *Ripariosida hermaphrodita.* For instance, the site of the A. W. Cusick 1986 collection from downtown Cincinnati is now a small parking lot. The populations near Beckjord Power Station along US Rt. 52 east of Cincinnati have likely been removed following the creation of manmade “lakes” between the Ohio River and the scenic byway to store the coal refinery’s biproducts. Additionally, maintenance decisions along this stretch of the US Rt. 52 embankment may have spelled demise for Virginia mallow, as the bank was heavily overgrown. This may also be the case for those collections made farther east along the same highway (M. A. Vincent 1989 and A. W. Cusick 1985).

Conservation and protected areas

Given that *Ripariosida hermaphrodita* is globally ranked G3 Vulnerable, and is possibly extirpated, imperiled and vulnerable throughout its range in the United States and southern Ontario, the conservation status of areas with Virginia mallow populations became an important final variable to analyze. No U.S. agency has reported on the conservation status of Virginia mallow, despite stewarding almost the entirety of its range. To address the claim made in the 1985 paper from Spooner et al. that no known populations of Virginia mallow occur in protected areas, we have mapped protected areas that intersect with populations of *Ripariosida hermaphrodita*. With data sourced from the U.S.G.S. Protected Areas Database 2.0 for relevant regions of northeast North America, in addition to known conservation status for the Ontario, Canada population, eighteen distinct protected lands were found to overlap with known Virginia mallow populations.

These eighteen protected areas span over 120,000 acres and encompass 31% of the populations demarcated during the georeferencing process. The protected areas vary considerably in management, access and longevity from one another. Ten of the areas are managed by the federal government, while an additional three are managed by the state and another two are managed by the local government. The remaining three areas are managed by private, private-federal and non-governmental entities. Importantly, only six of the eighteen areas have known mandates for biodiversity protection, while the remainder include highly transformed park systems such as the National Mall in Washington, D.C. where *Ripariosida hermaphrodita* is presumed extirpated and multi-use areas such as the Raystown Recreation Area in Pennsylvania. Since Spooner et al. published their findings of the distribution of Virginia mallow, five protected areas have been founded, including a Nature Conservancy easement in Virginia, the Cotton Hill Wildlife Management Area in West Virginia, the Ohio River Islands National Wildlife Refuge in Kentucky, an Natural Resource Conservation Services Emergency Watershed Protection Program floodplain easement in Indiana and the Goldsboro Access along the Lower Section of the Susquehanna River Water Trail in Pennsylvania. This wave of newly created protected areas indicates an optimistic trend towards increasing acreage designated for biodiversity protection, particularly important for an elusive native perennial like Virginia mallow. Nonetheless, there is necessity for a comprehensive assessment of critical habitat and conservation status of Virginia mallow throughout its United States range to accompany those reports produced by the government of Ontario, Canada (Environment Canada 2015, Bickerton 2011, COSEWIC 2010).

V. Recommendations

This niche analysis provides the foundation for the comprehensive field work necessary to clarify the demography and ancestral basin of Virginia mallow. Continued field reconnaissance to sites of collection as well as the discovery of yet unknown populations are both required. Tissue preservation can provide the basis for research into the population genetics of *Ripariosida hermaphrodita* to better understand the mechanisms and patterns of its dispersal and to distill native from adventive populations.

VI. Acknowledgements

This work was made possible by the guidance and expertise of Dr. Cynthia Skema and Dr. Timothy Block of the Botany Department of the Morris Arboretum of the University of Pennsylvania. Without online herbarium access from the collections included in this study, and in particular the additional data from Donna Ford-Werntz of West Virginia University and Michael Vincent of Miami University, a niche analysis would not have been possible. Observational evidence for the habitat of *Ripariosida hermaphrodita* was provided for by Chris Frye of the Maryland Department of Natural Resources. Funding for the position of Flora of Pennsylvania Intern, through which I was able to complete this work, is thanks to the Eli Kirk Price Endowment.

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