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Genetic and Morphometric Assessment of the Origin, Population Structure, and Taxonomic Status of *Anticlea vaginata* (Melanthiaceae)

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Abstract—*Anticlea vaginata* (Melanthiaceae) is a rare and endemic plant species restricted to hanging gardens in low-elevation desert regions of the Colorado Plateau. Its more widespread congener, *A. elegans*, is morphologically similar, but occurs in montane forests that encompass and extend beyond the natural range of *A. vaginata*. Here, we use morphometric and genetic analyses to investigate the biogeographic origin, population structure, and taxonomic classification of *A. vaginata* relative to *A. elegans*. Our results demonstrate that *A. vaginata* is closely related to and morphologically indistinguishable from *A. elegans* and likely represents remnant populations of *A. elegans* derived from a Pleistocene vicariance event. We conclude that *A. vaginata* warrants treatment as *Anticlea elegans* subsp. *vaginata*, since it exhibits a similar level of differentiation from *A. elegans* subsp. *elegans* as subsp. *glaucus*. Since *A. vaginata* occupies an ecologically unique niche, exhibits a distinct flowering period and harbors unique alleles, we suggest separate conservation management in order to protect this subspecies and its fragile habitat, which is currently threatened by climate change and the potential for groundwater development.

Keywords—AFLP, endemic, hanging garden, morphometrics, population genetics, vicariance.

Anticlea vaginata Rydb., Alcove Death Camas, is a poorly understood Colorado Plateau endemic that is restricted to hanging gardens, unique springs that occur in desert canyon alcoves. Due to its narrow distribution and dependence on perennial springs in a desert environment, it is a species of conservation concern (Schwartz 2002; NatureServe 2013). *Anticlea vaginata* represents the only hanging garden species within the genus *Anticlea* (Welsh and Toft 1981; Schwartz 2002; Spence 2008), which is largely distributed in high elevation montane forests. Populations of *A. vaginata* are highly disjunct from one another, distributed as isolated populations across 5° latitude from northern Utah and Colorado to northern Arizona.

Morphologically, *A. vaginata* closely resembles its widespread and variable congener, *Anticlea elegans* Pursh., which occupies montane forests surrounding the distribution of *A. vaginata* and extends across much of western North America. *Anticlea elegans* comprises an eastern and a western subspecies; subsp. *glaucus* (Nutt.) A. Haines and subsp. *elegans*, respectively (Hess and Sivinski 1995; Zomlefer 1997; Haines 2010). The latter is found in a wide variety of montane habitats from northern Alaska to southern New Mexico and includes the mountains surrounding the distribution of *A. vaginata*. Based on their morphological similarity, Cronquist et al. (1977) considered *A. vaginata* as a synonym of *A. elegans*. Schwartz (2002), however, treats the two taxa as separate species based on several floral and vegetative characteristics.

Morphologically, the two species are distinguishable from other members of the genus by having erect pedicels and rotate to rotate-campanulate corollas at anthesis. The characters historically used to distinguish *A. vaginata* from *A. elegans* are 3–6 mm white tepals, persistent, numerous loose sheaths at the base of the stem, and a large clumping growth form (vs. 7–12 mm cream to greenish tepals, no persistent leaf bases, and bulbs growing singly in *A. elegans*) (Rydberg 1912; Welsh et al. 1993; Hess and Sivinski 1995; Schwartz 2002). *Anticlea vaginata* also flowers later than *A. elegans* (August to October vs. June to August), and in a given geographic region, *A. elegans* populations will have finished flowering when *A. vaginata* begins. *Anticlea vaginata* typically grows on sandstone below 1,800 m in elevation, is always found in springs, and is usually in the deep shade of the associated alcove.

Anticlea elegans subsp. *elegans* occurs in a wide variety of montane habitats, but in the Intermountain West is usually found on limestone above 2,400 m, occasionally in springs, and often in shaded areas (Welsh et al. 1993; Schwartz 2002).

Given its morphological similarity, it is unclear whether *A. vaginata* represents a single, distinct species, or a low-elevation form of the widespread and variable *A. elegans*. If *A. vaginata* were a separate species, we would expect it to exhibit several distinct morphological characters and have considerable genetic divergence from *A. elegans*. Alternatively, if *A. vaginata* is morphologically and genetically similar to *A. elegans*, despite being ecologically isolated, it may be best treated as a subspecies.

The biogeographic origin of *A. vaginata* is also unclear, considering its geographic proximity to *A. elegans*. One possibility is that *A. vaginata* may have originated from *A. elegans* subsp. *elegans* via single or multiple long-distance dispersal events, followed by adaptation to the desert environment. Since *A. vaginata* and its congeners lack long-distance dispersal mechanisms, we consider multiple dispersal events unlikely (Spence 2008). In the case of a single dispersal scenario, we would expect populations to be morphologically similar and exhibit a subset of the total genetic variation observed in *A. elegans* subsp. *elegans* (e.g. via a founder event). Phylogenetically, we would also expect to see a nested (i.e. paraphyletic) group of all *A. vaginata* populations that are more closely related overall to each other than to geographically nearby populations of *A. elegans* subsp. *elegans*. In the unlikely case of multiple long-distance dispersal events, we would expect populations of *A. vaginata* to be nested within or paired with populations of *A. elegans* based on geographic proximity (i.e. paired *A. vaginata*/*A. elegans* populations occupying proximal locations) combined with reduced genetic diversity and subsets of genetic diversity.

Alternatively, *A. vaginata* may have originated through a vicariance event (Keate 1996; Spence 2008). Habitat fragmentation and gradual divergence from *A. elegans* may have occurred via warming and drying of the climate since the Pleistocene. Plant zonation on the Colorado Plateau during the late Pleistocene supports this hypothesis in that boreal trees and likely many herbaceous species occurred up to 1,000 m lower in elevation than they occur today (Cole 1982;

Betancourt 1984; Withers and Mead 1993; Spence 2008). Thus, the morphologically similar *A. elegans* subsp. *elegans* could have been part of a low-elevation Pleistocene flora, with the current distribution of *A. vaginata* representing remnant populations that have persisted within the cool, wet hanging garden habitat.

If vicariance best explains the origin of *A. vaginata*, we would expect to see a general overlapping pattern of morphological and genetic variation between the montane (*A. elegans* subsp. *elegans*) and desert populations (*A. vaginata*) with little to no differentiation between the two species. Phylogenetically, we would expect to see intermixed populations of *A. vaginata* and *A. elegans* (i.e. no clear species groups) with little to no explainable geographic pattern. Additionally, we would expect to see relatively high levels of genetic diversity retained in large hanging garden populations. We would also expect to detect genetic differentiation among the highly dissected, individually isolated populations of *A. vaginata* relative to *A. elegans* subsp. *elegans*, which is more continuously distributed (Martínez-Ortega et al. 2004, Schönswetter and Tribsch 2005).

Finally, if the two species have been ecologically isolated from one another since the Pleistocene (e.g. as in the case of vicariance), high-resolution genetic markers would likely show some unique differences between the populations in the form of private alleles (Martínez-Ortega et al. 2004, Schönswetter and Tribsch 2005). The presence of private alleles could be indicative of incipient divergence arising, in part, from the non-overlapping flowering times exhibited by the two species.

To investigate the taxonomic and biogeographic affinities of *A. vaginata* and *A. elegans* subsp. *elegans*, we used morphometric and genetic analyses to address two main questions: 1) Are *A. vaginata* and *A. elegans* taxonomically unique groups, recognizable as distinct species? Or should they be treated as a morphologically variable species or subspecies? 2) Is the biogeographic origin of *A. vaginata* best explained by one or a few, long-distance dispersal events from *A. elegans* subsp. *elegans*, or by in situ fragmentation via post-Pleistocene vicariance within hanging garden habitats on the Colorado Plateau?

MATERIALS AND METHODS

Morphometrics—Measurements were taken from 208 specimens (Appendix 2) from field collections and the following herbaria: ASC, ASU, ARIZ, BRY, CS, DES, NAVA, RM, UNM, UTC, UVSC, the herbarium for the Southeast Utah Group, and the herbarium at Glen Canyon National Recreation Area. Only correctly identified collections exhibiting many of the measured characteristics were used. Field collections were made between May 2008 and October 2009. Type collections were examined using high-resolution digital images available from the herbaria in which they are housed.

A total of 79 specimens of *Anticlea vaginata* spanning its known range were examined, including collections from ten previously unvouchered populations. A selection of 107 herbarium and field collections of *A. elegans* subsp. *elegans* were chosen to represent the geographic range of that species. Collections represented the full range of morphological variation exhibited by both species. Twenty collections of *A. virescens* (Kunth.) J. F. Macbr., a widespread, related species, were included as an outgroup.

Characters were chosen based on those used to delineate *A. vaginata* and *A. elegans* in previous treatments (Rydberg 1912; MacBride 1918; Preece 1956; Welsh et al. 1993; Hess and Sivinski 1995; Schwartz 2002). One-way analysis of variance (ANOVA) was assessed for seventeen vegetative and floral characters, 13 quantitative and four categorical (Appendix 3). Field observations confirmed that white to cream to greenish tepals occur regularly in both species, and flower color was not preserved on herbarium collections, so this feature was not used in the analysis.

Principal components analysis (PCA) was used to assess structure in the morphological data in PC-ORD 5.10 (McCune and Mefford 2006). The twelve characters with less than five missing values were used for this analysis (Appendix 3), and missing values were approximated with the average value for that character. A *p* value was generated using a randomization test. A canonical discriminant function analysis (DFA) (Klecka 1980) was conducted in SPSS v. 19 (SPSS IBM, Armonk, New York) using the same data matrix.

Genetics—Preliminary work indicated that sequence data from the *trnL* (UAA)-*trnF* (GAA) intergenic intron and spacer region (*trnL-F*, plastid) and the internal transcribed spacer region ITS-1, 5.8S, and ITS-2 (ITS) utilized by Zomlefer et al. (2001) for generic circumscriptions within Melantheae did not show variation between *A. vaginata* from *A. elegans* subsp. *elegans*. Thus, we generated data based on more rapidly evolving genetic markers using the Amplified Fragment Length Polymorphism (AFLP) technique (Vos et al. 1995). This technique has been successfully used to delimit species and subspecies in flowering plants (e.g. Saarela et al. 2003; Lihova et al. 2004; Ellis et al. 2009), assessing population genetic variation and structure (e.g. Campbell et al. 2003), and providing indirect estimates of gene flow (e.g. Schmidt & Jensen 2000; Tremetsberger et al. 2003; Huft & Richardson 2006; Coppi et al. 2008).

A total of 398 individuals were analyzed for AFLP variation, representing 15 populations of *Anticlea vaginata* and nine of *A. elegans* subsp. *elegans*. Two populations of *A. virescens* and one of *A. mogollonensis* were used as outgroups for phylogenetic analysis (Fig. 1, Appendix 1). Leaves were dried and stored in silica gel prior to DNA extraction. In order to avoid clones, only leaves from different clumps of plants were used for analysis. Sampled populations of *A. vaginata* spanned its known range and populations of *A. elegans* were chosen from nearby geographic regions of varying distance (Fig. 1).

Genomic DNA was extracted using the Qiagen DNeasy 96 plant kit and the associated protocol with minor adjustments (Qiagen, Valencia, California). DNA quality and quantity was measured using gel electrophoresis on a 2% agarose gel and a NanoDrop ND-1000 spectrophotometer (Thermo Fischer Scientific, Waltham, Massachusetts).

We used the AFLP protocol of Hersch-Green and Cronn (2009), with few modifications. For each individual, 15 ng of genomic DNA was digested by *EcoRI* and *MseI*, and ligation of corresponding adapters to

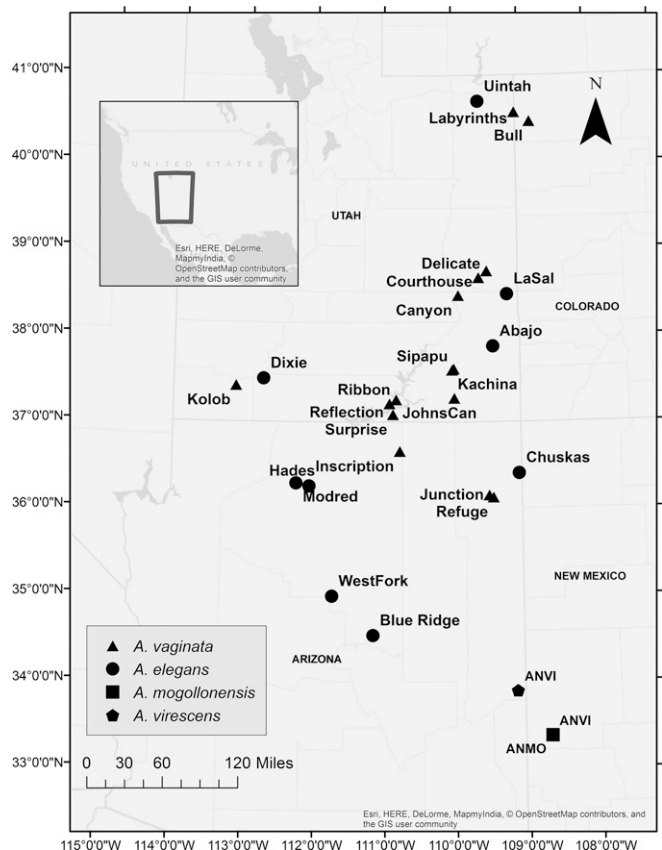


FIG. 1. Geographic locations of 27 *Anticlea* populations sampled.

the fragments occurred simultaneously. Primers complementary to the adaptor sequences plus one selective nucleotide (*EcoRI*+A and *MseI*+C) were used for preselective amplification. For the preamplification process, a 1:5 dilution of the restriction/ligation product were added to the preamplification master mix, which consisted of 1 × Mg-free PCR buffer, 0.1 mg/mL BSA, 1.5 mM MgCl₂, 0.2 mM each of dNTPs, 0.8 uM each of *EcoRI*+A and *MseI*+C, and 1.25 U/uL of *Taq* DNA polymerase.

For selective amplification, eight fluorescently labeled primer pairs containing the complement to the adaptor sequence plus three selective nucleotides were tested on ten individuals representing the four species and different geographic areas. The six primer pairs that produced the largest number of fragments across the samples were chosen: *EcoRI*-ACT-(FAM), *MseI*-CAG; *EcoRI*-ACT-(FAM), *MseI*-CAA; *EcoRI*-ACC-(NED), *MseI*-CAG; *EcoRI*-ACC-(NED), *MseI*-CAA; *EcoRI*-AAC-(NED), *MseI*-CAG; *EcoRI*-AAC-(NED), *MseI*-CAA. The selective amplification reaction consisted of undiluted preamplification product and 1 × MgCl₂ (15 mM) PCR Buffer, 0.2 mM each dNTPs, 0.375 uM *EcoRI*+3 primer, 1.0 uM *MseI*+3 primer, and 0.5 U/rxn *Taq* DNA polymerase.

A 1:10 dilution of the AFLP product was mixed with formamide and GeneScan 600 LIZ size standard following the associated protocol, and separated using capillary electrophoresis on an ABI 3730XL (Applied Biosystems, Foster City, California).

GeneMapper Software v.4.0 (Applied Biosystems) was used to analyze the AFLP fragments. Profiles were analyzed with automated scoring using a base pair range of 100–600 bp and a peak height minimum of 1,000 for all primer combinations. To minimize scoring noise, only larger peaks were used for bin generation. Bins were then hand-edited for consistency and usefulness. Bins that contained scored peaks in no template controls were removed from the analysis. Profiles were rescored using the edited bin set, a base pair range of 100–600 bp, and a peak height minimum determined separately for each primer combination. Primer combinations *EcoRI*-ACC-(NED), *MseI*-CAA and *EcoRI*-ACT-(FAM), *MseI*-CAA were assigned a minimum peak height of 750; all others were set to 500. Loci present in only one individual were removed from the data set. Loci with highly variable peak heights across samples were also removed from the data set, since these bands are often unreliable and may increase error (Piñeiro et al. 2009). A standard Euclidean error rate was calculated following Holland et al. (2008).

Estimates of genetic variation (%P, Nei's Gene Diversity (H_e) and structure (G_{ST} , AMOVA and Bayesian-based Structure analysis) were calculated for all populations. Indirect estimates of gene flow (Nm) and private alleles were also scored for all populations of *A. vaginata*, and *A. elegans* using PopGene 1.32 (Yeh et al. 1997). Non-metric multidimensional scaling (NMS) using Jaccard's distance measure and the slow and thorough method on Autopilot in PC-ORD 5.10 (McCune and Mefford 2006) was used to visualize the relationships among samples. A neighbor-joining (NJ) tree of all individuals and populations rooted with *A. virescens* and *A. mogollonensis* was created in MEGA5 (Tamura et al. 2011) using a Nei's genetic distance matrix created in GenAIE 6.4

(Peakall and Smouse 2006). Data matrices and associated trees were submitted to TreeBASE (study number 15824, <http://treebase.org/treebase-web/search/study/summary.html?id=15824>).

Four separate analyses of molecular variance (AMOVA, Excoffier et al. 1992) were run in GenAIE 6.4 (Peakall and Smouse 2006) using a Nei's genetic distance matrix and 9,999 permutations to calculate a *p* value. One was used to evaluate how total genetic variation was partitioned among species and among and within populations. The second used geographic region as a proxy for species to evaluate species-level partitioning. Two more assessed differentiation within *A. vaginata* and *A. elegans* separately.

Genetic isolation by distance was assessed using a Mantel test in GenAIE 6.4 (Peakall and Smouse 2006). Distances (km) among populations were compared to a pairwise population distance matrix of Φ_{PT} values using 9,999 permutations.

Finally, Structure 2.2 using ΔK as described by Evanno et al. (2005) was used to analyze individuals of *A. elegans* and *A. vaginata* in order to determine the number of genetic groups (Pritchard et al. 2000; Falush et al. 2007; Pritchard et al. 2007). The admixture model with 10,000 burnin followed by 100,000 iterations for each K from 1–10 was used. Ten replications were conducted for each level of K. Multiple runs were pooled using CLUMMP 1.1.2 (Jakobsson and Rosenberg 2007) and graphics were generated using Distruct (Rosenberg 2004).

RESULTS

Morphometrics—One-way ANOVAs indicate significant differences in means for eight out of 13 characters (Table 1). Capsule length, plant height, flower number, and bulb length and width did not significantly differ between the two species (Table 1). For all quantitative characters, the range of variation in *A. vaginata* largely overlaps that of *A. elegans* (Table 1, Fig. 2). All characters scored as present/absent were found regularly in both species (Table 1).

The morphological ordination (Fig. 3) shows *A. elegans* and *A. vaginata* mostly overlapping, while *A. virescens* remains as a separate group. The first two components from the PCA explained significantly more variation than would be expected by chance ($p = 0.0001$), 26.4% and 21.6% respectively, for a total of 48% of the variation explained. The first component represents gradients in flower diameter (0.5091), tepal length (0.4949), and tepal width (0.4637). The second component represents gradients in inflorescence structure (0.5214), flower number (0.5084), and leaf length (0.5040). Canonical discriminant function analysis (DFA) correctly classified 92.9% of *A. elegans* individuals, 69.6% of *A. vaginata* individuals, and

TABLE 1. Descriptive statistics of 16 morphological characteristics. Flower structure, persistent leaves, and persistent sheaths are categorical data, all values in millimeters (mm), except inflorescence height in centimeters (cm). *p* values are from one-way ANOVAs comparing *A. vaginata* and *A. elegans*.

Character	<i>A. elegans</i>					<i>A. vaginata</i>					<i>p</i>
	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD	
bract_length	84	8	25	13.8	3.7	56	6	75	11.5	9.4	0.0199*
bulb length	46	8	25	16.4	4.1	33	7	29.5	17.8	5.5	0.2025
bulb width	46	8	25	13.8	3.5	33	7	24	15.2	4.5	0.1413
capsule lgth	27	8	17	12.3	2.2	25	8	19	11.8	2.8	0.4827
flwr_diam	84	10	25	15.3	2.8	56	9.5	18.5	13.8	1.8	0.0002*
flwr_number	84	6	60	20.8	12.5	56	3	70	24.7	16.1	0.1055
height	44	180	535	364.2	95.1	31	135	580	332.6	107.7	0.1837
inflor. hgt	38	4.5	36	15.8	9.4	23	3.5	64	24.2	13.9	0.0068*
leaf_length	84	65	380	217.8	70.7	56	85	710	324.7	126.1	<.0001*
leaf_width	84	3	20	7.6	3.2	56	3.5	23	8.9	3.9	0.0261*
pedicel_lgth	84	4	36	15.1	6.4	56	8	27	15.0	4.8	0.0317*
tepal length	84	4.5	10.5	6.9	1.1	56	4	12.3	6.3	1.1	<.0001*
tepal width	84	2.5	8.5	4.1	0.9	56	2.5	7.5	3.6	0.9	0.0042*
inflor_struct	84	Presence	Absence	Racemose	Panicle	56	Presence	Absence	Racemose	Panicle	
persistent lvs	84	24	60	22	62	56	13	43	11	44	
present sheaths	84	64	20			56	45	11			

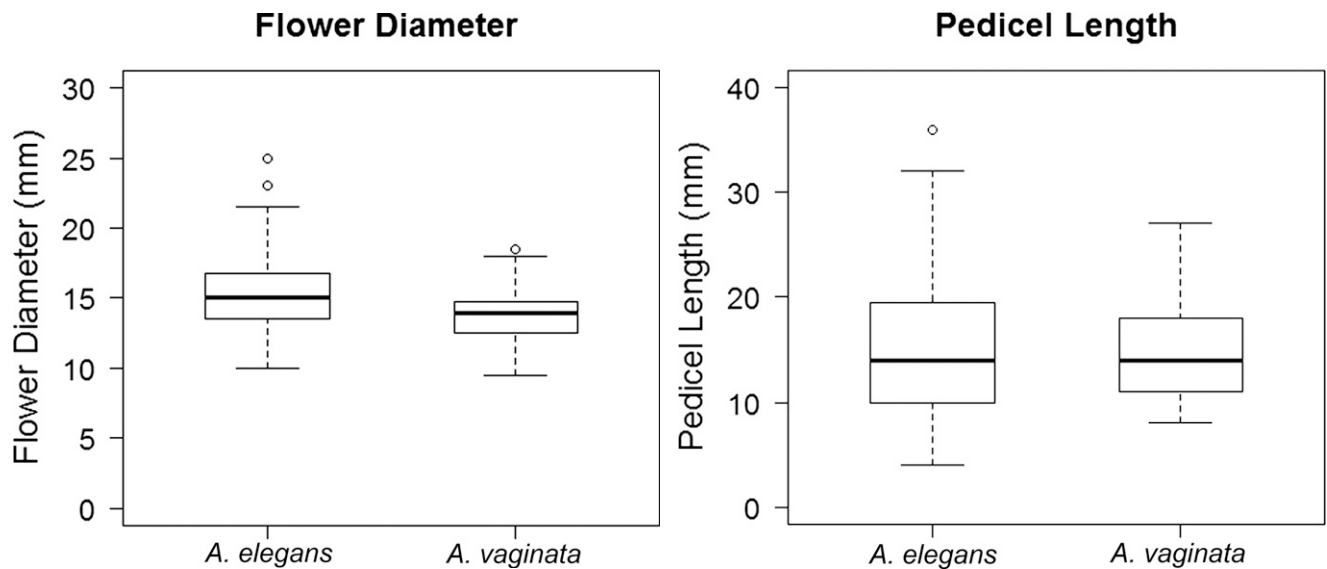


FIG. 2. Box plots showing variation of two morphological characters previously used to separate *A. vaginata* and *A. elegans*, flower diameter and pedicel length. Horizontal bars represent the median, boxes indicate the interquartile range, and whiskers extend to the most extreme data point that is not more than 1.5 times the interquartile range. Dots indicate outliers. Flower diameter and pedicel length are significant at $p < 0.05$.

86.7% of *A. virescens* individuals. Function one explained 74.3% of the variation and was composed primarily of flower diameter (0.786), tepal length (0.567), and tepal width (0.480). Function two explained 25.7% of the variation and was composed primarily of leaf length (0.698) and leaf width (0.426).

Genetics—From the 398 samples, 341 polymorphic loci were analyzed. Outlier analysis in PC-ORD 5.10 (McCune and Mefford 2006) indicated that 16 individuals varied more than two standard deviations from the mean and these were removed from the analysis.

Percentage of polymorphic loci was 87.1% for *A. vaginata* and 81.5% for *A. elegans* (Table 2). Nei's gene diversity ranged from 0.058–0.135 for *A. vaginata* populations and 0.072–0.126

for *A. elegans* populations (Table 2). Population differentiation (G_{ST}) was 0.299 for *A. vaginata*, which is high when compared to its widespread relative (0.251, Table 2) and to the mean values of other long-lived perennials (0.19), endemics (0.18), and narrow species (0.21) (Nybohm 2004). The combined G_{ST} for *A. vaginata* and *A. elegans* was 0.296.

TABLE 2. Genetic diversity, genetic structuring, and private alleles for *A. elegans* and *A. vaginata*. GD=Nei's Gene Diversity, Std. Dev. = Standard deviation of gene diversity, % poly = percent polymorphic loci, Pa=private alleles.

Group	GD	Std. Dev.	% poly	G_{ST}	Pa
All <i>A. vaginata</i> and <i>A. elegans</i>	0.1192	0.1532	97.4	0.296	
All <i>A. vaginata</i>	0.114	0.151	87.1	0.299	32
Bull	0.067	0.139	24.6		0
Canyon	0.073	0.142	29.3		0
Courthouse	0.067	0.135	27.0		0
Delicate	0.058	0.120	28.7		0
Inscription	0.074	0.153	25.8		2
JohnsCan	0.075	0.155	24.3		1
Junction	0.072	0.131	33.4		0
Kachina	0.114	0.155	48.1		1
Kolob	0.064	0.139	22.9		1
Labyrinths	0.083	0.153	29.6		0
Reflection	0.067	0.142	23.2		1
Refuge	0.087	0.159	31.1		1
Ribbon	0.087	0.159	30.2		1
Sipapu	0.135	0.183	50.2		0
Surprise	0.078	0.148	31.1		1
All <i>A. elegans</i>	0.121	0.160	81.5	0.251	22
Abajo	0.076	0.143	32.0		0
BlueRidge	0.093	0.164	31.7		1
Chuska	0.086	0.153	35.8		2
Dixie	0.085	0.153	31.7		0
Hades	0.100	0.167	34.0		0
La Sal	0.126	0.17	52.8		1
Modred	0.072	0.147	26.7		0
Uintah	0.083	0.142	37.5		1
WestFork	0.091	0.165	29.9		2

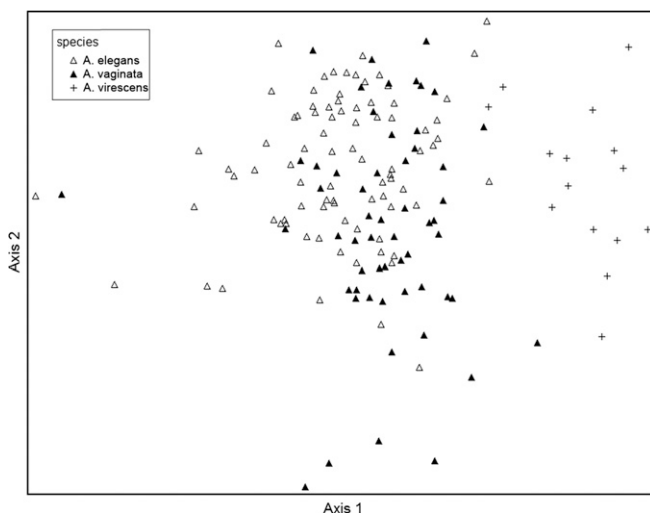


FIG. 3. Scatterplot of components 1 and 2 of the PCA of 12 floral and vegetative characters on 84 specimens of *A. elegans*, 56 specimens of *A. vaginata*, and 15 specimens of *A. virescens*. The first two axes explained 34.6% and 20% of the variation, respectively. The first component mostly represents gradients in flower diameter (0.5091), tepal length (0.4949), and tepal width (0.4637). The second component largely represents gradients in inflorescence structure (0.5214), flower number (0.5084), and leaf length (0.5040).

Nm for combined *A. vaginata* and *A. elegans* was 1.19, which is relatively high for herbaceous perennials (Wolf and Soltis 1992). The two species shared a large majority of alleles, but also had small percentages of unique ones. *Anticlea vaginata* had 32 private alleles while *A. elegans* had 22 (Table 2).

The final NMS 3-dimensional solution had a stress of 19.28 and final instability of 0.00088. An overlay of species shows individuals of *A. elegans* and *A. vaginata* largely overlapping, but grouped separately from individuals of the other two species (Fig. 4).

A neighbor-joining (NJ) tree consisting of all 398 individuals shows that 22 of 27 populations form distinct, individual clusters (supplementary Fig. S1). A separate NJ tree based on pairwise population genetic distance shows that populations of the two species form a largely overlapping pattern of inter-related groups, none of which formed individual monophyletic clades for either *A. vaginata* or *A. elegans* (Fig. 5).

The AMOVA partitioning of total genetic variation for species and geographic regions, respectively, attributed 64% and 65% of genetic variation to within population differences, 28% and 22% to among population differences, and 8% and 13% to species or regional differences (Table 3). The AMOVA conducted only with *A. vaginata* attributed 34% of genetic variation to among population differences and 66% to within population differences. The AMOVA for *A. elegans* attributed 28% of genetic variation to among population differences and 72% to within population differences.

Isolation by distance analyses showed no significant correlation between geographic and genetic distance for either montane (*A. elegans*, $R^2 = 0.0024$) or hanging garden (*A. vaginata*, $R^2 = 0.0115$) populations. There was also no correlation when montane and hanging garden populations were combined ($R^2 = 0.0092$).

Structure analysis indicated that the most appropriate number of genetic groupings represented by the *A. vaginata* and *A. elegans* samples was $k = 2$, but these did not corre-

spond with delineation of the two species (Fig. 6). Individuals from the same population have similar membership probabilities (Fig. 6), particularly Ribbon, Reflection, Delicate, Dixie, and West Fork.

Taken together, the genetic data show substantial amounts of structuring at the population level with some populations forming distinct geographic groups, but not species groups. The populations in Glen Canyon National Recreation Area, Ribbon and Reflection are unusual for having the latest flowering time by far (early October), forming a well-defined group in the genetic analyses (Fig. 6, Fig. S1), showing the lowest genetic diversity (Table 2), and containing a high number of private alleles (5). The Natural Bridges National Monument hanging gardens, Sipapu and Kachina, are genetically admixed (Fig. S1), show the highest levels of genetic diversity (Table 2), and contain a high number of private alleles (5). The Moab region hanging garden populations, Delicate and Courthouse, stand out in the Structure Analysis as being the only ones that have affiliation primarily with the genetic group identified in gray (Fig. 6). Individuals from the Mogollon Rim, BlueRidge, and WestFork, are admixed and set apart in the cluster analyses (Fig. 5, Fig. S1) and have the highest number of private alleles (6). Of the remaining populations, many do not form distinct groups, but are instead genetically admixed.

DISCUSSION

Taxonomic Status of *Anticlea vaginata*—Despite being geographically separate, living in very different habitats, and having different phenology, the morphological and genetic analyses do not separate *Anticlea vaginata* as a distinct species. Morphological traits that have historically been used to distinguish *A. vaginata* from *A. elegans* exhibit large within-species variation and their ranges mostly overlap (Table 1, Fig. 2, Fig. 3), making montane and hanging garden individuals too similar to reliably distinguish. Genetic analyses also did not separate individuals by current species delineations (Figs. 4–6); rather genetic differences were better explained, albeit weakly, by geographic groupings (Table 3). In addition, indirect estimates of gene flow based on Nm suggest somewhat high gene flow between the hanging garden and montane populations ($Nm = 1.19$), though it is unclear whether this is ongoing gene flow or evidence of past gene flow retained in the largely clonal hanging garden populations.

Although these two groups cannot be reliably distinguished, significant differences in means of morphological traits and the ability of the DFA to correctly classify 92.9% of *A. elegans* and 69.6% of *A. vaginata* suggest that montane populations and hanging garden populations are in the process of morphological differentiation. Hanging garden plants tend to have smaller flowers but bigger, more robust vegetative structures (Table 1, Figs. 2, 3). Hanging garden plants also regularly form large, clumped clonal mats, which is very uncommon for montane individuals. Robust vegetative structures and clonal mats may reflect the age of the individual and the stability of the hanging garden habitat.

Additionally, genetic analyses suggest that hanging garden populations are in the early stages of genetic differentiation. High among-population differentiation ($G_{ST} = 0.296$) and evidence that some groups of hanging garden populations are distinct from the others suggests that some hanging garden populations may be on separate evolutionary trajectories.

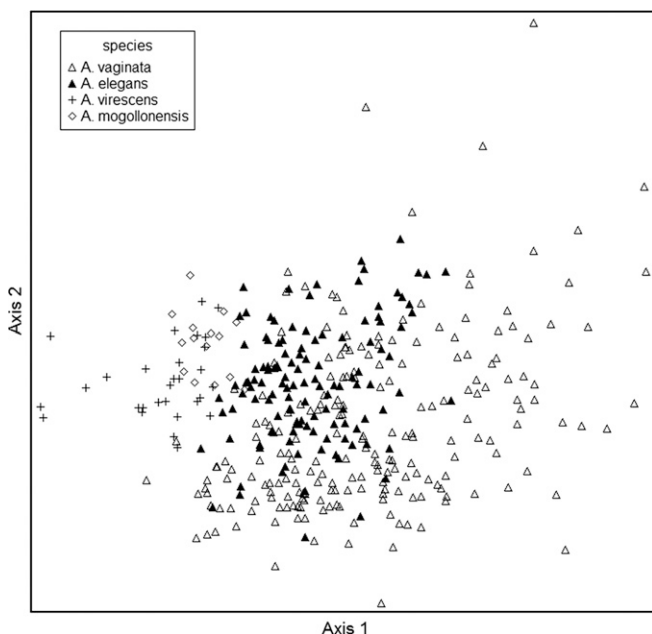


FIG. 4. Non-metric multidimensional scaling ordination of species using Jaccard's distance measure comparing samples of *A. vaginata*, *A. elegans*, *A. virescens*, and *A. mogollonensis* based on AFLP markers.

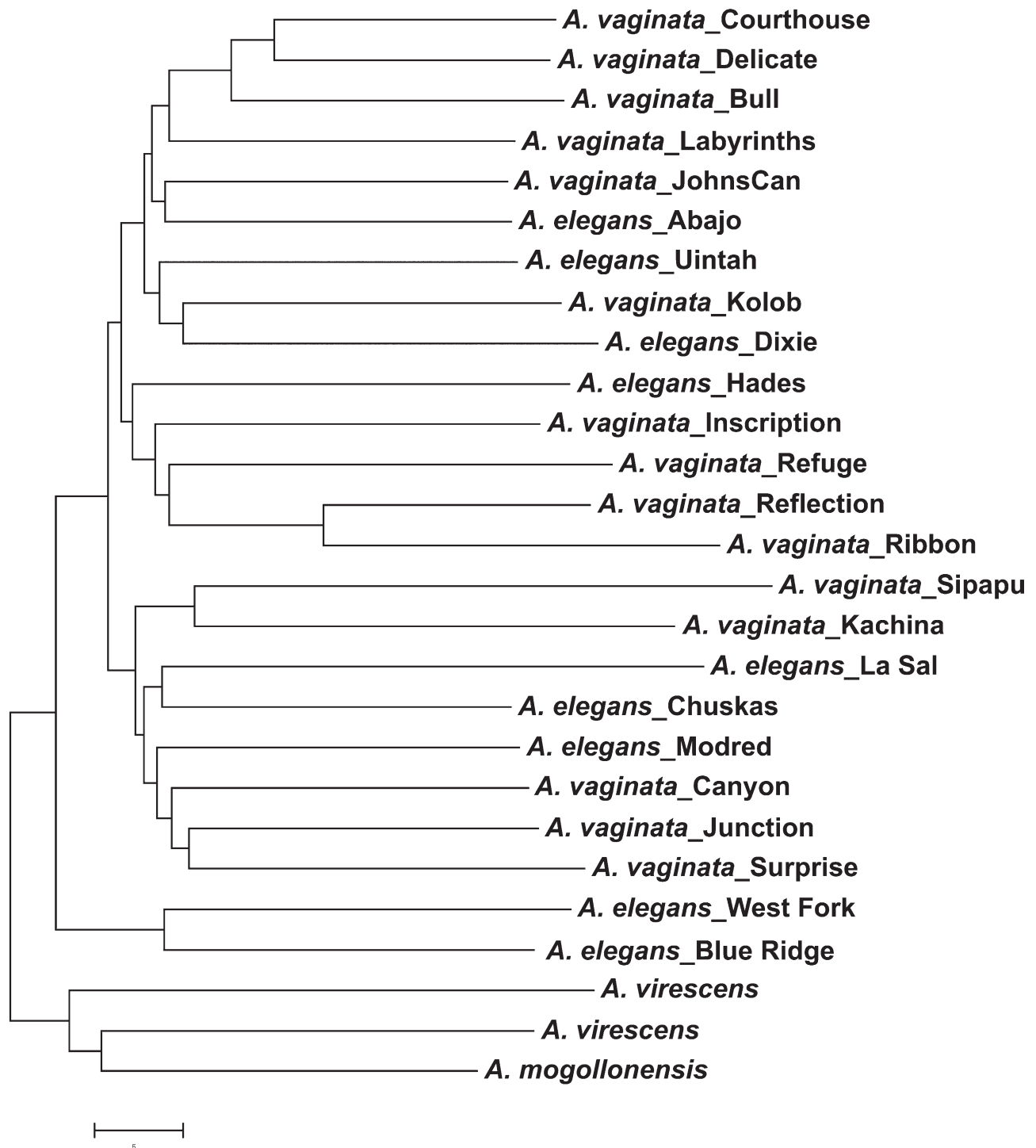


FIG. 5. Neighbor-joining tree of all 27 populations using Nei's genetic distance of AFLP markers. Each population is labeled with species and population code. Tree is rooted with *A. virescens* and *A. mogollonensis*.

Our results, based on morphological and genetic analyses, suggest that *A. vaginata* should be combined with *A. elegans* to reflect the current morphological and genetic similarity, but should be treated as a subspecies, since analyses indicate early differentiation in the hanging garden populations. These indications, combined with ecological differences in elevation, geographic distances, and phenology also suggest that subspecific status is warranted. This circumscription is consistent with other studies evaluating morphologically

similar species (e.g. Martínez-Ortega et al. 2004; Perny et al. 2004) and with the current treatment of *A. elegans*. *Anticlea vaginata* displays as much morphological distinction as the current two subspecies of *A. elegans* whose morphologies also show a great deal of overlap (Zomlefer 1997).

The relationships among *A. elegans* populations occurring on the Mogollon Rim in Arizona, *A. virescens*, and *A. mogollonensis* require further study. The genetic study presented here (Figs. 4, 5) and some shared morphological characteristics

TABLE 3. Analysis of molecular variance summary tables showing the partitioning of genetic variance among either species or geographic region, among populations, and within populations.

Summary AMOVA Table – Species						
Source	df	SS	MS	Est. Var.	%	<i>p</i>
Among Species	3	887.732	295.911	2.107	8%	0.0001
Among Pops	23	3098.808	134.731	7.945	28%	0.0001
Within Pops	371	6593.207	17.771	17.771	64%	0.0001
Total	397	10579.746		27.823	100%	

Summary AMOVA Table - Regions						
Source	df	SS	MS	Est. Var.	%	<i>p</i>
Among Regions	10	2282.185	228.218	3.394	13%	0.0001
Among Pops	16	1704.355	106.522	6.026	22%	0.0001
Within Pops	371	6593.207	17.771	17.771	65%	0.0001
Total	397	10579.746		27.191	100%	

Summary AMOVA Table – <i>Anticlea vaginata</i>						
Source	df	SS	MS	Est. Var.	%	<i>p</i>
Among Pops	14	1998.923	142.780	8.475	34%	0.010
Within Pops	208	3493.014	16.793	16.793	66%	
Total	222	5491.937		25.268	100%	

Summary AMOVA Table – <i>Anticlea elegans</i>						
Source	df	SS	MS	Est. Var.	%	<i>p</i>
Among Pops	8	994.551	124.319	7.310	28%	0.010
Within Pops	121	2275.526	18.806	18.806	72%	
Total	129	3270.077		26.116	100%	

(Hess and Sivinski 1995; Palmquist pers. obs.) suggest that these populations are very closely related.

Origins of the Hanging Garden *Anticlea*—Distinguishing between hypotheses of vicariance and dispersal can be difficult, but overall patterns of genetic variation and divergence can provide insight into which is the most likely explanation for an observed disjunct distribution. In the case of *Anticlea*, two scenarios are possible: 1) a once widespread species adapted to wet, mesic environments became fragmented with the warming and drying of a post-Pleistocene climate, with populations surviving in wet, hanging garden habitats (ecological vicariance); or 2) *Anticlea* dispersed from high-elevation montane sites into low-elevation hanging garden sites – the only habitat where an already mesic-adapted species could survive in a post-Pleistocene climate.

We find our data to be most consistent with a scenario of ecological vicariance involving fragmentation and survival of a once widespread, montane *Anticlea* in disjunct hanging gardens on the Colorado Plateau. Evidence consistent with this hypothesis includes: 1) non-monophyletic, but closely inter-related populations of both species, suggesting gradual fragmentation with some gene flow; 2) an extensive overlapping pattern of morphological and genetic variation with montane populations, which could arise from secondary (post-Pleistocene) contact between the two species, but is

unlikely given the non-overlapping flowering times currently observed between them; 3) relatively similar levels of within population genetic diversity, coupled with higher among population differentiation in *A. vaginata*; 4) a larger number of private alleles in *A. vaginata*, which is likely due to isolation of the hanging garden habitat and observed differences in flowering time; and 5) no correlation between geographic and genetic distance, which would be expected if individual hanging garden populations arose via dispersal from more distant montane populations.

We found no evidence for a single dispersal event and little to no evidence supporting multiple dispersal events. For example, we did not find considerably lower levels of genetic diversity in the hanging garden populations (e.g. as a result of individual founder events), as compared to *A. elegans*, which would be indicative of either dispersal scenario. Instead, levels of diversity were quite similar between the two species (0.114 vs. 0.121). We also did not observe a monophyletic *A. vaginata* nested within *A. elegans* or a monophyletic sister group to *A. elegans*, which would suggest a single dispersal event or independent dispersal events, respectively. We did, however, find some evidence for localized dispersal events, despite the fact that multiple dispersal events seem unlikely. Two populations, Kolob and JohnsCan, show an equivocal result that could be interpreted as dispersal. Both of these populations occupy atypical habitats, along springs at the bottom of canyons rather than the archetypal alcove, and have somewhat reduced levels of diversity. Kolob and JohnsCan are also most closely related to their montane geographic neighbors, Dixie and Abajo, respectively. However, both populations have private alleles and levels of diversity comparable to a few of the other hanging garden populations that show little to no affinity to nearby montane populations, making the biogeographic origin of the Kolob and JohnsCan populations unclear. We emphasize, however, that no obvious long-distance dispersal mechanism has been identified for *Anticlea*. Thus, taken together we argue that vicariance, not dispersal, is a more likely explanation for the overall disjunct pattern of distribution of *Anticlea* in hanging garden habitats.

Our finding that hanging garden populations are the result of population fragmentation in response to a warming, drying climate following the Pleistocene is consistent with other studies of plant species whose disjunct distributions have been influenced by ice age events (e.g. Tribsch et al. 2002; Saarela et al. 2003; Schönswetter and Tribsch 2005; Michalczyk et al. 2010). An important additional test of the vicariance hypothesis will be to determine whether a post-Pleistocene climate had similar effects on unrelated species that also occupy hanging garden habitats in the southwestern U.S. It is likely that further research on hanging garden endemics will show similar results. The probable closest

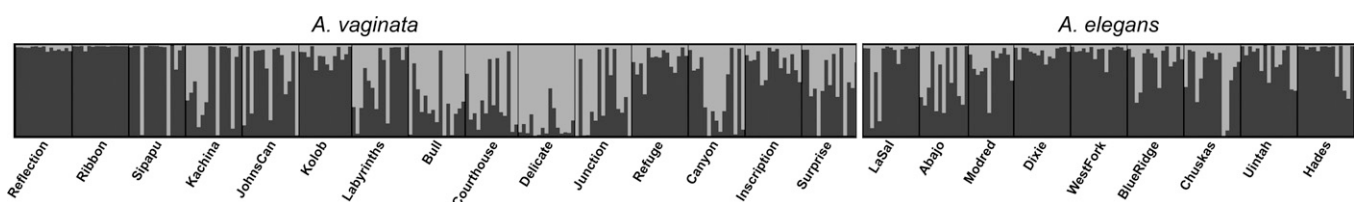


FIG. 6. Distruct graph of Structure analysis of *A. vaginata* and *A. elegans*. Each vertical bar represents one individual. The different shades of gray represent the proportion of shared genetic profiles from the 2 clusters identified by Structure.

congeners of sixteen of the other 20 Colorado Plateau endemic spring species occur in boreal temperate habitats, most lack long-distance dispersal mechanisms, and they represent nine different families; Asteraceae, Boraginaceae, Cyperaceae, Hydrangeaceae, Orchidaceae, Phrymaceae, Primulaceae, Ranunculaceae, and Violaceae (Spence 2008). Additionally, two of those endemics, *Dodecatheon pulchellum* (Raf.) Merr. var. *zionense* (Eastw.) S. L. Welsh and *Jamesia americana* Torr. & A. Gray var. *zionis* N. H. Holmgren & P. K. Holmgren, are hanging garden varieties of morphologically variable, montane species (Welsh et al. 1993; Reveal 2009), much like the hanging garden variety of *A. elegans*. Very few studies of these species have been published, so little is known about their population genetics, morphological variation, and evolutionary history (Spence 2008).

Conservation Implications—As a subspecies, the hanging garden populations still warrant management separate from *A. elegans* subsp. *elegans*. Following the methodology for defining management units of Crandall et al. (2000), *A. vaginata* falls into category 5: recent ecological distinction. The hanging garden populations were likely part of a widespread, low-elevation species during the Pleistocene and are now ecologically isolated. As such, attempts to conduct artificial crosses between the two ecological groups, montane and hanging garden populations, are not recommended (Crandall et al. 2000). Following the recommendations of Crandall et al. (2000), crosses between hanging garden populations would be acceptable, though unnecessary in this case. The hanging garden populations exhibit relatively high levels of genetic diversity and typically have a large number of individuals that form clonal patches. It is therefore unnecessary to transplant individuals among regions or supplement gene pools with genetic material from dissimilar groups, particularly

when considering the importance of genetic adaptation to microhabitats (Huenneke 1991).

Currently, the primary threats to these hanging garden populations are the potential impact of climate change on the water availability for the hanging garden communities (Spence 2008) and, in some places, grazing and trampling by livestock (Palmquist pers. obs.). Human impacts on these populations, including the use of spring water at the source for livestock or other purposes, should be minimized to avoid damaging the hanging gardens. Beyond these measures, reproductive success should be assessed and long term monitoring of populations should be implemented.

TAXONOMIC TREATMENT

ANTICLEA ELEGANS (Pursh) Rydb. Bull. Torrey Bot. Club 30: 273. 1903. *Zigadenus elegans* Pursh., Fl. Am. Sept. 1: 241. 1814.—TYPE: U. S. A., Montana. Lewis and Clark Co., along the Blackfoot River west of Lewis and Clark Pass, 7 Jul 1806, *Lewis s. n.* (lectotype: PH-LC!).

Plants 1.8–8 dm, from bulbs. Bulbs clumped, in pairs, or occurring singly, tunicate, narrowly ovoid, 0.5–3 × 0.5–3 cm. Stems with or without persistent leaf bases. Leaves mostly basal, alternate, narrow, keeled, proximal blades 7–75 cm × 3–30 mm, distal ones reduced. Inflorescences loosely racemose to paniculate, 5–70 flowered, with 1–6 branches; bracts sometimes tinged with purple, ovate, 6–25(75) mm. Pedicel erect at anthesis, 3–23 mm. Perianth perigynous, rotate to rotate-campanulate, 9–25 mm diam.; tepals persistent in fruit, white to cream colored, sometimes tinged green, bronze, or purple, narrowed at base, outer tepals ovate, inner tepals spatulate to obovate, 4–12 × 2.5–6 mm; gland 1, obcordate. Capsules 8–19 mm.

KEY TO THE SUBSPECIES

1. Bracts with scarios margins and tips; leaves usually sharply pointed; tepals typically white or cream, sometimes with green or purple abaxially; capsule lance-conic 1–2.2 cm long. 2
2. Flowers larger, (10-)13.5–17(-25) mm in diameter; leaves shorter, (6.5-) 19.5–24(-38) cm; plants of high-elevation forests. 1. *A. elegans* subsp. *elegans*
2. Flowers smaller, (9.5-)12.5–14.5(-18.5) mm in diameter; leaves longer, (8.5-)30–35(-71) cm; plants of desert canyon springs. 2. *A. elegans* subsp. *vaginata*
1. Bracts herbaceous with subulate tips; leaves blunt to subacute; tepals typically colored green, bronze, or purple abaxially; capsule ovoid-conic, 1–1.4 cm long. 3. *A. elegans* subsp. *glauca*

1) *ANTICLEA ELEGANS* (Pursh) Rydb. subsp. *ELEGANS*, Bull. Torrey Bot. Club 30: 273. 1903. *Anticlea elegans* (Pursh) Rydb. var. *elegans* (Nutt.) Zomlefer & Judd, J. Bot. Res. Inst. Texas 3: 159–160. 2009. *Zigadenus elegans* Pursh, Fl. Am. Sept. 1: 241. 1814.—TYPE: U. S. A., Montana. Lewis and Clark Co., along the Blackfoot River west of Lewis and Clark Pass, 7 Jul 1806, *Lewis s. n.* (lectotype: PH-LC!).
Zigadenus alpinus Blankinship, Sci. Stud. Montana Coll. Agric., Bot. 1: 44. 1905. *Anticlea alpina* (Blankinship) A. Heller, Muhlenbergia 6: 12. 1910.—TYPE: near tree limit, Spanish Peaks, July 20, 1901 *J. Vogel s. n.* (lectotype: MONT!).
Zigadenus chloranthus Richards., Frankl. Narr. first Journ. App. 736. *Anticlea chlorantha* (Richards.) Rydb., Bull. Torrey Bot. Club 30: 273. 1903.—TYPE: *Richardson s. n.* (type not designated).
Zigadenus coloradensis Rydb., Bull. Torrey Bot. Club 27: 534. 1900. *Anticlea coloradensis* Rydb., Bull. Torrey Bot. Club

30: 273. 1903. *Zigadenus elegans* var. *coloradensis* M. E. Jones., Bull. Univ. Mont. Biol. Ser. 15: 22. 1910.—TYPE: U. S. A., Colorado, Idaho Springs, 26 Aug 1895, *Rydberg s. n.* (isotype: NY!).
Zigadenus dilatatus Greene, Pl. Baker. 1: 51. 1901.—TYPE: U.S.A., Colorado, La Plata Mts frequent everywhere within hills, 13 Jul 1898, *C. F. Baker 522* (isotypes: CAS!, DS, GH, MO!, NY, POM, RM!, UC, US!).
Zigadenus gracilentus Greene, Pittonia 4: 241. 1901. *Anticlea gracilenta* (Greene) R. R. Gates, J. Linn. Soc., Bot. 44: 155. 1918.—TYPE: Mexico, Chihuahua, slopes of Sierra Madre, 2,743 m, 1 Oct 1887, *C. G. Pringle 1383* (holotype: ND, isotypes: F!, GH!, MICH!, NY, LL!, US!, WIS!).
Zigadenus longus Greene, Pittonia 4: 240. 1901. *Anticlea longa* (Greene) A. Heller, Muhlenbergia 6: 12. 1910.—TYPE: U. S. A., Oregon, Blue Mountains, mountain stream banks, 4,000 ft, 28 Jul, *W. C. Cusick 2060* (isotypes: MO!, RM!, UC, US!, WS).

Zigadenus mohinorensis Greenm., Proc. Amer. Acad. Arts 39: 71. 1903. *Anticlea mohinorensis* (Greenm.) R. R. Gates, J. Linn. Soc., Bot. 44: 155. 1918.—TYPE: Mexico, Chihuahua, Mount Mohinora, 1 Sep 1898, E. W. Nelson 4875 (isotype: US!).

Zigadenus washake A. Nelson, Publ. Sci. Univ. Wyoming Bot. 1: 124. 1926.—TYPE: U. S. A., Wyoming, Fremont Co., Washakie National Forest, 10,000 ft, 15 Aug 1924, A. M. Cook s. n. (holotype: RM!).

Plants 1.8–5.4 dm. Bulbs usually occurring singly or in pairs, occasionally clumped, tunicate, narrowly ovoid, 0.8–2.5 × 0.8–2.5 cm. Stems usually without, sometimes with persistent leaf bases. Proximal leaf blades 6.5–38 cm × 3–20 mm. Inflorescences usually racemose, sometimes paniculate, 5–60 flowered, with 1–3(6) branches; bracts sometimes tinged with purple, ovate, 8–25 mm with scarious margins. Pedicels 3–20 mm. Flowers 10–25 mm diam.; tepals white to cream, sometimes with green stripe on abaxial side, sometimes tinged with purple, 4.5–12 × 2.5–6 mm. Capsules 8–17 mm long.

Distribution—*Anticlea elegans* subsp. *elegans* is found from Alaska south to central Arizona and New Mexico, with a few populations in northern Mexico and west Texas, and west into eastern Washington, Oregon, and Nevada, and east to North Dakota, South Dakota, and Minnesota. It grows in alpine wet meadows, mountain springs, exposed mountain slopes, deep shade of coniferous forest, and typically on limestone.

Phenology—Plants flowering from Jun – Jul (early Aug).

2) **Anticlea elegans** (Pursh) Rydb. subsp. **vaginata** Palmquist & T. Ayers, comb. nov. *Anticlea vaginata* Rydb., Bull. Torrey Bot. Club 39: 108. 1912. *Zigadenus vaginatus* (Rydb.) Macbride, Contrib. Gray Herb. 53:4. 1918.—TYPE: U. S. A. Utah, San Juan Co., Armstrong Canyon near the Natural Bridges, 1,600–1,800 m, 4 August 1911, P. A. Rydberg & A. O. Garrett 9407 (holotype: NY!; isotype: GH!, MO!, NY!).

Plants 1.4–5.8 dm, often hanging from cliff faces. Bulbs usually clumped and in large mats, sometimes occurring singly or in pairs, tunicate, narrowly ovoid, 0.7–3 × 0.7–2.5 cm. Stems with or without persistent leaf bases. Proximal leaf blades 8.5–71 cm × 3.5–23 mm. Inflorescences racemose or paniculate, 3–70 flowered, with 1–6 branches; bracts sometimes tinged with purple, ovate, 6–18(75) mm. Pedicels 3.5–23 mm. Flowers 9.5–18.5 mm diam.; tepals persistent in fruit, white to cream colored, sometimes with green stripe on abaxial side, sometimes tinged with purple, 4–8 × 2.5–5 mm. Capsules 8–19 mm.

Distribution—*Anticlea elegans* subsp. *vaginata* is endemic to hanging gardens and occurs in a patchy distribution from northeast Utah and western Colorado south to northeast Arizona. It grows in the deep shape of the hanging garden alcove or, less typically, on exposed, saturated slopes outside the alcove. It grows from the hanging garden walls and on the talus slope, but only where there is sufficient water. It almost always grows on sandstone, but very occasionally on limestone, where limestone underlies a layer of sandstone.

Phenology—Plants flowering from (late-Jul) Aug – Oct.

3) **ANTICLEA ELEGANS** (Pursh) Rydb. subsp. **GLAUCUS** (Nutt.) A. Haines, Stantec Bot. Notes 13: 2. 2010. *Anticlea glauca* (Nutt.) Kunth, Enum. 4. 192. 1843. *Melanthium glaucum* Nutt. Gen. 1:232. 1818. *Zigadenus elegans* Pursh var. *glauca* (Nutt.) Preece ex Cronq., Man. Vasc. Pl. NorthE.

U.S. Canad. ed. 2, 864. 1991. *Anticlea elegans* var. *glauca* (Nutt.) Zomlefer & Judd, J. Bot. Res. Inst. Texas 3(1): 159. 2009.—TYPE: On the gravelly banks of the St. Laurence in calcareous soil; around the Cataract of Niagara, on the borders of Lakes Erie, Huron and Michigan and up the Missouri to Fort Mandan. T. Nuttall s. n. (Type not designated, potential lectotypes: BM!, PH!).

Plants 3–8(10) dm. Proximal leaf blades darker green, 20–40 cm × 3–12 mm, coriaceous, glaucous, mostly blunt or subacute. Inflorescences usually paniculate, rarely racemose; bracts usually with purple tinge, lance-ovate, herbaceous, tapering to firm subulate tips. Tepals strongly colored with green, bronze, or purple, 8–12 mm long. Capsules ovoid-conic, 10–14 mm long. From Rydberg (1912), Fernald (1935), Gleason and Cronquist (1963), and Zomlefer and Judd (2009).

Distribution—*Anticlea elegans* subsp. *glauca* is found from Quebec, New York, and Pennsylvania west to the eastern Dakotas, and in scattered locations in the southern Appalachian Mountains and the Ozark Mountains (Zomlefer 1997). It grows in bogs in coniferous forests, calcareous wetlands, and along rivers and lakeshores (Zomlefer 1997; Zomlefer and Judd 2009).

Phenology—Plants flowering from late Jun–Sep (Fernald 1935).

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APPENDIX 1. DNA vouchers. DNA vouchers are listed alphabetically with taxon name, population code, population locality, number of individuals analyzed, and collector number (herbarium acronym). Herbarium abbreviations follow Index Herbariorum (Thiers 2014) with these exceptions: GLCA = the herbarium at Glen Canyon National Recreation Area, SEUG = the herbarium for the Southeast Utah Group, ZION = the herbarium at Zion National Park.

Anticlea elegans subsp. *elegans*. Abajo, U. S. A. Utah: Abajo Mountains, Manti-La Sal National Forest, 13, *L. Hannon Williams* 6 (ASC). BlueRidge,

U. S. A. Arizona: Barbershop Canyon, Coconino National Forest, 15, *M. Sommer* 2 (ASC). Chuska, U. S. A. Arizona: Chuska Mountains, Navajo Nation, 15, *E. Palmquist* 40 (NAVA). Dixie, U. S. A. Utah: Markaguant Plateau, Dixie National Forest, 15, *E. Palmquist* 33 (ASC). Hades, U. S. A. Arizona: Hades Lake, Grand Canyon National Park, 15, *G. Rink* 7676 (ASC). La Sal, U. S. A. Utah: La Sal Mountains, Manti-La Sal National Forest, 15, *L. Hannon Williams* 10 (ASC). Uinta, U. S. A. Utah: Uinta Mountains, Ashley National Forest, 15, *E. Palmquist* 45 (ASC). Modred, U. S. A. Arizona: Modred's Abyss, Grand Canyon National Park, 12, *G. Rink* 4877 (ASC). West Fork, U. S. A. Arizona: West Fork of Oak Creek Canyon, Coconino National Forest, 15, *M. Sommer* 1 (ASC). *Anticlea elegans* subsp. *vaginata*. Courthouse, U. S. A. Utah: Courthouse Wash, Arches National Park, 15, *E. Palmquist* 46 (SEUG). Delicate, U. S. A. Utah: Near Delicate Arch, Arches National Park, 15, *Welsh, Harrison, Moore* 2335 (SEUG). Junction, U. S. A. Utah: Junction of Canyon del Muerto and Canyon de Chelly, Canyon de Chelly National Monument, 15, *G. Rink* 1366 (ASC). Refuge, U. S. A. Utah: near Refuge Rock, Canyon de Chelly National Monument, 15, *G. Rink* 1371 (ASC). Canyon, U. S. A. Utah: Canyonlands, 14, *N. Boschen* S1-84 (SEUG). Bull, U. S. A. Utah: Bull Canyon, Dinosaur National Monument, 15, *E. Palmquist* 44 (ASC). Labyrinths, U. S. A. Utah: Labyrinths, Dinosaur National Monument, 15, *E. Palmquist* 43 (ASC). Reflection, U. S. A. Utah: Reflection Canyon, Glen Canyon National Recreation Area, 15, *E. Palmquist* 46 (GLCA). Ribbon, U. S. A. Utah: Ribbon Canyon, Glen Canyon National Recreation Area, 15, *E. Palmquist* 36 (GLCA). Inscription, U. S. A. Arizona: Inscription House Spring, Navajo Nation, 15, *D. Roth* 830 (NAVA). Johns, U. S. A. Utah: Johns Canyon, 15, *E. Palmquist* 39 (ASC). Kolob, U. S. A. Utah: Kolob Canyons, Zion National Park, 14, *E. Palmquist* 41 (ZION). Kachina U. S. A. Utah: Near Kachina Bridge, Natural Bridges National Monument, 15, *E. Palmquist* 38 (SEUG). Sipapu, U. S. A. Utah: Near Sipapu Bridge, Natural Bridges National Monument, 15, *E. Palmquist* 37 (SEUG). Surprise, U. S. A. Arizona: Surprise Valley, Navajo Nation, 15, *D. Roth* 822 (NAVA). *Anticlea mogollonensis*. ANMO, U. S. A. New Mexico: Mogollon Mountains, 15, *C. Huff* 693 (UNM). *Anticlea virescens*. ANVII, U. S. A. New Mexico: Mogollon Mountains, Gila National Forest, 15, *E. Palmquist* 42 (ASC). ANVI2, U. S. A. Arizona: White Mountains, Apache-Sitgreaves National Forest, 15, *L. Hannon Williams* 16 (ASC).

APPENDIX 2. Specimens examined for morphological analyses. Herbarium abbreviations follow Index Herbariorum (Thiers 2014), with the following exceptions: GLCA = herbarium at Glen Canyon NRA, SEUG = the herbarium for the Southeast Utah Group, ZION = the herbarium at Zion National Park.

Anticlea elegans subsp. *elegans*. CANADA. Mackenzie: vicinity of Brintnell Lake, *H. M. Raup* and *J. H. Soper* 9445 (RM); Northwest Territories: vicinity of Aubry Lake, *R. Rieve* & *J. Marsh* 181 (ASU); vicinity of Aubry Lake, *R. Rieve* and *J. Marsh* 448 (ASU). U. S. A. Alaska: Denali Co., Denali National Park, Wonder Lake, *A. Nelson* and *R. A. Nelson* 3878 (RM); Alaska Range, mi 254.3 Richardson Highway, *H. J. Lutz* 101758 (RM); Borg Creek at Glacier Creek Rd, Kateel River Merid. 19 mi N of Nome, *R. V. Harris* 8869RH (ASC). Arizona: Apache Co., Navajo Nation, Chuska Mts, south of Buffalo Pass, *D. Roth* 252 (NAVA); Apache Co., Big Cienega, White Mts, *M. Schmidt* 160 (ARIZ); Apache Co., Fort Apache Indian Reservation, *C. E. Granfelt* 69-177 (ARIZ); Apache Co., Apache National Forest, *T. Ayers* 1615 (ASC); Apache Co., White Mountains, Forest Service Rd 117A, 4.2mi NE of the junction of FS Rd 117 and 1mi SW of the junction with FS rd 118, and 1.8 mi NE of Camero Lake turnout, *B. D. Parfitt* & *D. Rickel* 3879 (ASU); Apache Co., Apache National Forest, in neighborhood of Spruce Dale Ranch, *D. M. Snyder* s. n. (ASU); Apache Co., Sheeps Crossing Campground, Mt. Baldy Wilderness, *T. Reeves* R601 (ASU); Apache Co., McKays Peak springs, *J. C. Watt* s. n. (ASU); Apache Co., Chuska Mts, south of Buffalo Pass, Navajo Nation, *E. C. Palmquist* 40 (NAVA); Coconino Co.; near Hole-In-Ground campground, ca. 3 mi W of Woods Canyon Lake, *T. Mason* & *C. T. Mason* 2608 (ARIZ); Coconino Co., Quaking Aspen Canyon, Kaibab Plateau, *L. Gooding* 173-48 (ARIZ); Coconino Co., Inner Basin, San Francisco Peaks, *Hevly* et al. s. n. (ASC); Coconino Co., SW slope of Agassiz, *L. Paulik* SA-70 (ASC); Coconino Co., Kaibab National Forest, 100 m below Kendrick Peak, trail side, *L. T. Greene III* G175 (ASC); Coconino Co., Willow Valley, 9 mi SE of Happy Jack, FR211B, *J. M. Rominger* 1673 (ASC); Coconino Co., Kendrick Peak, on east Newman hill, SW of lookout tower, *J. Ricketson* 597 (ASC); Coconino Co., Milk Spring along Pt Sublime Rd, *G. Rink* 7633 (ASC); Coconino Co., Robbers Roost spring, *G. Rink* 7691 (ASC); Coconino Co., Hades Lake, *G. Rink* 7676 (ASC); Coconino Co., Inner Basin of the San Francisco Peaks,

Coconino National Forest, *G. Rink* 4325 (ASC); Coconino Co., San Francisco Peaks, Inner Basin, *P. Johnson* s. n. (ASC); Coconino Co., Sycamore Canyon Wilderness, Tule Canyon, 500 m SW of fork, 6 km NW of its confluence with Sycamore Canyon, *M. Baker* 9854 (ASU); Coconino Co., Sitgreaves National Forest, Bear Canyon Lake, *L. R. Landrum* 5562 (ASU); Coconino Co., West Fork Canyon of Oak Creek, 9 mi N from Sedona, ca. 2.25 mi from canyon mouth along trail, *E. Gilbert* 732 (ASU); Coconino Co., Lake #1 east of Woods Canyon lake, *Taylor and Pinkava* 4563 (ASU); Coconino Co., West Fork Oak Creek, along stream, *Pinkava et al.* L18780 (ASU); Coconino Co., Inner Basin, San Francisco Peaks, *T. Reeves* and *D. Keil* K11574 (ASU); Coconino Co., Volunteer Canyon, 9 mi SE of Parks, ½ mi SE of Railroad Tank, *M. Schilling* 303(364) (ASU); Coconino Co., Inner Basin of San Francisco Mts above water facility buildings on Pipeline rd., *R. Romans* and *E. Lehto* 26 (ASU); Coconino Co., San Francisco Peak, Inner Basin, 3-4 km above water facility buildings, *R. Hevly* et al. R5241 (ASU); Coconino Co., Coconino National Forest, upper west fork of oak creek, about 0.8mi downstream from where FR 231 crosses West Fork, *E. Gilbert* 90 (ASU); Coconino Co., Coconino National Forest, 6 mi s of Flagstaff, Upper Walnut Canyon, *L. R. Landrum* 6959 (ASU); Coconino Co., Buck Springs Ranger Station, *R. E. Collom* 776 (ASU); Coconino Co., West Fork of Oak Creek, *Pinkava* et al. L20208 (ASU); Coconino Co., Inner Basin, San Francisco Peaks, *Keil* et al. P13762 (ASU); Coconino Co., Hole-In-Ground campground, 3 mi W of Woods Canyon Lake turnout, rim rd, *T. Mason* and *C. T. Mason Jr.* 2608 (ASU); Coconino Co., Inner Basin, San Francisco Peaks, *M. Strauss* s. n. (ASU); Coconino Co., Brookbank, San Francisco Peaks, *D. J. Pinkava* 6224 (ASU); Coconino Co., West Fork of Oak Creek, *C. F. Deaver* 3177 (ASU); Coconino Co., Fort Valley, Flagstaff, *C. F. Deaver* 3381 (ASU); Coconino Co., West Fork of Oak Creek, *E. Lehto* 18238 (ASU); Coconino Co., West Fork of Oak Creek, *R. B. Oxford* and *E. L. Smith* 483 (ASU); Coconino Co., West Fork of Oak Creek, *E. C. Palmquist* 34 (ASC); Coconino Co., West Fork of Oak Creek, *M. D. Sommer* 1 (ASC); Coconino Co., Mesa above E. Clear Creek and Barbershop Canyon junction, Blue Ridge area, *M. D. Sommer* 2 (ASC); Coconino Co., Mesa above E. Clear Creek and Barbershop Canyon junction, Blue Ridge area, *E. C. Palmquist* 35 (ASC); Gila Co., on rd to Valentine Creek, 4 mi from State Route 288 junction, *M. Mittleman* and *W. Hodgson* H852 (ASU). Colorado: Alamosa Co., Rio Grande National Forest, Sangre de Cristo Range, vicinity of south Zapata lake, *T. Hogan* 3435 (ASC); Clear Creek Co., FR7020 to St. Mary's Glacier, *J. Ackerfield* 1255 (CS); Dolores Co., Eastern San Miguel Mts, East Fork Trail, 1.5 mi south of Colo. 145, *R. L. Powell* 1997-30 (CS); El Paso Co., Mt. Manitore, *L. S. Ehlers* 366 (ASU); Huerfano Co., Huerfano river local, *H. MacKay* 7C-91 (ASU); Garfield Co., 1.2 mi N of Bar H-L Guard Station, *S. O'Kane Jr.* 476 (CS); Gillpin, Co., Gamble Gulch near Bee Vee mine, s. of Rollinsville, *G. N. Jones* 33414 (CS); Grand Co., Arapaho National Forest, Williams Fork Mts, between Henderson Tunnel and Williams Fork, ca 6 air mi SW of Byers Peak, ca 13.5 air mi SW of Winter Park, *E. Foley* 2602 (CS); Gunnison Co., 13 mi NW Crested Butte, North Pole Basin, White River National Forest, *E. R. Olgeirson* 128 (CS); Gunnison Co., Gothic area, *M. Kalil* s. n. (ASU); Gunnison Co., U.S. 50 at Monarch Pass, 40 mi E of Gunnison, *N. H. Russell* 10220 (ASU); Jackson Co., Medicine Bow Mts, Jack Creek and vicinity, 0.5 air mi. SW of Calamity Pass, ca 6 air mi S of Gould, *R. L. Hartman* 69354 (RM); Jackson Co., Never Summer Mts, along old logging road-trail between Illinois River and Illinois Pass, ca 10 air mi S of Gould, ca 29 air mi SE of Walden, *B. E. Nelson* 50313 (RM); Las Animas Co., Wilkens creek, parallel to hwy 12, ca ¼ mi NW of Stonewall Gap, *B. E. Neely* 4625 (CS); Larimer Co., Rocky Mountain National Park, spec. Mt. Trail, *J. M. Rominger* 1126 (ASC); Larimer Co., Mummy Pass trail in Roosevelt National Forest, *A. Shultz* s. n. (ASU); Mineral Co., 4-6 mi North and West of Creede, *J. Lewis* s. n. (ASU); Pitkin Co., White River National Forest, Rt 82 just below Roaring Fork River, *D. J. Pinkava* and *E. Leto* 6279 (ASU); Routt Co., near head of Summit Creek, SW of City Mtn, SE of Nipple Peak, Elkhead Mtns, *D. H. Wilken* 14813 (CS); San Miguel Co., west side of Ophir pass, ¼ mi below, *G. Goodwin* 2008 (ASC). Idaho: Bonneville Co., Caribou Mt, *E. B. Payson* and *G. M. Armstrong* 3590 (RM); Custer Co., Bear Canyon, *A. Nelson* and *J. F. MacBride* 1492 (ASC); Elmore Co., 1 mi east of Atlanta, Sawtooth Primitive Area, headwaters of Middle Fk. Boise River above Atlanta, *C. L. Hitchcock* and *C. V. Muhlick* 10195 (RM); Idaho Co., 12 mi SW of Riggins alongside trail 123, *R. T. Bingham* & *C. J. Miller* 84 (ASU); Lemhi Co., east slope of Lemhi Range, vic. Blue Dome, 23 mi N of Hwy 22 on Hwy 28, 10 mi N of Blue Dome, just south of FS rd to Meadow Canyon and Coal Kiln Canyon, *D. and M. Henderson* 1045 (ASU); East Fork, Wood River, *C. N. Woods* and *I. Tidestrom* 2786 (RM). Montana: Gallatin Co., Flathead Creek, *B. J. Jones* s. n. (ARIZ); Gallatin Co., Snowflake springs, 31 mi N of west Yellowstone, *D. Patten* and *E. Lehto* 35 (ASU); Granite Co., 3 km N of Drummond, *C. Schaack* 986 (ASC); Beaverhead National Forest,

O. Sparrow 169 (RM); Deerlodge National Forest, C. E. Fleming 40 (RM); Flathead National Forest, Echo Lake, C. H. Kauffman & G. B. Cummins 73 (ASU). Nevada: Elko Co., head of Dave creek on Jack Creek Mesa Rd, 15 mi NE of Jarbidge, P. Train 850 (ARIZ); Elko Co., Ruby Mountain, S of Harrison Pass, J. L. Gentry Jr. and G. Davids 1829 (ASU); Lander Co., Toiyabe National Forest, Toiyabe Range, Big Creek, S. Goodrich s. n. (RM); Lander Co., Toiyabe National Forest, Toiyabe Range, Big Creek, 14 mi from Austin, S. Goodrich 13368 (ASU); Nye Co., Hot Creek Range, North Canyon, 2 rd mi W of the site of Morey, A. Tiehm 14039 (ASU); Pershing Co., West Humboldt Mts, Star Creek Canyon on the east side of the range, west of the Silver State mine, A. Tiehm 9194 (DES); White Pine Co., Monte Neva hot springs NW of McGill, A. Atwood et al. 20877 (ASU); White Pine Co., Ruby Mts, Sherman Mt, N. H. Holmgren 3897 (ASU). New Mexico: Lincoln Co., Sierra Blanca, at northern border of Mescalero Indian Reservation, 10 mi NW of Ruidoso, M. Baad 991 (DES); McKinley Co., south tributary of little water creek, se of Asaayi Lake, B. Sivinski et al. s. n. (NAVA); Mora Co., Santa Fe National Forest and vicinity: Sangre de Cristo Mts: Pecos Wilderness: trail 251 along Horsethief Creek, 2.5 air mi WSW of Pecos Baldy, B. Reif 7534 (UNM); Rio Arriba Co., San Pedro Peaks and its surrounding meadows, A. Fleck s. n. (ASU); San Juan Co., Navajo Nation, Chuska Mountains, about 4.5 miles south of Todadena Lake, A. Clifford 00-728 (NAVA); San Miguel Co., Pecos River, 1 mi north of Terraro, B. Hutchins 8389 (UNM); Taos Co., upper Long Canyon trail, R. D. Worthington 32624 (UNM). Oregon: Wallowa Co., Wallowa-Whitman National Forest, slopes close beside Falls Creek, above the falls, approx 8 mi S of Enterprise, C. Feddema 3599 (RM); Wallowa Co., Jewett Lake, Wallowa Mts, about 10 mi S of Wallowa Lake, G. Mason 7978 (ASU). Utah: Duchesne Co., Uinta Mts, south fork of Rock Canyon, ca 3 mi NW of turnout to Upper Stillwater Dam, D. Barnes 2563 (UVSC); Garfield Co., by Wildcat Ranger Station in the Boulder Mts., R. D. Huish s. n. (UVSC); Grand Co., La Sal Mts, south along road from Geyser Pass to Blue Lake, J. G. Harris 2618 (UVSC); Iron Co., near Cedar Breaks National Monument, L. Higgins 4595 (ASU); Juab Co., Deep Creek Mts, head of Indian Farm Creek Canyon, J. G. Harris 4005 (UVSC); Kane Co., Cascade Falls Trail, Dixie National Forest, Markaguant Plateau, E. C. Palmquist 33 (ASC); Piute Co., Tushar Mts, Big Flat, J. G. Harris 2322 (UVSC); Summit Co., north slope Uinta Mts, Wasatch National Forest, east fork Bear River, ca 28 air mi SSE of Evanston, WY, C. H. Refsdal 7404 (RM); Utah Co., Santaquin Canyon, ca 1.6 km above Trumbolt Picnic Area, J. G. Harris 2963 (UVSC); Uintah Co., East park reservoir, 30 mi NE of Vernal, R. Graybosch 347 (ASC); Uintah Co., 0.3 mi east of Kaler Hollow bathroom and table on the Red Cloud Loop FR018, Ashley National Forest, E. C. Palmquist 45 (ASC); Wasatch National Forest, Whitney Ranger Station pasture, District 6, C. H. McDonald 315 (RM). Wyoming: Albany Co., Cummins, A. Nelson 1453 (RM); Albany Co., Snowy Range along Brooklyn Ridge and Lake, S. F. Glassman 7123 (ASU); Fremont Co., Wind River Range, about 25 mi W of Lander, on the Moccasin Lake Rd, H. G. Fisser 707 (RM); Fremont Co., ca 8.2 air mi SSE of Duboise, ca 6.9 mi s on Trail Lake Rd, J. Haines 5083 (RM); Fremont Co., meadows on north facing slope of Bold Mountain, D. Van Denbos 7312 (RM); Park Co., Absaroka Mts, Eleanor Creek N to Ridge, R. L. Hartman 19329 (RM); Teton Co., Teton Mts, A. Nelson and E. Nelson 6486 (RM); Yellowstone National Park, Mammoth Hot Springs, A. Nelson and E. Nelson 6055 (RM).

Anticlea elegans subsp. *glaucaus*. U. S. A. Iowa: Dickenson Co., Manhattan Slough, 1.5 mi N of lakeside laboratory, Lakeville Township, R. F. Thorn 12501 (ASU). Michigan: Grand Ledge, *Devey* s. n. (ARIZ). Minnesota: Sibley Co., about 3 mi east of the junction of State Highways 15 and 19 in Winthrop, W. R. Smith 4284 (RM); Mahanomen Co., along Hwy 200 west of Zerkel, S. E. Hamilton 70 (ASU). North Dakota: Benson Co., prairies, Leed, J. Lunell s. n. (RM); McHenry Co., 4 mi west of Towner, prairie along railroad right of way, J. E. Bare & R. L. McGregor 1033 (ASU). South Dakota: Custer Co., near Sylvan Lake, Black Hills, G. E. Osterhout 7849 (RM); Custer Co., ¼ mi south of Custer, O. Degener and L. Peiler 16325 (RM). Wisconsin: Green Lake Co., Boy Scout Camp Tichora on Green Lake, R. Peters 40 (ASU).

Anticlea elegans subsp. *vaginata*. U. S. A. Arizona: Apache Co., hanging garden on north side of Coyote Creek about 2 km upstream of Wheatfields Creek, G. Rink 1312 (UNM); Apache Co., hanging garden 2/3 of the way up the Lady White Route near the Junction, G. Rink 1466 (NAVA); Apache Co., hanging garden at the upper end of the Selah Spring route in Canyon de Chelly, G. Rink 1369 (NAVA); Apache Co., hanging garden at the end of the alcove north of the White Lady route in Canyon del Muerto just above the Junction, G. Rink 1366 (NAVA); Apache Co., Canyon del Muerto, just up from the junction with Canyon de Chelly, NW-facing alcove with small seep, D. Roth 1396 (NAVA); Apache Co., hanging garden on north side of Coyote creek about 2 km upstream of Wheatfields Creek, G. Rink 1312 (NAVA); Apache Co., hanging

garden at the upper end of the Selah Springs route in Canyon de Chelly, G. Rink 1396 (ARIZ); Apache Co., hanging garden at the upper end of the Selah Springs route in Canyon de Chelly, G. Rink 1396 (UNM); Apache Co., west of Refuge Rock in Canyon de Chelly, G. Rink 1371 (ASC); Apache Co., hanging garden 2/3 of the way up the Lady White Route near the Junction, G. Rink 1466 (ASC); Apache Co., hanging garden at the upper end of Selah Springs Route in Canyon de Chelly, G. Rink 1396 (ASC); Apache Co., hanging garden on north side of Coyote Creek about 2 km upstream of Wheatfields Creek, G. Rink 1312 (ASC); Apache Co., north side of Coyote Creek about 2 km upstream of Wheatfields Creek confluence, G. Rink 1312 (BRY); Apache Co., upper end of the Selah Springs trail, in north-side tributary to Canyon de Chelly about one mi upstream of the Beehive trail, G. Rink 1396 (BRY); Coconino Co., along the Inscription House Ruin trail, extensive seep area just N of the trail, D. Roth 836 (NAVA); Coconino Co., Inscription House Ruin spring, seep/spring area along sandstone seam at the canyon head, D. Roth 830 (NAVA); Coconino Co., Inscription House Ruin spring, seep/spring area along sandstone seam at the canyon head, D. Roth 830 (ASC); Coconino Co., along the Inscription House Ruin Trail, extensive seep area just N of the trail, D. Roth 836 (ASC). Colorado: Moffat Co., above Harding Hole, S side of Yampa River, T. Naumann 182 (CS); Moffat Co., ravine below Signature Cave at Harding Hole, N side of Yampa River, T. Naumann 277 (RM); Moffat Co., Bull Canyon, Dinosaur National Monument, E. C. Palmquist 44 (ASC). Utah: Grand Co., along the seep line above Delicate Arch trail, K. S. Forsythe 18 (SEUG); Grand Co., lower Delicate Arch seeps, D. Fagan 4 (SEUG); Grand Co., lower Delicate Arch seeps, D. Fagan 2 (SEUG); Grand Co., lower Delicate Arch seeps, D. Fagan 5 (SEUG); Grand Co., hanging garden north of trail to Delicate Arch, Arches National Monument, S. L. Welsh, B. F. Harrison, G. Moore 2335 (SEUG); Grand Co., lower Delicate Arch seeps, D. Fagan 1 (SEUG); Grand Co., lower Delicate Arch seeps, D. Fagan 3 (SEUG); Grand Co., lower Delicate Arch seeps, D. Fagan 6 (SEUG); Grand Co., side canyon off Kane Springs Canyon, ca 4 mi SW of Moab, J. Tuhy 3775 (ARIZ); Grand Co., side of canyon off Kane Springs Canyon ca 4 mi SW of Moab, J. Tuhy 3775 (ASU); Grand Co., Arches National Park, Courthouse wash, B. Franklin 3766 (RM); Grand Co., hanging garden north of trail to Delicate Arch, Arches National Park, Welsh et al. 2335 (BRY); Grand Co., side of canyon off Kane Springs Canyon, ca 4 mi SW of Moab, J. Tuhy 3775 (BRY); Grand Co., Moab Utah, Cottam 2165 (BRY); Grand Co., Arches National Park, NE side of Courthouse Wash past Ring Arch, E. C. Palmquist 46 (SEUG); Kane Co., Glen Canyon National Recreation Area, Fence hanging garden, Fence Canyon, J. Fowler 1030b (RM); Kane Co., Pool garden, Reflection Canyon, west of the confluence of San Juan and Colorado Canyons, S. L. and S. L. Welsh 11878 (BRY); Kane Co., Cottonwood Canyon off Reflection Canyon, Glen Canyon National Recreation Area, E. C. Palmquist 27 (ASC); Kane Co., Cottonwood Canyon off Reflection Canyon, Glen Canyon NRA, E. C. Palmquist 47 (ASC); Kane Co., Fence Canyon, Glen Canyon NRA, J. Fowler 1030 (GLCA); San Juan Co., Colorado River lateral canyon, 76 mi above Lee's Ferry, H. C. Cutler 3181 (ASC); San Juan Co., Natural Bridges National Monument, seep above Kachina Bridge, R. Fleming 1114 (SEUG); San Juan Co., Natural Bridges National Monument, seep above Kachina Bridge, R. Fleming 1114 (SEUG); San Juan Co., Natural Bridges National Monument, alcove near Sipapu Bridge, Heil and Fleming 5669 (SEUG); San Juan Co., Navajo Nation, Cliff Canyon, west of Navajo Mountain, D. Roth and K. McCoy 793 (NAVA); San Juan Co., Navajo Nation, Surprise Valley, north of Navajo Mountain, along the Rainbow Bridge trail, large alcove just before trail climbs out of the canyon towards the bridge, D. Roth 732 (NAVA); San Juan Co., Lower John's Canyon, S. of Poll Mesa on the canyon bottom, A. Clifford 93-148 (NAVA); San Juan Co., Surprise Valley, north of Navajo Mountain, along the Rainbow bridge trail, D. Roth 822 (NAVA); San Juan Co., Navajo Nation, Surprise Valley, North of Navajo Mountain, along the Rainbow Bridge Trail, D. Roth 732 (ASC); San Juan Co., Cliff Canyon west of Navajo Mountain, ca ¾ mi downstream from the junction with Redbud Pass Canyon, D. Roth and K. McCoy 793 (BRY); San Juan Co., Armstrong Canyon between Kachina Bridge and Owachomo Bridge, Natural Bridges National Monument, S. L. Welsh and G. Moore 2496 (BRY); San Juan Co., Surprise Valley, north of Navajo Mountain, along the Rainbow Bridge Trail, D. Roth 822 (BRY); San Juan Co., second hanging garden on the east side of the Colorado River just north of the San Juan River confluence, N. D. Atwood, S. L. Welsh, J. Murdock 3229 (BRY); San Juan Co., second hanging garden on the east side of the Colorado River just north of the San Juan River Confluence, N. D. Atwood, S. L. Welsh, J. Murdock 3229 (GLCA); San Juan Co., second hanging garden up the San Juan River from its confluence with the Colorado River, along west side on an east exposure, N. D. Atwood and R. Allen 3180 (BRY); San Juan Co., vicinity of Kachina Bridge, Natural Bridges National Monument, S. L. Welsh and G. Moore 2409 (BRY); San Juan

Co., John's Canyon drainage, 3.0 mi N of Muhley Point, *A. Clifford and K. Heil* 03-1080 (BRY); San Juan Co., Natural Bridges National Monument, alcove near Sipapu Bridge, *Heil and Fleming* 5669 (BRY); San Juan Co., Navajo Nation, John's Canyon, 14 mi WNW of Rd 316, *K. Heil and A. Clifford* 22897 (BRY); San Juan Co., Ribbon Canyon, Granddaddy Garden, Glen Canyon NRA, *E. C. Palmquist* 36 (ASC); San Juan Co., alcove near Sipapu Bridge, Natural Bridges National Monument, *E. C. Palmquist* 37 (SEUG); San Juan Co., John's Canyon, 15 mi from Hwy 316 on the John's Canyon rd, *E. C. Palmquist* 39 (ASC); San Juan Co., hanging garden in alcove N of trail that exits White Canyon from Kachina Bridge, Natural Bridges National Monument, *E. C. Palmquist* 38 (SEUG); San Juan Co., Natural Bridges, *L. C. Higgins and S. L. Welsh* 14258 (GLCA); Uintah Co., hanging garden in Labyrinths, Dinosaur National Monument, *E. C. Palmquist* 43 (ASC); Washington Co., partway up Kolob Arch canyon along creek, Zion National Park, *E. C. Palmquist* 41 (ZION); Canyonlands National Park, along Syncline trail in an alcove with a permanent spring, *N. S. Boschen* 51-84 (SEUG);

Anticlea virescens. MEXICO. Arteaga, Sierra Los Camargos, *G. B. Hinton* 17880 (ASU); Chihuahua, in the Sierra Madres near Colonia Garcia, *C. H. T. Townsend and C. M. Barber* 184 (RM); Chihuahua, Municipio de Ocampo, Parque Nacional Cascada de Basaseachi, Cañón La Zorra plots, Sierra Madre Occidental, *M. Joe* 640 (ASC); Chihuahua, Municipio de Ocampo, Parque Nacional Cascada de Basaseachi, Cañón del Pájaro plots, Sierra Madre Occidental, *M. Joe* 994 (ASC); Tamaulipas, on east and south slope and summit of Pena Nevada, *Stanford, Lauber, Taylor* 2549 (RM). U. S. A. Arizona: Apache Co., Mt. Baldy, edge of forest to ca 1/8 mi toward summit of Mt. Baldy along Sheep Crossing Trail, *T. Reeves* R5278 (ASU); Apache Co., along Mt. Baldy trail at Sheep's Crossing, White Mts, *C. and B. Schaack* 1264 (ASC); Apache Co., Reservation Ranch on Apache Indian Reservation, *L. N. Goodding and Shields* 406-41 (ASU); Cochise Co., upper Carr Canyon, Huachuca Mts, *T. R. Van Devender* s. n. (ARIZ); Cochise Co., Split Rock Canyon Game preserve, Huachuca Mts, *L. N. Goodding* 894-49 (ARIZ); Cochise Co., southwest flank of Huachuca Peak, *J. E. Bowers* 3375 (ARIZ); Greenlee Co., White Mts below Willow Creek, weir #1, end of FR564A, *T. Reeves* 8600 (ASU); Greenlee Co., Davis Creek, 1/2 mi up from Forest Service rd 275, *J. Cordts and W. Hodgson* 3138 (DES); Greenlee Co., n. of Clifton, *O. M. Clark* 12937

(UNM). Colorado: Gunnison Co., West Elk Mts, Summit of McClure Pass, 0.8 mi from main hwy along dirt rd following the ridge eastward, *W. A. Weber and R. C. Whittmann* 19070 (UNM). New Mexico: Catron Co., Mogollon Mts, drainage W. of National Forest Trail 195, Stub Trail, N. slopes of Bearwallow Mt, S. of Deep Creek, *S. Nelson et al* s. n. (DES); Catron Co., Gila National Forest, along drainage flowing NW into BS canyon on N slope of Bearwallow Mt, *T. F. Daniel and S. Nelson* 3598 (ASU); Catron Co., Mogollon Mts, FR159 between SilverCreek Divide and Sandy Point, *R. Sivinki & K. Lightfoot* 2518 (UNM); Catron Co., Mogollon Mts, FR 159 between Silver Creek Divide and Sandy Point, *E. C. Palmquist* 42 (ASC); Socorro Co., Magdalena Mts, South Baldy, *C. R. Hutchins* 4736 (UNM).

APPENDIX 3. Morphological characters and states used in for taxa in this study. *Used for Principal components analysis and canonical discriminant function analysis.

1. Proximal leaf length (mm)*: from divergence point on stem.
2. Proximal leaf width (mm)*
3. Capsule length (mm): persistent tepals to apex.
4. Number of flowers (approx)*: buds, flowers, pedicels, and capsules.
5. Pedicel length (mm)*: average of lowest two to three pedicels on main inflorescence axis.
6. Bract length (mm)*: average of lowest two or three subtending bracts.
7. Bulb length (mm): base of bulb (not including roots) to where bulb width equaled stem width.
8. Bulb width (mm): widest part of bulb.
9. Plant height (cm): apex of bulb to base of first flower pedicel or first branch; only measured for individuals that had reached anthesis.
10. Inflorescence height (cm): base of first flower pedicel or first branch to base of pedicel of uppermost flower; only measured for inflorescences with open flowers or capsules at apex of inflorescence.
11. Flower diameter (mm)*.
12. Tepal length (mm)*.
13. Tepal width (mm)*.
14. Persistent leaves* (retained entire leaves, bases and blades): 0 = absent, 1 = present.
15. Sheaths at base of stem* (retained sheaths that never had blades): 0 = absent, 1 = present.
16. Inflorescence structure*: 0 = racemose, 1 = one branch, 2 = two branches, 3 = three branches, 4 = four branches, 5 = five branches, 6 = six branches.
17. Flower shape*: 0 = rotate, 1 = campanulate.