Friends of Plant Conservation, Inc.

Fall 2020 Volume XII, Number 3
Editor: Katherine Schlosser

"That which we call a Rose By any other Name would smell as sweet "

Marshallia pulchra (Beautiful Barbara's Buttons), photo by John Burkhart

Knapp, Wesley M., Poindexter, Derick B., Weakley, Alan S. "The true identity of *Marshallia grandiflora*, an extinct species, and the description of *Marshallia pulchra* (Asteraceae, Helenieae, Marshalliinae)." *Phytotaxa* [Online], 447.1 (2020): 1–15. Web. 23 Oct. 2020

Juliet was certain of Romeo's lineage, but it mattered little to her (Romeo and Juliet, Shakespeare). In the proper naming of plants, however, lineage does matter. There has been some confusion over *Marshallia* species, now put to rest.

The plant we in North Carolina have long known as *Marshallia grandiflora* is no more. *M. grandiflora* is now recognized as the 53rd extinct plant species in North America.

In its stead is the newly re-named *Marshallia pulchra* which, it turns out, is the plant we have been identifying as *M. grandiflora*.

Published in the June 2020 issue of *Phytotaxa*, Wesley Knapp, Derek Poindexter, and Alan Weakley explain the evolution and resolution of the identification of *Marshallia*, and without the tragedy of the Montagues and Capulets.

From the Abstract:

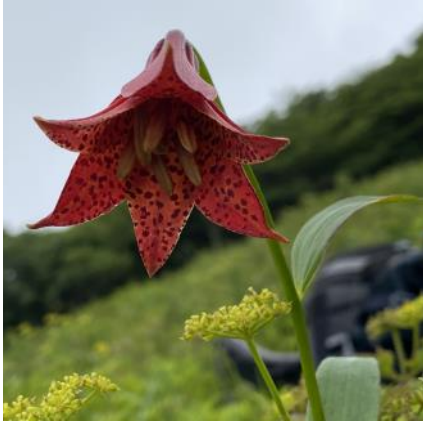
Marshallia grandiflora (Asteraceae, Helenieae, Marshalliinae) is recognized as the 53rd extinct plant species to North America, and an endemic species of the Blue Ridge physiographic province of North Carolina. A new name, *Marshallia pulchra*, is provided for the more broadly ranging species that has been confounded with and generally considered conspecific with *M. grandiflora.* Morphologic and biogeographic data clearly separates two species within the previous concept of *M. grandiflora. Marshallia grandiflora and M. pulchra* are compared with their sister species, *M. legrandii*. Cytological data is given for *M. legrandii*, including the first report of B-chromosomes in the genus.

The full article is attached at the end of this newsletter.

Update from Tater Hill

Howdy Folks

I wanted to provide a brief update on the Tater Hill Plant Preserve and some of the work we are trying to accomplish. Covid has put a damper on many of our public outreach activities, but we hope to return to those efforts as soon as it is safe for everyone involved.



This year, one of our main foci has been Gray's Lily (*Lilium grayi*). This species is endemic to the Southern Appalachians and many state, and federal agencies are currently working on re-evaluating its conservation status. On Tater Hill, we have a large population and have been conducting demographic studies (counting individuals) and using deer exclusion plots (we call them lily-domes) to measure the effect deer have on reproductive success. This kind of primary data is critical to inform how conservation agencies respond to threats to Gray's lily.

Many of you know that Tater Hill is part of a unique botanical region in our state, known as the Amphibolite mountains. In this region, many rare and threatened species occur, and the Tater Hill Plant Preserve's primary mission is to protect those species. One way we accomplish that work is by monitoring the health of known populations across the preserve. This year, due to covid restrictions, we could refocus some of our energies towards finding new populations of rare plants and/or re-locating populations that have not been seen in many years. We were able to update our records on *Micranthes pensylvanica* (Swamp Saxifrage). We also found several new populations of *Aconitum reclinatum* (Trailing Wolf's Bane). Most of the new Trailing Wolf's Bane populations were located on properties recently added to the preserve. We searched for several other species with historical records in the area but have not yet located individuals.

This fall, we are working to mark or remark the preserve boundaries. We have an upcoming service-learning event with the Appalachian State forestry club. During this activity, we will discuss the importance of plant preserves to protect biodiversity and why they often need to be closed to the public. We will then spend some time walking boundaries and updating our signage and markings so that people understand when they cross property lines.

In closing, we hope that covid recedes in the spring so we can host wildflower walks and share the extraordinary botanical diversity of the preserve.

~ Matt Estep

Boundary marking at Tater HIll

Lesley Starke, PCP Director

Staffing changes 2019-2020 in the Raleigh office:

Katherine Culatta has just completed her first year as PCP Research Specialist.

Lori Wright is Ginseng Coordinator & Admin Assistant who keeps PCP running (as well as handling FoPC items which come to PCP).

Geoff Austin is onboard as the PCP Plant Ecologist.

Temporary field technicians:

Miller & HL are out on the Coastal Plain (Boiling Spring Lakes & Hog Branch Pond) are getting burning goals accomplished.

Lysimachia asperifolia at Boiling Springs

Olivia Dannemiller works part-time with PCP and part-time with FoPC from her base in Asheville.

Fisher Stines, still an NC State student, is working mainly in Piedmont preserves and is focused on Durham County preserves.

Projects:

Lesley explained the tremendous opportunity presented the possibility of adding plants to the NC Wildlife Action Plan. Significant new funding is expected to soon become available for this program. It will be important to work with the NC Wildlife Resource Commission. https://www.ncplantfriends.org/uploads/5/7/1/0/57102531/plants_in_the_ncwap.pdf

This year's biggest accomplishment was getting the Protected Plant List updated and out for public comment on October 1, 2020. PCP and the PC scientific committee has been working on this since 2017.

Alan Weakley strongly encouraged FoPC members to comment during the Public Comment period which runs from October 1- November 30, 2020. The deadline for demanding a public hearing is 16 October, 2020. To view these proposed changes visit the NC Register.

To submit your comments email phil.wilson@ncagr.gov or mail to Phil Wilson, 1060 Mail Service Center, Raleigh, NC 27699-1060.

Olivia Dannemiller, Vol. Coord.

In a time when we have to limit our social lives and distance from others, we've found some of our workdays are the perfect opportunity for folks to get outdoors and be social in a responsible way! While we have not been able to have our usual year of volunteer activities, we have found ways to adjust and start holding workdays again.

This fall we have engaged volunteers at Cedar Mountain Bog Preserve to remove *Microstegium vimineum* and to cut back woody species in the core bog to open up sunlight. We included volunteers in our annual Schweinitz's sunflower monitoring at Mineral Springs Barrens Preserve and Redlair Preserve. And we had volunteers out to Dupont State Forest to help on a special grant project we have to perform stewardship tasks in swamp pink and pitcher plant habitats, including thinning the bog area, allowing the plants more sunlight, and removing non-native invasive species.

We look forward to continuing to find ways for you to participate in conserving North Carolina's imperiled species and unique habitats.

More photos on page 9 ……

Plant of interest at Tater Hill…

Aconitum reclinatum, Trailing Wolf's Bane, Trailing White Monkshood. ©Kerry Wixted, iNaturalist. CC-BY-NC

As Matt Estep mentioned (see page 2), Tater Hill is home to a number of species, many particular to amphibolite areas. *Aconitum reclinatum* is one of those, appearing in "Rich cove forests, particularly along brook banks, in seepages, and in periglacial boulder fields with seepage, primarily over mafic rocks," as described by Alan Weakley (2020).

It does not appear on the NC Plant Conservation Program's Protected Species list, though it is recognized by the NC Natural Heritage Program as Rare to Uncommon in North Carolina (S3). Where it appears, it is sometimes in large populations and the largest known population is in North Carolina (Zhou *et al*, 2018). A North Carolina population was the basis for the paper by Zhou on microsatellites (used for DNA profiling and also known as "genetic fingerprinting") in *A. reclinatum* for further study of its genetic diversity and population structure.

Aconitum is best known to gardeners as the species *A. napellus,* Monkshood or Wolf's Bane, which is native to western and central Europe. It is known for its beautiful blue flowers that remind some of a monk's cowl, or hood. It is better known as a powerful poison implicated in the killing of animals and enemies as early as the 1st century.

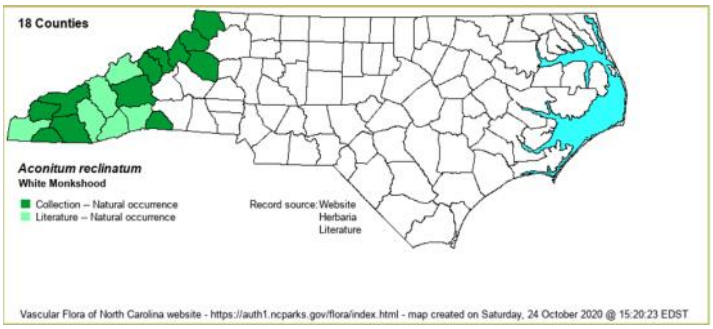
Aconitum reclinatum contains some of the same chemistry, though probably not as much as other *Aconitum* species. Caution is still a good idea, as the more toxic species can cause serious problems just from handling the plant.

The leaves of *A. reclinatum* are deeply palmately lobed with five coarsely-toothed segments, and racemes of the slightly helmeted appearance of the flowers on thin undulating stems. The flowers appear to be white, but are actually more of a cream color and are downward facing.

The plants have been recorded in 12 counties in North Carolina. They are threatened by many of the usual dangers to plant populations: logging; hydrology changes; soil compaction by hiking, biking; excessive grazing. Seed germination may be impacted by these threats.

It is worth looking for this plant when in likely habitat. If you find it, be sure to report to Lesley Starke at NCPCP and to the NC Natural Heritage Program.

~ Kathy Schlosser



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Zhou, J., W. Liu, H. Kong, and W. Gong. 2018. Identification and characterization of microsatellites in *Aconitum reclinatum* (Ranunculaceae), a rare species endemic to North America. *Applications in Plant Sciences* 6(6): e1161.

2020 FOPC Award Honorees

Mt. Mitchell

Marilyn K. Westervelt (Mimi)

Outstanding Service

Mimi served as Treasurer from 2015—2018. During that time, she spent many hours of her time to organize FOPC books to meet accounting standards. She also served as Harvest Field Co-Steward, assisting with boundary checks, invasives removal, and *Helianthus schweinitzii* propagation. She retired at the end of 2018.

At the end of 2019, she graciously agreed to return for a short time to again bring FOPC books up to date, including bringing State and Federal returns up to date and preparing books for a new treasurer. Again, she spent many hours getting FOPC back into good financial shape and helping the new interim treasurer to understand the system used by FOPC, staying longer than she thought would be necessary.

Mimi was always available to answer questions and provide guidance to assure that FOPC remained within legal compliance and proper operating procedures, always looking toward fulfilling the Mission and Vision of the organization.

Wupatki Natl. Monument, AZ

Katherine K. Schlosser

Outstanding Service
Honorary Member

Kathy was on the initial FOPC Board in 2008, serving as Secretary. She continued on, in many roles including President. Among her accomplishments were establishing the newsletter and editing for 12 years, participating in the funds to complete the purchase of Caraway Preserve, the sale of confiscated ginseng to bring in $136,000, serving as co-steward of Harvest Field, visiting almost all of the PCP Preserves, arranging events for members and dedications for newly acquired or expanded preserves. Kathy was appointed to the NCPC Board, serving from 2012 to 2019, and serving as chair for several years.

Personal Note: As this is my last newsletter, I want to say thank you to all of the friends I have made through this organization. Some of you I know mostly from electronic communication, some I see regularly at events, some I know well, and some I have frequently counted on for advice and guidance. I treasure each of you.

I hope all of us stay in touch, and that I will see you often at FOPC events. I will remain active as long as I can, but not in an official position.

Thank you for what I count as among the best 12 years of my life.

~ Kathy

Boundary Marking

One of the tasks of stewarding a Preserve is an annual walk of the boundary and assuring that those boundaries are marked as best as possible. This group of volunteers at Tater Hill Preserve, under the guidance of Matt Estep, undertook the task of posting diamond shaped yellow metal signs and/or painting 4" x 8" purple blazes on trees along the boundary edges. They completed 1/2 mile of new marking and touched up marking on 1 mile of previous work on this foray.

Our thanks to each of the volunteers, including 4 members of the Forestry Club and 3 members of the lab group; all are students at ASU:

Rachel Milkereit (FOPC summer intern)
Cathy Parham
Bubba Pfeffer
Gabe Hernandez
Jennifer Belk
Ben Brewer
David Camp

Greetings from an old friend

Those of you who have been members for a few years will remember **Camille Collins**, who served on the FoPC board of directors as Secretary 2012—2014. She was always a willing and positive volunteer and we still miss her. Not long after she left, she and her husband had their first son, and not long after that another came along (see photo).

Catching up with her, I learned that Camille and family sold their home, purchased and re-purposed one of the huge school buses (dubbed "Cowabungabus"), and have been travelling the country since. I attached two photos showing her creativity:

The chevron wall is made from old pallets she sanded, stained, and pieced, and in the lower right corner of the photo below is a table top she made using "pecky cy-press" filled with pigmented epoxy. GORGEOUS!

Autumn in PCP Preserves

Pond at Eastwood, December 2012.

Rhexia aristosa seed pod. Pondberry, November 2011.

Snapping turtle baby at Eno Diabase,11-2016

Eno Diabase picnic, Nov. 2016

Schweinitz's seedhead at Harvest Field, Nov. 2010

***Symphoricarpos orbiculatus*, Eno Diabase, Nov. 2016**

***Celastrus scandens*, Cedar Cliff, Nov. 2012**

Swamp pink at DuPont Forest.

Tackling woody overgrowth at Cedar Mountain Bog..

Removing woody debris.

FoPC Website: www.ncplantfriends.org

New Editor

Having been responsible for the FOPC newsletter since the beginning of our organization in 2008, I am delighted to turn it over to a new editor: **Terri Buckner**. Terri will begin with the Winter issue and I am sure she would appreciate any photos, articles, or suggestions for the newsletter. We have established an Editorial Board to provide her with guidance and assistance. Terri can be reached at:

tbuckner@ibiblio.org

Welcome, Terri; we wish you all the best!

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The true identity of *Marshallia grandiflora*, an extinct species, and the description of *Marshallia pulchra* (Asteraceae, Helenieae, Marshalliinae)

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Abstract

Marshallia grandiflora (Asteraceae, Helenieae, Marshalliinae) is recognized as the 53rd extinct species to North America, and an endemic species of the Blue Ridge physiographic province of North Carolina. A new name, *Marshallia pulchra*, is provided for the more broadly ranging species that has been confounded with and generally considered conspecific with *M. grandiflora.* Morphologic and biogeographic data clearly separates two species within the previous concept of *M. grandiflora. Marshallia grandiflora and M. pulchra* are compared with their sister species, *M. legrandii*. Cytological data is given for *M. legrandii*, including the first report of B-chromosomes in the genus.

Keywords: *Marshallia grandiflora, Marshallia pulchra, Marshallia legrandii, endemic, extinct, Asteraceae, conservation concern, morphology, cytology*

Introduction

Marshallia Schreber (1791: 810, Asteraceae) is a genus of 7–11 species ranging from Pennsylvania and West Virginia, south to Florida and west to Nebraska, Oklahoma and Texas (Watson 2006; Weakley 2015). Beadle and Boynton (1901) completed the first comprehensive study of the genus recognizing a total of 11 species and naming three; *M. grandiflora* Beadle & Boynton (1901: 7), *M. mohrii* Beadle & Boynton (1901: 8), and *M. ramosa* Beadle & Boynton (1901: 8). Channell (1957) completed a monograph recognizing 8 species and 2 varieties. More recently Watson (2006) recognized 7 species, while Weakley (2015) recognized 8 species and 1 variety, including *M. legrandii* Weakley (2012: 2), the most recently described species in the genus (Weakley & Poindexter 2012). Contemporary phylogenetic work in the genus (Hansen and Goertzen 2014) utilizing nrDNA ITS recovered a sister relationship between *M. grandiflora* and *M. legrandii.*

Marshallia grandiflora has been regarded as a species "vulnerable to extinction" (ranked as G3) (NatureServe 2019). It is a conservation target in every US State where it is known to occur. Kentucky and Pennsylvania assign conservation ranks of S1—Critically Imperiled while Tennessee and West Virginia assign the rank of S2—Imperiled. North Carolina considers this species as State Historical having not been seen since 1919 (*Crayton s.n.*, NY!).

In 2016, the first author was studying images of *Marshallia grandiflora* from North Carolina, when he was struck at the apparent morphologic disparity between the North Carolina specimens and plants he had seen in the wild in Pennsylvania. He reached out to the second and third authors (who had been involved in *Marshallia* systematic studies) to see if they had critically examined *M. grandiflora* across its range, which they had not. After discussions and examinations of images available online at the New York Botanical Garden C.V. Starr Virtual Herbarium (2019), SERNEC data portal (SERNEC 2019), and Smithsonian National Museum of Natural History type database (2019), we decided to conduct a critical examination of *M. grandiflora s.l.* and its sister species, *M. legrandii*.

Here we present the results of a morphologic analysis of *M. grandiflora* from throughout the geographic range of the species. We then present a taxonomic revision and include a key to species, descriptions, and representative

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specimens. We recognize two species within *M. grandiflora s.l.* In addition, we provide chromosomal data for *M. legrandii*.

Materials and methods

We studied the morphologic variation and habitat of *M. grandiflora s.l.* from across its entire geographic range. We examined specimens at NCU and loaned additional herbarium specimens from CM, EKU, GH, NY, and US. We studied additional specimens through the SERNEC Data Portal (SERNEC 2019) from WVU. Herbarium acronyms follow Index Herbariorum (Thiers 2019). We created distribution maps from these specimens. Thus, each dot on the map represents at least one voucher specimen.

We selected a representative subset of specimens for statistical analysis. Specimens were selected from throughout the geographic and morphologic range of *Marshallia grandiflora s.l.* Measurements were taken only from mature complete specimens with ripened cypselae. Ripened cypselae are often not present in herbarium specimens as *Marshallia* is usually collected in flower, making most specimens incomplete for this study. Specimens measured for statistical analysis are denoted by an asterisk (*) after the herbarium acronym in the representative specimens examined section. Types measured or referenced are denoted with a superscript letter representative of their status (e.g., ^{H} =Holotype, ^{I}=Isotype).

Statistical Analysis—We selected 16 specimens (5—North Carolina *M. grandiflora*, 9—non-North Carolina *M. grandiflora*, and 2—*M. legrandii*) for detailed morphologic analysis. These specimens represent the entire extant geographic range of *Marshallia grandiflora* (4 States), 5 apparently distinct populations from 2 counties of western North Carolina, and the entire known global range of *M. legrandii,* a 2-site endemic from North Carolina and Virginia (Weakley & Poindexter 2012). After careful study of numerous herbarium specimens, we selected a set of morphologic characters for analysis. These were cypselae length, cypselae (pappus) scale length, leaf length, leaf width, and culm width. Summary statistics including means, standard deviation, ranges, as well as component loadings for select variables are provided for these characters (Table 1 & 2).

TABLE 1. Morphologic characters measured on *M. grandiflora, M. pulchra,* and *M. legrandii* showing mean ± 1 standard deviation and range (in parentheses) for each character. Superscripts following standard deviations denote statistically significant differences in means between individual taxa. *n* = sample size. All measurements in mm, unless otherwise noted. An asterisk precedes characters utilized within the PCA.

Characters Examined	*M. grandiflora (n=4)*	*M. pulchra (n=9)*	*M. legrandii (n=3)*	Anova F
*Cypselae Length	4.6 ± 0.65^{a}	3.08 ± 0.49^{b}	3.33 ± 0.31^{b}	10.1965
	(4–5.5)	(2.3–3.5)	(3–3.6)	
*Cypselae Scale Length	2.24 ± 0.3^{a}	1.66 ± 0.36^{b}	1.27 ± 0.29^{b}	8.4625
	(2–2.7)	(0.9–2)	(1.1–1.6)	
Leaf Length (cm)	22.25 ± 2.55^{a}	12.86 ± 1.47^{b}	15.77 ± 1.42^{a}	38.8613
	(18.7–24.5)	(10.5–14.5)	(14.5–17.3)	
Leaf Width (cm)	2.85 ± 0.3^{a}	1.91 ± 0.4^{b}	0.97 ± 0.23^{c}	24.5869
	(2.4–3)	(1.3–2.5)	(0.7–1.1)	
*Leaf Length/Width Ratio	7.81 ± 0.32^{a}	6.94 ± 1.4^{a}	17.33 ± 6.41^{b}	21.4904
	(7.4–8.17)	(5.43–9.33)	(13.18–24.71)	
Culm Width	2.04 ± 0.44^{a}	1.81 ± 0.39^{a}	1.77 ± 0.4^{a}	0.5411
	(1.4–2.5)	(1.2–2.5)	(1.3–2)	

When multiple individuals were present on a single herbarium sheet, we measured all characters from a single individual. When measuring a character that was present more than once per individual (i.e. cypselae) we measured the one with the greatest value. Measurements were made from only mature specimens. Leaf length was measured from the leaf tip to the base of the sheath. Leaf width was measured at the widest point. Cypselae length refers to only

the achene body. Cypselae scales were measured from the tip to the meeting location on the cypselae. Culm width was measured below the inflorescence but below the portion of obvious flaring where the inflorescence heads meet the culm.

TABLE 2. List of morphologic characters examined with component loadings and percent variance (in parentheses) explained by the first two Principal Components.

Characters Examined	Component 1 (48.7%)	Component 2 (34.5%)
*Cypselae Length	0.6521	0.6509
*Cypselae Scale Length	0.8700	-0.01381
*Leaf Length/Width Ratio	-0.52744	0.7819

We conducted statistical tests using JMP® Pro 13 (SAS 2016). We initially assessed Goodness-of-fit for each measured character with the Shapiro-Wilk W Test to determine if any variable deviated from a normal distribution. In addition, we performed a univariate Bartlett Test of homogeneity to examine the potential occurrence of unequal variances between taxa for a given character.

An Analysis of Variance (ANOVA) was conducted to test the null hypothesis that there is no morphological discontinuity between the *M. grandiflora* populations from North Carolina vs. non-North Carolina, and *M. legrandii*. The difference in means of each character by taxon were also tested for significance.

Lastly, we utilized multivariate Principal Components Analysis (PCA) to visually inspect the amount of morphological variation in the data set as projected in ordination space and to elucidate which characters were most diagnostic for the three taxa. Before conducting the PCA, we conducted a correlation analysis on all characters. To avoid weighing potentially redundant morphologic variables, we excluded those that were highly correlated ($r > 0.7$) from our PCA. The dataset was standardized so each variable had a mean of 0 and a standard deviation of 1. We also investigated the potential for character variation associated with a latitudinal gradient. Similar methods have been useful in numerous morphologic studies (Naczi *et al.* 1998, Saarela *et al.* 2003, Kjaer *et al.* 2004).

An additional examination of the PCA was conducted to see if there were any morphologic distinction between the two geographically disjunct areas of *M. grandiflora* from Pennsylvania/West Virginia and Kentucky/Tennessee.

Chromosomal Study—We collected heads of *Marshallia legrandii* from plants occurring at the type location in Granville Co., North Carolina. These were fixed in Carnoy I solution for 24 hours, then transferred to 70% ethanol. Anthers were extracted from material positioned in the intermediate zone between nearly open and closed flowers. These anthers were then stained with acetocarmine and squashed in Hoyer's mounting medium. We isolated multiple cells at metaphase with a trinocular Leica phase contrast microscope at 1000x magnification and imaged representative meiotic figures with a Canon Rebel T3i camera attachment.

Geographic Distribution—We obtained latitude and longitude for each specimen examined using Google Earth™. These data were subsequently used to create range-wide distribution maps using ArcMap 10.4 for Desktop (ESRI 2015).

Ecology—Habitat information for each putative taxon was qualitatively compiled based on herbarium label information, personal observations, and discussions with experts who have observed these species in the field.

Results

Morphological Studies

Of the characters assessed (Table 1) leaf length (and by association, leaf length/width ratio) demonstrated significant deviation from a normal distribution. Based on these findings, as well as the low sample numbers included within our assessment, we used the nonparametric Wilcoxon method to compare means for each pair of taxa across variables. Likewise, leaf length/width ratio was found to have significantly unequal variances as informed by the Bartlett Test of homogeneity, thus requiring log transformation of this variable prior to performing an ANOVA and testing of means (Table 1). Culm width was excluded from further analysis as it was not statistically different among any of the putative taxa (Table 1). North Carolina plants of *Marshallia grandiflora* are statistically different from non-North Carolina *M. grandiflora* in cypselae length, cypselae scale length, leaf length, leaf width, and leaf length/width ratio (Table

1). *Marshallia grandiflora* from North Carolina differs statistically from *M. legrandii* in cypselae length, cypselae scale length, leaf width, and leaf length/width ratio (Table 1). Non-North Carolina *Marshallia grandiflora* differs statistically from *M. legrandii* in leaf length, leaf width, and leaf length/width ratio (Table 1).

Our correlation analyses indicated that leaf length and leaf width were strongly correlated (r >0.7) and were thus excluded from further PCA as independent characters. All remaining characters retained were not highly correlated and were thus included in downstream analyses. Furthermore, no correlation of morphologic characters with latitude was observed. Our PCA represents the three most significant morphological characters: cypselae length, cypselae scale length, and leaf length/width ratio.

The characters with the two highest *F*-values were plotted graphically (Fig. 1) and reveal no overlap between groups. This shows that by using just leaf length/width ratio and cypselae length *M. grandiflora* from North Carolina can be distinguished from non-North Carolina *M. grandiflora*. A scatterplot of scores of components 1 and 2 from the PCA clearly shows three distinct groups (Fig. 2). Principal component 1 accounts for 48.7% of the variation and component 2 accounts for 34.5%, for a combined total of 83.2% of the variation explained (Table 2). All specimens of *M. grandiflora* from North Carolina cluster together. All specimens outside of North Carolina cluser in a distinct group, as do *M. legrandii*.

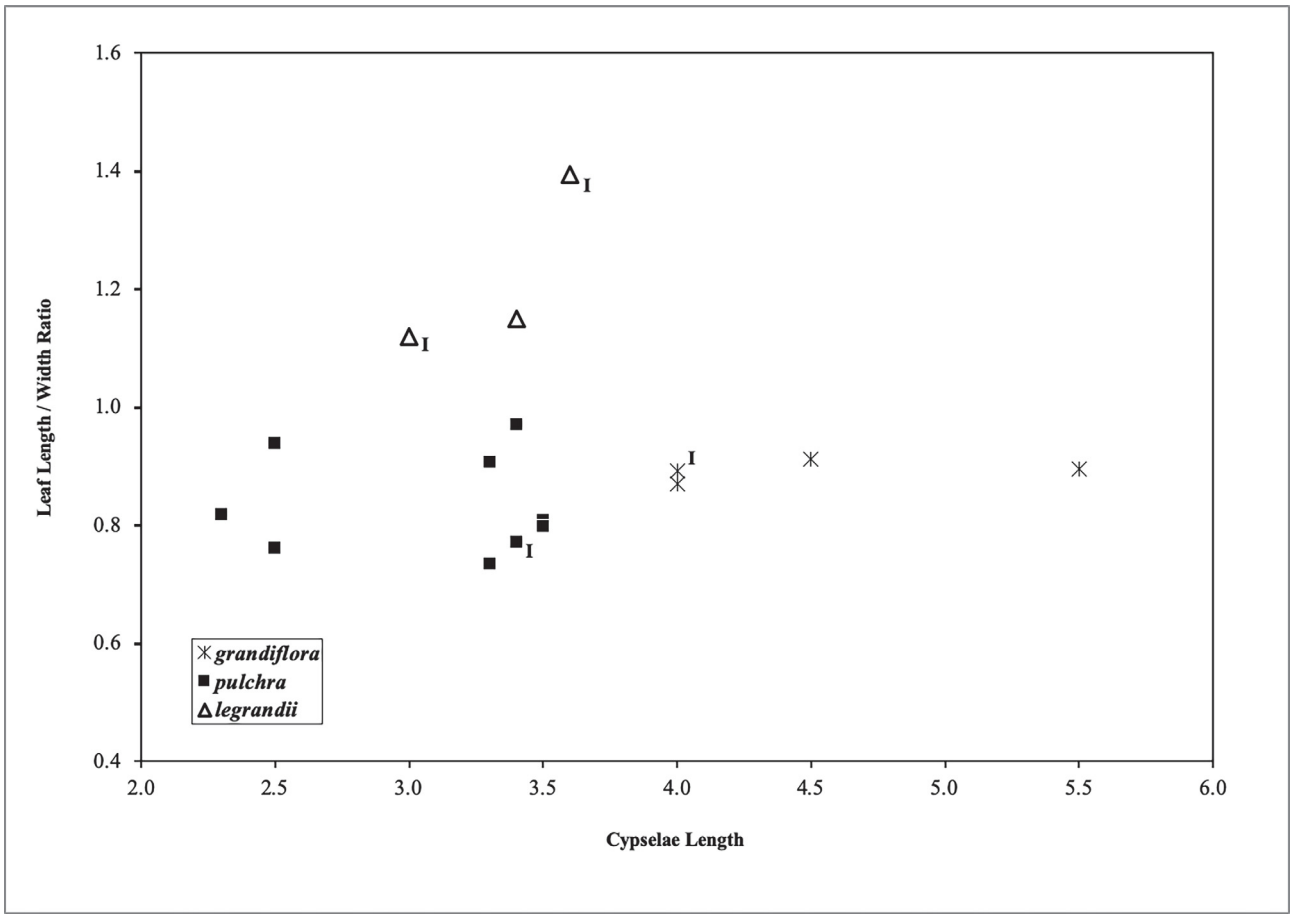

FIGURE 1. Scatterplot of the two most important characters (cypselae length vs. leaf length/width ratio) for distinguishing *Marshallia grandiflora s.s.* as revealed by ANOVA.

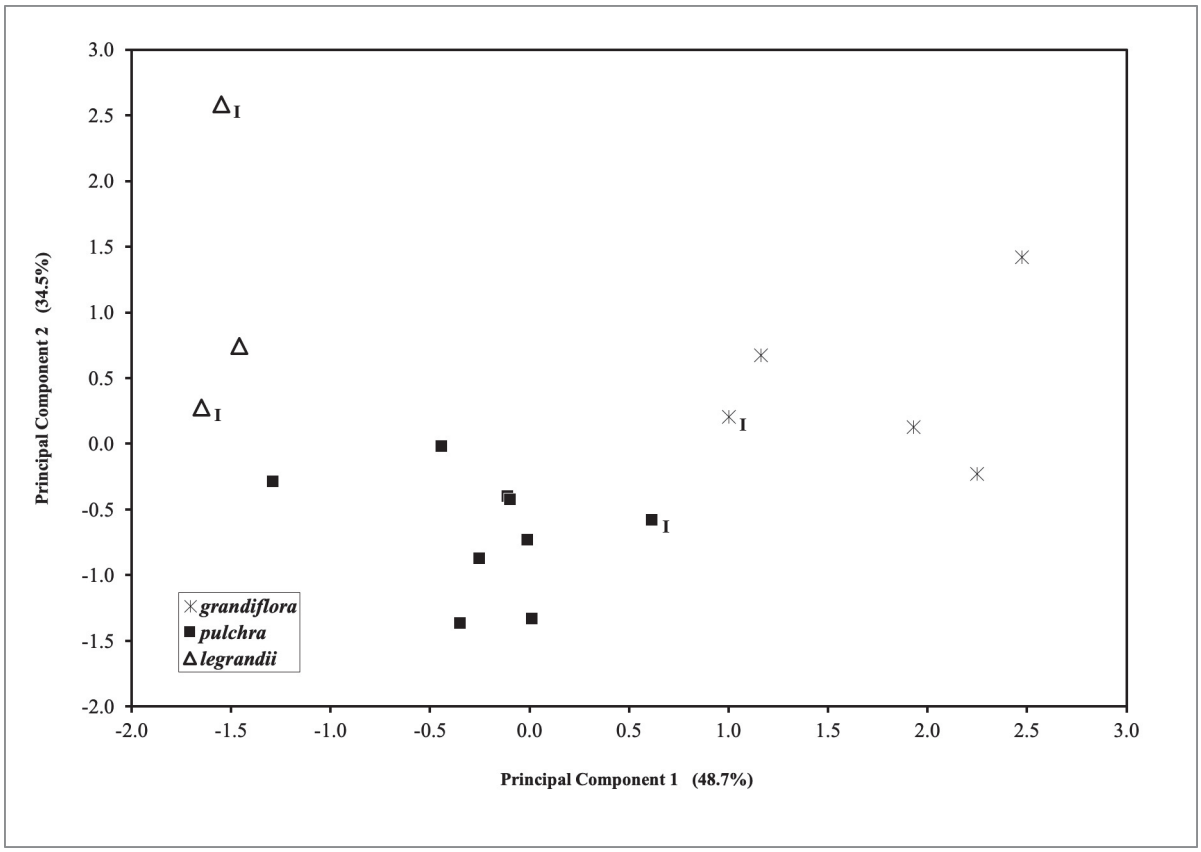

FIGURE 2. Principal components analysis of the *Marshallia "grandiflora*" species complex.

FIGURE 3. Cypselae of A) *Marshallia grandiflora* (Henderson Co., NC, *Smith s.n.*, NY), B) *M. pulchra* (Cumberland Co., TN, *Patrick 1290*, EKY), and C) *M. legrandii* (Granville Co., NC, *Weakley 7274*, NCU, Isotype). Scale = 1 mm.

No morphologic distinction was found between the two geographically disjunct populations of *M. grandiflora* occurring in Pennsylvania/West Virginia and Kentucky/Tennessee. Specimens from each geographic region are embedded within and throughout the cluster of specimens from these geographic regions in the PCA.

FIGURE 4. Image of *M. grandiflora* Holotype.

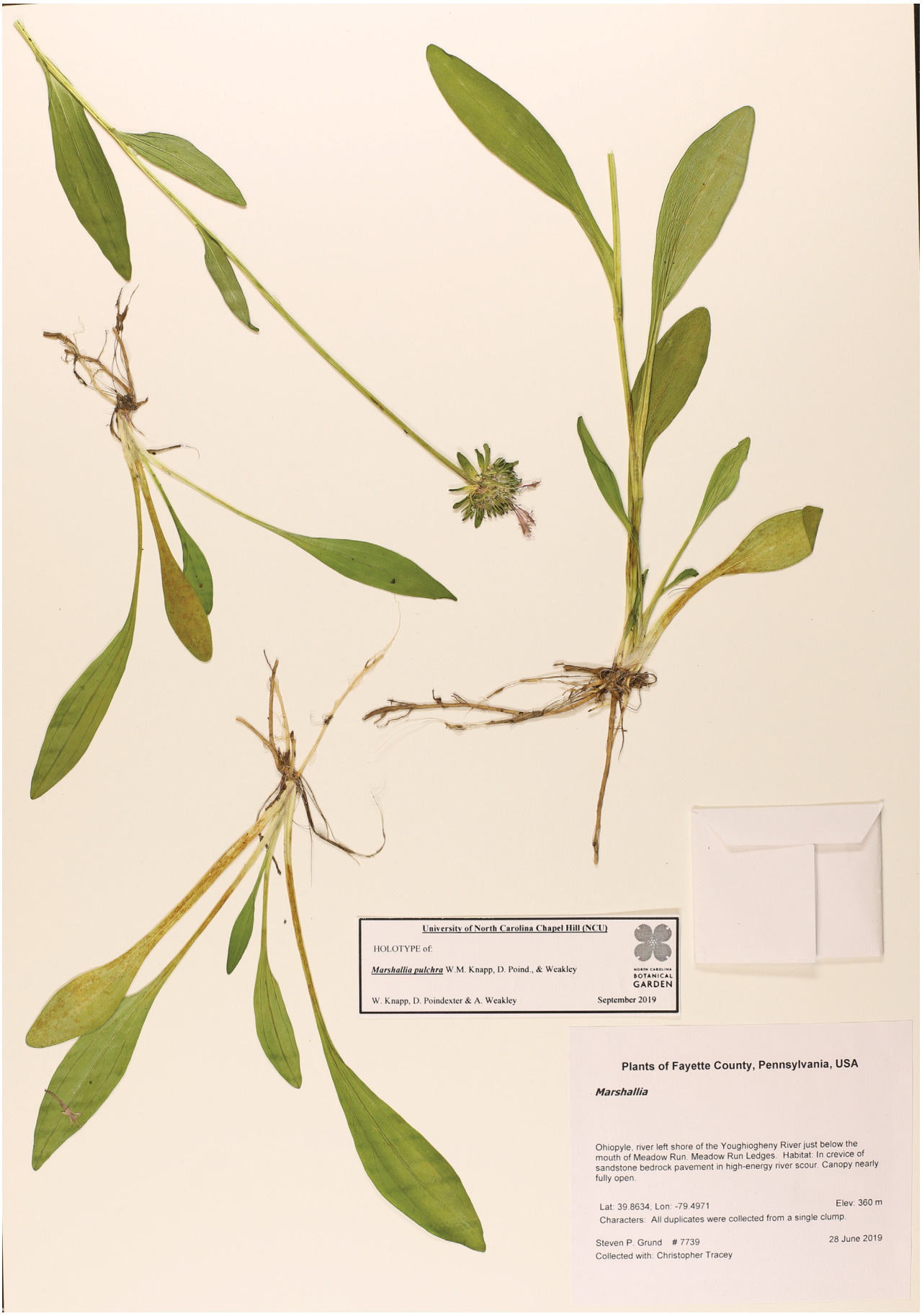

FIGURE 5. Image of *M. pulchra* Holotype.

FIGURE 6. Image of *M. legrandii* Holotype

The western North Carolina plants attributed to *Marshallia grandiflora* can be easily distinguished from the non-North Carolina material by the size of the cypselae and the cypselae size and shape (Fig. 3). The cypselae of *M. legrandii* are most similar to non-North Carolina *M. grandiflora*, but the cypselae scales are shorter (Fig. 3). The leaves of *Marshallia grandiflora* from North Carolina are much longer and wider than those found outside of North Carolina (Fig. 4 & 5). *Marshallia legrandii*, has much longer and thinner leaves easily separating it from *M. grandiflora s.l.* (Fig. 6).

Chromosomal Analysis

Our single count for *M. legrandii* is diploid (n=9; Fig. 7). The majority of cells with meiotic figures were at late diakinesis-early metaphase in all of our preparations. Interestingly, all clearly countable cells presented two satellite chromosomes (a.k.a, B chromosomes). No anomalous observations were observed.

Geographic Distribution

In western North Carolina *Marshallia grandiflora* was known only from Henderson and Polk Counties, from the Blue Ridge Physiographic Province (Fig 8). The non-North Carolina *M. grandiflora* is found from Pennsylvania and West Virginia then again in Kentucky and Tennessee (Fig. 8), where it is restricted to the Appalachian Plateau Physiographic Province with the exception of one location in Greenbriar Co., WV, where it appears to reach into the Ridge and Valley Physiographic Province (*Wilson s.n.*, WVU!). The sister species to *M. grandiflora s.l.*, *M. legrandii*, is known from two counties, Granville Co., North Carolina and Halifax Co., Virginia, where it is endemic to the Piedmont Physiographic Province (Fig. 8).

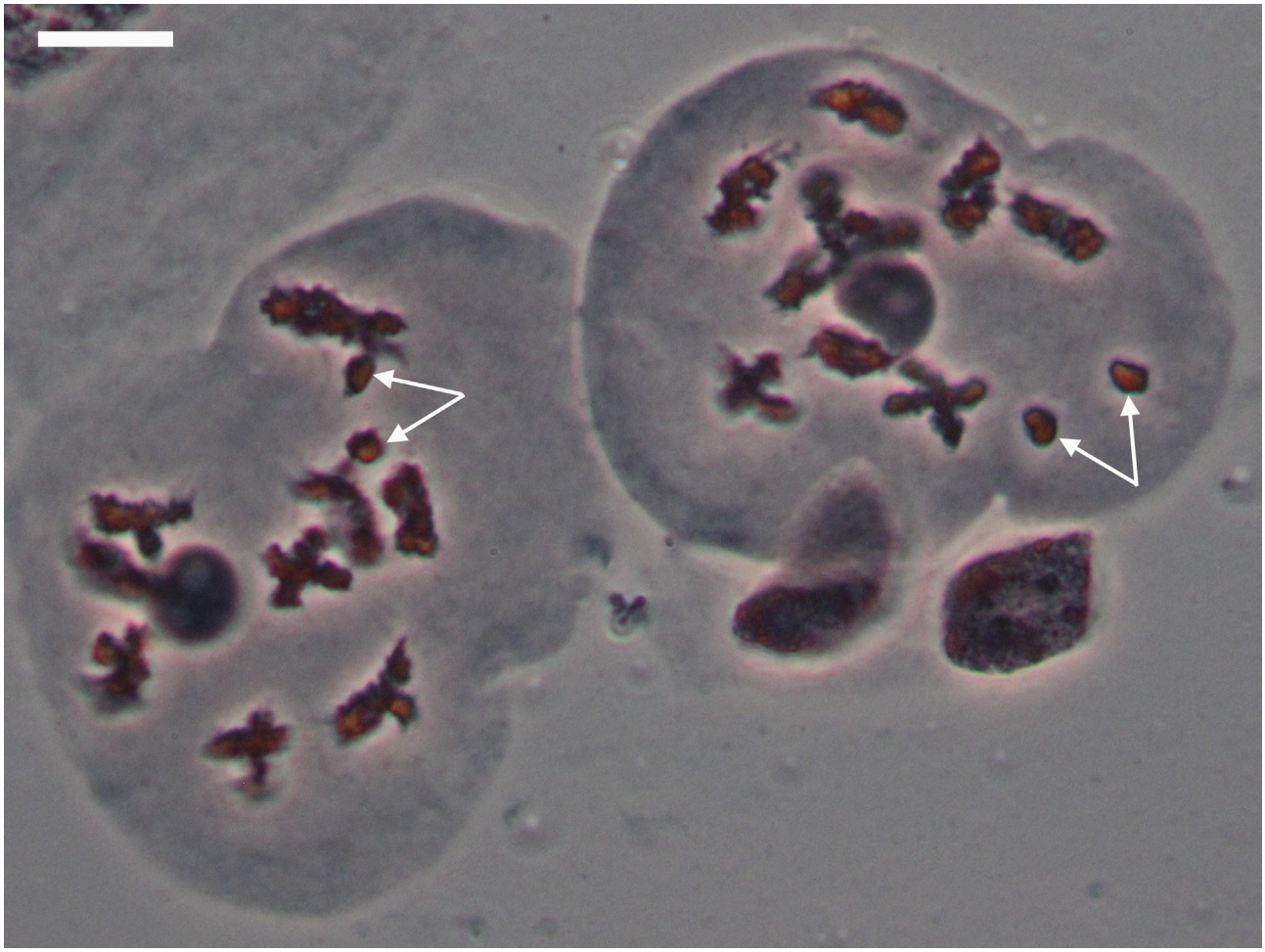

FIGURE 7. Meiotic chromosomes of *Marshallia legrandii* (n=9II+2B) at late diakinesis-early metaphase (nucleoli visible) with 2 cells present. Note: arrows indicate B-chromosomes. Scale = 10 µm. Sampled live material is from the holotype population (Granville Co., NC).

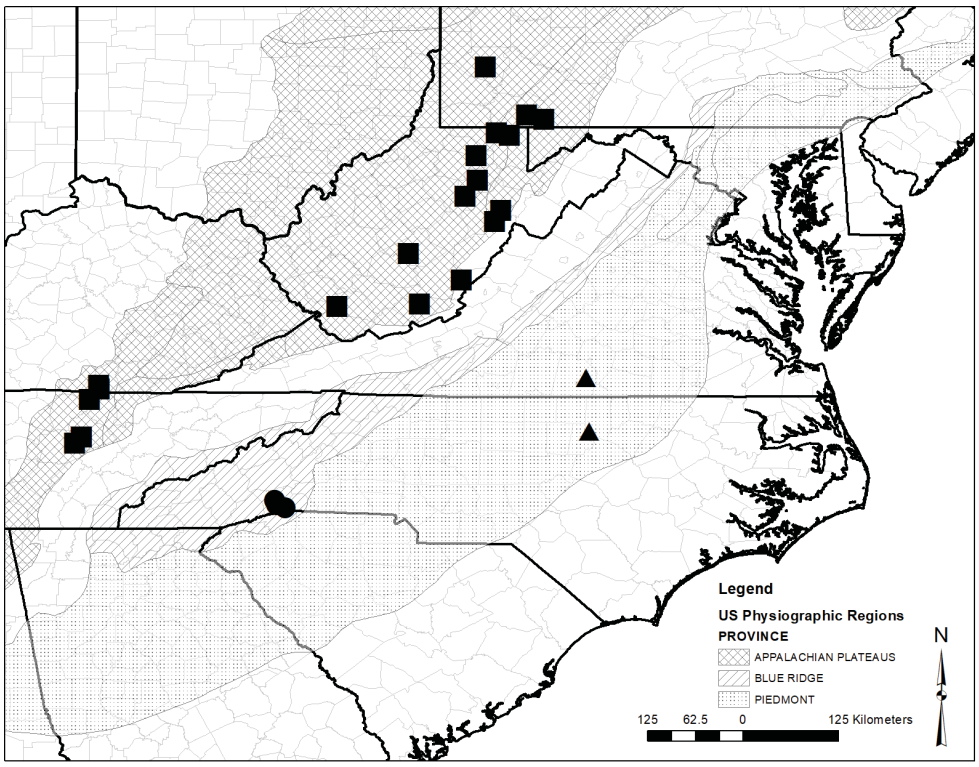

FIGURE 8. Geographic range of *M. grandiflora* (circles), *M. pulchra* (squares) & *M. legrandii* (triangles) within eastern North America and their respective occurrence within major United States physiographic provinces. Inset map with gray box depicts focal geographic area in context of North America.

Ecology

There are apparent ecological differences between the western North Carolina specimens of *M. grandiflora* and the non-North Carolina material. Though data is often scant on old herbarium records, the following habitat data is listed on herbarium labels from North Carolina material: borders of swamp (*Smith s.n.,* NY!), damp soil (*Beadle & Boynton 4215b*, NY!), dry soil (*Beadle & Boynton 4215a,* NY!). Habitat from specimens from outside of North Carolina include rock crevices along high-energy river scour (*Grund 7739*, NCU!), boulder bars (*Patrick & Perkins 1290,* EKU!), cobble bars (*Campbell s.n.,* BEREA!), and gravel bars (*Patrick et. al 1264*, EKU!). These data argue for two distinct ecological species. The ecological distinction between *M. grandiflora s.l.* and *M. legrandii* is likewise striking as *M. legrandii* occupies Diabase Barrens and glades of the Piedmont of north-central North Carolina and south-central Virginia.

Discussion

Marshallia grandiflora, as currently circumscribed, includes two morphologically, ecologically, and biogeographically distinct taxa. The holotype of *M. grandiflora* (*Beadle & Boynton 1215a,* US!) is from southwestern North Carolina, where it is now presumably extinct. We hypothesize that the populations of *Marshallia* in southwestern North Carolina represented a narrowly endemic species. As such, a new name is required for the remaining members of the more widespread, rare taxon formerly attributed to *M. grandiflora*, known from west of the Blue Ridge in northeastern Tennessee, southeastern Kentucky, eastern West Virginia, and southwestern Pennsylvania. To this end, we here propose "*Marshallia pulchra*".

The extremely limited two county geographic range of *M. grandiflora s.s.* (Henderson and Polk Counties, North Carolina) represents a previously unrecognized extinction hotspot, which was also once home to two additional extinct plant species; *Narthecium montanum* (Small) C.H. Grey (1938: 446) and *Orbexilum macrophyllum* (Rowlee) Rydberg (1919: 5). Interestingly, a syntype of *N. montanum* was also collected by Crayton in 1919 (*s.n.*, NY!), last collected

in 1933 (*Clement s.n.*, PH!), one month prior to the last known collection he made of *M. grandiflora* from the general area. *Orbexilum macrophyllum* was first discovered in 1897 (*Rowlee s.n.*, NY!), with the last known collection made a mere two years later in 1899 (*Townsend s.n.*, WTU!).

Marshallia grandiflora s.s. represents the 53rd extinct plant species to be identified in North America north of Mexico (Knapp *et al.*, 2019). It is surprising to note that after its last collection 101 years ago, it is only now being recognized as a putatively extinct two county endemic. Discoveries such as this underscore the scientific importance of natural history collections. No other species of *Marshallia* are known from the Blue Ridge of North Carolina for this taxon to be confused with, and given the high level of anthropogenic disturbance in these counties, our extinction hypothesis seems most likely. Reports of *M. trinervia* (Walter) Trelease (1891:196) from the Blue Ridge of North Carolina in Weakley (2015) are based on cultivated material (*Foust s.n.*, NCU!).

Watson and Estes (1990) found populations of *M. pulchra* [reported as *M. grandiflora*] from Tennessee, Pennsylvania, and West Virginia to be diploid (2n=18), as is our sample *M. legrandii*. Interestingly, all cells of *M. legrandii* presented two satellite chromosomes (a.k.a., B chromosomes; Fig. 3). To our knowledge, this is the first documentation of the presence of supernumerary chromosomes in the genus, though they are most frequent and well-known in the highly diverse Poaceae and Asteraceae (Jones et al. 2008). Unfortunately, no counts can be made of the presumably extinct populations of *M. grandiflora* from North Carolina.

Taxonomic Treatment

1. ***Marshallia grandiflora*** Beadle & Boynton. TYPE:—U.S.A. North Carolina. Polk Co.: Dry soil near Saluda, 22 Jul 1898, *C.D. Beadle & F.E. Boynton 4215a* (Holotype: US!; Isotypes: GH!, NY!).

Perennial 59–89 cm, fibrous-rooted from caudices. **Stems** erect, unbranched, striate, becoming non-striate towards base, sparsely to densely short pubescent. **Leaves** basal and cauline, alternate; basal petiolate, upper sessile or reduce; blades 3-nerved, elliptical, broadly oblanceolate, or spatulate, 18.7–24.5 cm long × 2.4–3.0 cm wide; apex acute to obtuse. **Heads** single, 2.0–2.8 cm in diameter. **Peduncle** 2.5–28.5 cm. **Phyllaries** 8–14 mm long × 2–4 mm wide, apices acute. **Paleae** ± linear, apices acute. **Corollas** light pink to purple, 12–15 mm long, tubes 8–10 mm, lobes 2.5–5.2 mm long × 0.3–0.5 mm wide. **Anthers** White-gray, 3.3–3.5 mm long, the column exerted beyond the corolla throat. **Cypselae** 4.0–5.5 mm long, 0.4–0.6 mm wide, hairy on the ribs at maturity. **Pappi scales** 2.0–2.7 mm long, attenuate, scale margins serrulate (Fig. 4).

The type specimens listed above have usually been treated as syntypes or isotypes. For the sake of clarity, the US collection represents the intended holotype as indicated by "type" on the label.

Geographic Range—*Marshallia grandiflora* was historically restricted to the southern Blue Ridge Physiographic Province of the Southern Appalachians in Henderson and Polk Counties, North Carolina (Fig. 8).

Etymology—The specific epithet "*grandiflora*" implies large flowers, though this descriptor does not differ significantly in comparison with its two extant sister species.

Common Name—Appalachian Barbara's-buttons or Large-flowered Barbara's-buttons.

Ecology—Bogs, swamp margins, and adjacent drier soils (this description is somewhat speculative, based on interpretation of the very limited herbarium collections).

2. ***Marshallia legrandii*** Weakley. TYPE:—U.S.A. North Carolina. Granville Co.: Picture Creek Diabase Barren, north of Butner, 22 July 2003, *A.S. Weakley, L.M. Giencke, & J.P. Perry III 7274* (Holotype: NCU!).

Perennial 60–80(–100) cm, fibrous-rooted from caudices. **Stems** erect, unbranched, striate, especially immediately below the head. **Leaves** basal and cauline, alternate; the first basal (most proximal) leaves withering by anthesis, basal petiolate, blades 3-nerved, lanceolate to oblanceolate (sometimes linear-oblanceolate), 15–30 cm long × 7–20 mm wide; apex acute to acuminate. **Heads** single, 26–45 mm in diameter. **Peduncle** 21–36 cm. **Phyllaries** 8–11 mm long × 2.5–3.7 mm wide, apices acute to broadly acute. **Paleae** ± acute-acuminate and only gradually and slightly dilated towards the apex. **Corollas** pale to deep pink, 10–13 mm long, tubes ca. 10 mm, lobes 5–7.5 × 0.7–1.1 mm. **Anthers** Purple, 3–3.5 mm long, the column exerted beyond the corolla throat. **Cypselae** 3.0–3.2 mm long, 1.5–1.6 mm wide, hairy on the ribs at maturity. **Pappi scales** 1.1–1.3 mm long, attenuate, scale margins entire to scabridulous (Fig. 6).

Geographic Range—*Marshallia legrandii* is restricted to Granville Co., North Carolina and adjacent Halifax Co., Virginia within the Piedmont Physiographic Province of these States (Fig. 8).

Etymology—The specific epithet "*legrandii*" commemorates the original discoverer, Harry Legrand, and emphasizes his immense contributions to natural heritage conservation.

Common Name—Oak Barrens Barbara's-buttons, Legrand's Barbara's-buttons, or Tall Barbara's-buttons.

Ecology—Restricted to fire-maintained mafic (diabase, greenstone) rock barrens, savannas, and woodlands.

3. ***Marshallia pulchra*** W.M. Knapp, D.B. Poind. & Weakley ***sp. nov.*** (Fig. 5) TYPE:—U.S.A. Pennsylvania. Fayette Co.: Ohiopyle, river left shoreline of the Youghiogheny River just below the mouth of Meadow Run. Meadow Run Ledges. Habitat: In crevice of sandstone bedrock pavement in high-energy river scour. Canopy nearly fully open. 39.8634, -79.4971, 28 June 2019, *S.P. Grund 7739 w/ C. Tracey* (holotype: NCU!; isotype: BRIT!, CM!, GH!, MO!, NY!, US!).

Perennial 20–90 cm, fibrous-rooted from caudices. **Stems** erect, unbranched, striate, becoming non-striate towards base, sparsely to densely short pubescent. **Leaves** basal and cauline, alternate; basal petiolate, upper sessile or reduce; blades 3-nerved, elliptical, broadly oblanceolate, or spatulate, 12–25 cm long × 14–24 mm wide; apex acute to obtuse. **Heads** single, 20–25 mm in diameter. **Peduncle** 10–20 cm. **Phyllaries** 8–11 mm long × 2–4 mm, apices acute. **Paleae** ± linear, apices acute. **Corollas** pink to lavender, 10–15 mm long, tubes ca. 10 mm, lobes 3–7 × 0.5–0.8 mm. **Anthers** Purple, 3–3.7 mm long, the column exerted beyond the corolla throat. **Cypselae** 2.3–3.5 mm long, 0.4–0.6 mm wide, hairy on the ribs at maturity. **Pappi scales** 0.9–2.0 mm long, attenuate, scale margins serrulate (Fig. 5).

Geographic Range—*Marshallia pulchra* is found in two distinct geographic areas: a more northern area in southwestern Pennsylvania south to southern West Virginia, and a more southern area in the Cumberland Plateau region of se. Kentucky and northeastern Tennessee (Fig. 8). Reports from Maryland are unvouchered and excluded. The northern extant limit of the species is Fayette Co., Pennsylvania with a historical collection from Allegheny Co., Pennsylvania from "near Pittsburgh" (*H. Leighton s.n.*, PH).

Etymology—The specific epithet "*pulchra*" was selected to emphasize the beauty of the species.

Common Name—Beautiful Barbara's-buttons or Monongahela Barbara's-buttons.

Ecology—Restricted to high energy river scour or ice scour of, but not limited to, the Big South Fork, Casselman, Cumberland, Obed, Tygart, and Youghiogheny.

Key to the Grandiflora Species Complex.

Supplemental key to Weakley's Flora beginning at key lead 8 (Weakley 2015).

8. Basal and lower cauline leaves (2–) 3–13 (–20) cm long (including the petiole), (5–) 10–20 (–30) mm wide, averaging about 6× as long as wide (including the petiole), the apex obtuse to rounded, length × width ration of 5.4–9.3; pappus scales 1.5–2.2 mm long; plants (2–) 3–5 (–8.5) dm tall; cypselae with absent or scattered resin-dots between the ridges; [Blue Ridge Mountains and more western, sedimentary Appalachians].
9. Cypselae 4.0–5.5 mm long, pappus scales 2.0–2.7 mm; longest leaves 18.7–24.5 cm long × 2.4–3.0 cm wide; [Blue Ridge of southwestern NC] ..... ***M. grandiflora***
9. Cypselae 2.3–3.5 mm long, pappus scales 0.9–2.0 mm long; longest leaves 10.5–14.5 cm long × 1.3–2.5 mm wide;[sedimentary rock Appalachians of TN, KY, WV, and PA] ..... ***M. pulchra***
8. Basal and lower cauline leaves (8–) 15–25 (–32) cm long (including the petiole), (3–) 7–12 (–15) mm wide, averaging about 10× as long as wide (including the petiole), the apex acute to acuminate, length × width ratio of 13.2–24.7; pappus scales 1.0–1.3 mm long; plants (4–) 6–9 (–10) dm tall; cypselae with copious resin-dots between the ridges; [Piedmont of NC and VA] ..... ***M. legrandii***

Scientific Conservation Ranking: In North America, the primary scientific conservation ranking system for plant species is that of NatureServe, similar to the international ranking system, IUCN (2010), widely used globally. *M. grandiflora* should be given a GX NatureServe rank and Extinct (EX) IUCN Red List rank. The rank of *Marshallia legrandii* is unaffected by these changes and G1 NatureServe rank should be retained. We propose an IUCN rank of Critically Endangered given the extremely narrow Area Of Occupancy (<10 km^2), and Extent of Occurrence (of <100 km^2), a severely fragmented distribution, and obvious habitat degradation caused by fire exclusion causing the majority of plants to be restricted to small woodland openings and artificial habitats (i.e. powerline rights-of-ways). The newly named *M. pulchra* (largely representing a broader concept of "*M. grandiflora*") retains a NatureServe G-rank of G3 and an IUCN Red List rank of Vulnerable (Walter & Gillett 1998).

US Legal Conservation Status: *M. grandiflora* is "under review" for federal listing at the time of this publication. The taxonomic separation of the previous concept of the species *M. grandiflora s.l.* into two species, *M. grandiflora s.s.* and *M. pulchra*, impacts a potential listing decision of both species, with *M. grandiflora* potentially extinct, and *M. pulchra* with a narrower distribution and more limited ecological situation. *Marshallia legrandii* has not been considered for federal listing under the US Endangered Species Act (USEAS) but is unquestionably more imperiled than *M. pulchra*.

Representative Specimens Examined:

***Marshallia grandiflora*. U.S.A. North Carolina. Henderson Co.:** Borders of swamp, Muddy Creek, 24 Aug 1881, *J.D. Smith s.n.* (NY*, US); Damp soil near Hendersonville, 14 Sep 1898, *C.D. Beadle & F.E. Boynton 4215b* (US*); May 1902, [no collector indicated], (NCU); Near Flat Rock, Aug 1919, *F.M .Crayton s.n.* (NY*). **Polk Co.:** Dry soil near Saluda, 22 Jul 1898, *C.D. Beadle & F.E. Boynton 4215a* (GH[I], NY*[I], US[H]). **[no county indicated]**, Nickens, [no date] *M. Hyams s.n.* (NY*).

***Marshallia pulchra.* U.S.A. Kentucky. McCreary Co.:** Big South Fork of the Cumberland River near gaging [sic] station. End of KY 1324. Southern part of Barthell, KY Quad, 9 July 1978, *M.E. Medley, R. Cranfill, & J. MacGregor 208-78* (EKU*); Found near Big Shoals of Big South Fork on Cobble Bar on west side, 28 July 1989, *J. Campbell s.n.* (BEREA). **Pennsylvania. Allegheny Co.:** Near Pittsburgh, 1915, *H. Leighton s.n.* (PH). **Fayette Co.:** Ohiopyle Falls, July 1886, *J.A. Shafer s.n.* (CM); Ohiopyle Falls, sandy river bank above falls, 1 July 1900, *J.A. Shafer & O.P Medsgar s.n.* (CM); Ohiopyle, 27 June 1902, *J.A. Shafer 44* (CM, NCU); Ohiopyle, 3 July 1905, plentiful on river, *B.H. Patterson s.n.* (CM); Ohiopyle, 6 September 1915, *J. Bright s.n.* (CM); Ohiopyle, 17-18 May 1939, *O.E. & S.K. Jennings s.n.* (CM); Ohiopyle, 21 June 1923, *J. Bright* (CM); Vicinity of Ohiopyle, 23-24 June 1923, *O.E. Jennings & E.H. Graham s.n.* (CM); Ohiopyle, 21-22 June 1930, *W.R. Van Dersal 1686* (CM); Ohiopyle, 23-24 June 1934, *O.E. Jennings s.n.* (CM); Ohiopyle, 22-23 June 1929, *O.E. Jennings s.n.* (CM); Ohiopyle, 21 June 1942, *O.E. Jennings s.n.* (CM); Ohiopyle, rock crevices along banks of Youghiogheny River, 13 July 1962, *D.H. Ross 48* (CM); Ohiopyle State Park, below Fern Cliff Bridge, Rocky floodplain among rock crevices and boulders in sandy soil, 13 Sept 2001, *J. Polonoli FG92* (CM); Youghiogheny River below Ohiopyle, just below mouth of Meadow Run, crevices of sandstone pavement subject to frequent scour, 23 July 2009, *S.P. Grund 4889* (CM*); Vicinity of Ohiopyle, 3 July–5 Oct [no year indicated], *J.A. Shafer & G.E. Kinzer s.n.* (CM); **Somerset Co.:** 2.4 km NW of the mouth of the Casselman River, river scour under partial shade of *Rhododendron arboreum*, 10 July 2009, *S.P. Grund, P.G. Wiegman* & *A. Kasicky 4875* (CM). **Tennessee. Cumberland Co.:** Sandy boulder bar 40m long by 12m wide on the west bank of a major bend in Daddys Creek, ca. 0.25 downstream from Antioch Bridge, Ozone Quad, 35°59'57"W, 84°49'10"W, elevation ca. 1440 ft, *T.S. Patrick & B.E. Perkins 1290* (EKU*). **Morgan Co.:** Scattered on boulder bar at mouth of Turkey Creek, S. bank of the Obed River, a large open area subject to flooding and with many grasses like *Calamovilfa arcuata, Andropogon gerardii, Sorghastrum nutans*, Lancing Quad, 30 Sep 1980, *T.S. Patrick & P.A. Schmalzer 1633* (EKU*); Open, bouldery margin Obed River, northwest of Hatfield Mountain, Lancing Quad, an area of extensive bouldery shrub margins ca. halfway between Obed Junction and Clear Creek, 24 June 1981, *T.S. Patrick & P.A. Schmalzer 3116* (EKU). **Scott Co.:** Scattered plants on open, sandy gravel bar along east bank of the South Fork Cumberland River between Stevens Branch and Blevins Branch, Barthell SW Quad, 30 June 1980, *T.S. Patrick et. al 1264* (EKU*). **West Virginia. Barbour Co.:** Sandy east bank of the Tygart's Valley River, downstream near Tygart Junction, 24 Sept 1904, *A.H. Moore 2556* (GH)*; 2568* (GH); Near Tygart Junction, 24 September 1904, *J.M. Greenman 217* (GH). **Greenbriar Co.:** On rocks along the Greenbrier River, Blue Bend, 3 Aug 1965, *D. Wilson s.n.* (WVU). **Marion Co.:** Valley Falls, 14 Aug 1957, *E.L. Core & W.R. Lenhart s.n.* (WVU). **Monongalia Co.:** Gorge of Cheat River in cracks of rock in river one mile southeast of Mont Chateau, 4 June 1909, *O.E. Jennings s.n.* (CM*); Mont Chateau, Cheat River east of Morgantown, 10 June 1914, *B.H. Patterson s.n.* (CM); Cheat River at Mt. Chateau, 2 June 1918, *J.Bright s.n.* (CM); Cheat River among rocks on island at head of Lake, 18 July 1943, *Mr. & Mrs. H.A. Davis 5892* (CM). **Nicholas Co.:** Gauley River National Recreation Area, ca 2.2 air km SSW of the Summersville Dam along the Gauley, 20 June 2006, *Streets 1916 w/C.D. Good* (WVU). **Preston Co.:** Big Sandy Creek on rocky creek bank, 2 July 1943, *Mr. & Mrs. H.A. Davis 5853* (CM*). **Randolph Co.:** Along Shavers Fork at mouth of Stone Coal Run, 12 July 1956, *Mr. & Mrs. H.A. Davis & E.E. Hutton 11614* (CM); Cheat Bridge, in sandy soil along Cheat River, 12 July 1941, *Eugene Hutton & Robert Whitlach 4968* (CM*). **Summer Co.:** Sandy soil, E bank of Greenbriar River, below cliff at Bacon's Falls, 3 July 1949, *W.B. Fox 2479* (WVU). **Upshur Co.:** Near Sago, 24 June 1895, *W.M. Pollock s.n.* (GH); 21 June 1895, *W.M. Pollock* (NCU); 1 July 1895, *W.M. Pollock* (NCU); 4 July 1896, *W.M. Pollock 189* (GH, NCU). **Wyoming Co.:** Simon, [no date indicated], *D.S. Evans s.n.* (WVU).

Marshallia legrandii. **North Carolina. Granville Co.:** Picture Creek Diabase Barren, north of Butner, 22 July 2003, *A.S. Weakley, L.M. Giencke, & J.P. Perry III* 7274 (NCU*). **Virginia. Halifax Co.:** Difficult Creek Heritage Preserve, mafic flats east of County Road 719, 27 June 2003, *A.S. Weakley, J.C. Ludwig, M. Leahy, L.C. Gastinger 7255* (NCU*).

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