PHYLOGENY AND EVOLUTIONARY HISTORY OF *ANTICLEA VAGINATA* RYDB. (MELANTHIACEAE): A HANGING GARDEN ENDEMIC

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ABSTRACT

This study examines the taxonomic status, population genetics, and evolutionary history of the rare, endemic plant species, *Anticlea vaginata* Rydb. (Melanthiaceae). Although this species is considered rare, very little research has been conducted on it. Doubts about its distinctness as a species have been raised, which has implications for its status as a rare species. An assessment of morphological distinction was conducted on *A. vaginata* and its closest relative, *Anticlea elegans*, using PCA, PERMANOVA, and one-way ANOVAs of key characteristics. Genetic variability and population structure were assessed with AFLPs. An NMS ordination, AMOVAs, cluster analyses, gene diversity estimates, and Structure 2.2 were used in combination to determine the level of genetic differentiation between the two species, if any. Four main patterns emerge: 1) *A. vaginata* and *A. elegans* exhibit large amounts of overlap in morphological and genetic variability. 2) Despite this overlap, there are indications of ongoing diversification. 3) Populations within *A. vaginata* exhibit relatively high levels of structuring and do not group as species during analyses. 4) Populations of *A. vaginata* show genetic patterns consistent with those expected for relictual populations. I conclude that since there are no consistent distinguishing characteristics between *A. vaginata* and *A. elegans*, *A. vaginata* should become a subspecies of A. *elegans*. Populations of *Anticlea vaginata* should be managed as units distinct from *A. elegans* subsp. *elegans*. It is likely that there is little gene flow between *A. vaginata* and *A. elegans*, and the populations of *A. vaginata* may be on their own evolutionary trajectories.

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CHAPTER 1 – BACKGROUND AND LITERATURE REVIEW

The canyon lands of the Colorado Plateau are situated in an arid to semi-arid environment. Springs and rivers provide an important habitat for many plant species that otherwise could not occur in these arid landscapes. Hanging gardens are one type of these springs, and they contain unique plant communities (Welsh & Toft 1981; Spence 2008). Hanging gardens occur in the extensive network of canyons that crisscross Utah, western Colorado, and northern Arizona (Spence 2008). In these canyons, hanging gardens are formed by perched aquifers that seep out of the permeable sandstone walls (May et al. 1995). As water seeps out of the walls, the rock is slowly eroded away, forming alcoves in the canyon walls (May et al. 1995).

Hanging gardens can vary from simple seeps following the bedding planes of the underlying rock to large alcoves with dense vegetation hanging from the walls and a plunge pool formed from drainages above (Welsh & Toft 1981). Two primary microhabitats occur in these gardens: the wet backwall habitat and colluvial-detritus floor of the garden. Some gardens contain a third habitat, a plunge pool basin that hosts a small wetland community (Spence 2008). The amount of light and exposure a garden experiences is determined by the structure of the garden, ranging from very little to fairly exposed (Welsh 1989, personal observation). Most gardens, however, receive very little sunlight and are buffered from the aridity and heat of the surrounding landscape (Welsh 1989).

Hanging garden vegetation is composed of both widespread, common riparian species as well as species that are only found in hanging gardens (Welsh 1989; Fowler et al. 2007; Spence 2008). Despite the relatively small areal extent hanging gardens cover, they contain 15% of the endemic species for the northern and central portions of the Colorado Plateau (Spence 2008). An assessment of vegetation associations in hanging gardens (Fowler et al. 2007) found that although hanging garden endemics are not widespread, some of them tend to be the dominant vegetation type when they occur. Many hanging garden endemics are likely derived from boreal-temperate relatives, though some have ties to southern species (Spence 2008).

Sheathed Death Camas, *Anticlea vaginata* Rydb. (Liliales: Melanthiaceae) is one of these hanging garden endemic species. It is found in the backwall habitat and colluvial-detrius habitat in hanging gardens, in fact it sometimes hangs from alcove ceilings (Welsh 1989, personal observation). Welsh & Toft (1981) noted that the disjunct distribution that is common for backwall vegetation is more pronounced in *A. vaginata*. Due to *A. vaginata*'s affinity to hanging gardens with high endemic species diversity, it has been suggested that studying *A. vaginata* would further the understanding of hanging garden endemics (Keate 1996).

Anticlea vaginata occurs primarily in southeastern Utah and northeast Arizona with a few outlying populations (Welsh 1981; Welsh & Toft 1989; Rink 2005; Fowler et al. 2007; ASC 2010; Roth pers. comm.). It occurs in Arches National Park, Canyonlands National Park, Canyon de Chelly National Monument,

Dinosaur National Monument, Glen Canyon National Recreation Area, Natural Bridges National Monument, Zion National Park, the Navajo Nation, and BLM lands. Much of its current known distribution is relatively protected in NPS units, though multiple hanging gardens have already been lost to the waters of Lake Powell (Welsh & Toft 1981, Welsh 1989). Although many hanging gardens seem to have appropriate habitat, Sheathed Death Camas only occurs in a relatively small number of them and in a seemingly sporadic distribution (Welsh & Toft 1981). One garden will contain large amounts of Death Camas, while the hanging garden in the next canyon will not (Welsh 1989). When it does occur, it is often present in very high numbers (Harrison et al. 1964; personal observation). This species requires the microclimates of the alcoves for survival (Welsh & Toft 1981; Keate 1996; Spence 2008), which makes it vulnerable to changes in climate and disturbance of habitat.

Anticlea vaginata Rydb. was originally described in 1912 from a collection near Natural Bridges National Park (Rydberg 1912) in southeastern Utah. It was subsequently transferred to *Zigadenus vaginatus* (Rydb.) Macbr. (Macbride 1918), and then recently returned to *A. vaginata* Rydb. (Zomlefer & Judd 2002). This most recent change is based on the results of a molecular examination of the tribe Melanthiaceae including the genus *Zigadenus* s.l. (Zomlefer et al. 2001). This study showed that *Zigadenus* s.l. is not monophyletic. The segregate genera *Anticlea* and *Toxicoscordion* were reinstated, *Stenanthella* was collapsed into

Anticlea, some species of *Zigadenus* were moved into *Stenanthium*, and some species of *Stenanthium* were moved into *Anticlea* (Zomlefer & Judd 2002).

Anticlea vaginata is sometimes called Sheathed Death Camas and sometimes Alcove Death Camas (Welsh 1993; Schwartz 2002). The first common name is presumably a reference to the original description's mention of numerous loose sheaths at the base of the stem. This is reflected in the scientific name as well, since "vaginata" is latin for "sheathed" (Stearn 2004). "Alcove" refers to the habitat of *A. vaginata*; the alcoves containing hanging gardens. The term "Death Camas" is a reference to its similar appearance to Camas, genus *Camassia*, which has an edible bulb (J.M.C. 1915). Death Camas has been mistaken for the edible Camas and injested (Heilpern 1995). Unfortunately for the hungry victim, species in the genus *Zigadenus* s.l. contain poisonous alkaloids, including amianthine, germine, jervine, veratradine, zygacine, and zygadenine (Zomlefer 1997). Though most people recover after 24 to 48 hours, the time in between ingestion and recovery is characterized by nausea, vomiting, abdominal cramping, diarrhea, an increase in nerve and muscle excitability, and an extremely low resting heart rate (Heilpern 1995). The toxic affects of *Zigadenus* on livestock have been well studied and the losses due to *Toxicoscordion paniculatum* (syn. *Zigadenus paniculatus*) have been considered enough of a problem to require chemical control (Hyder & Sneva 1962). Species within *Anticlea* are purportedly much less toxic than those species now included in *Toxicoscordion* (Walsh 1940).

Although *Anticlea vaginata* has generally been recognized as a distinct species (e.g. Welsh et al. 1993; Schwartz 2002), it has sometimes been treated as a synonym of *Anticlea elegans* Pursh (Cronquist et al. 1977). Rydberg's original description of *A. vaginata* (1912) states that its unique characteristics consist of "its habit of growing in big clumps, and its numerous loose sheaths at the base of the stem." He states that it is similar to *A. elegans* in the shape of the tepals and similar to *A. virescens* in the few veins on the tepals. However, the tepals were reported to be smaller than *A. elegans*, but broader than those of *A. virescens*. He also noted that *A. vaginata* resembled *A. virescens* in its branched inflorescence, but differed in the length of the pedicels and width of the leaves.

MacBride (1918) in his revision of *Zigadenus* and Gates (1918) in his revision of Melanthiaceae recognized *A. vaginata* as a distinct species. They also recognized many species that are now collapsed into either *A. elegans* or *Anticlea virescens* Kunth. MacBride (1918) indicated he had seen *A. elegans* exhibiting a clumped habit as described for *A. vaginata*, but that *A. vaginata*'s white flowers are noteable and different. Walsh (1940), in an unpublished taxonomic revision of *Zigadenus*, did not discuss *A. vaginata* and included it as a synonym of *A. volcanica,* though he apparently only looked at one *A. vaginata* specimen. Preece (1956), in an unpublished cytotaxonomic treatment of *Zigadenus* treated *A. vaginata* as its own species, but stated that it should be considered a synonym of *A. volcanica*. He retained *A. vaginata* as a distinct species because of differing habitats and distribution. Welsh et al. (1975) included *A. vaginata* in their list of

endangered Utah plants, and emphasized its distinctness and its relationship to *A. elegans* rather than *A. volcanica*. Cronquist et al. (1977) considered it similar enough to *A. elegans* to be included with that species in the Intermountain Flora as a "lowland phase." They did, however, note that *A. vaginata* is sometimes larger and clustered, as well as having smaller tepals. Welsh et al. (1993) continued to treat *A. vaginata* as its own species in The Utah Flora, using flower color, flower size, inflorescence structure, and habitat as the primary distinguishing characteristics. In the descriptions of the species, *A. vaginata* was taller, had longer leaves, a paniculate inflorescence (vs. usually racemose), white tepals (vs. green to cream), smaller tepals, and smaller capsules than *A. elegans*. Hess & Sivinksi (1995), in their assessment of *Anticlea*, discussed *A. vaginata* as a separate species and argued that its closest relative is *A. elegans* rather than *A. volcanica*. In their key to the American taxa of *Anticlea*, they separated *A. vaginata* from *A. elegans* with the "lower stem sheathed with numerous hard, persistent leaf bases," slightly smaller tepals, and the hanging garden habitat. Schwartz (2002) in the Flora of North America retained *A. vaginata* as a separate species, as well, using tepal color and the presence of persistent leaf bases as the primary distinguishing characters. The species descriptions of *A. vaginata* and *A. elegans* also indicated that *A. vaginata* had longer leaves, a paniculate inflorescence (vs. racemose to paniculate), bigger inflorescence, smaller pedicels, smaller flowers, smaller tepals, smaller bracts, and smaller capsules.

Though some authors suggest that *Anticlea volcanica* (Bentham) J. G. Baker is the closest relative of *A. vaginata* (Welsh & Toft 1981; Spence 2008), this is unlikely since *A. volcanica* is morphologically similar to the *Anticlea virescens* complex, rather than the *Anticlea elegans* complex (Hess & Sivinski 1995). *Anticlea volcanica* is a poorly understood and often misrepresented species (Hess & Sivinski 1995), which has lead to much confusion. It seems that the persistence in the literature to link *A. volcanica* and *A. vaginata* stems from a 1925 flora (Tidestrom 1925). All other researchers who link the two species either only looked at one specimen of one of the species or cited one of those authors. The authors that have looked at more than one specimen of both species conclude that *A. vaginata* is more similar to *A. elegans* (Hess & Sivinski 1995).

Very little research has been conducted on *Anticlea vaginata*. Welsh & Toft (1981) discuss *Anticlea vaginata*'s possible ties to *A. volcanica* as well as its disjunct distribution in their descriptive paper on the hanging gardens of southeastern Utah. Welsh (1989), in his summary of hanging garden types, mentions *A. vaginata*, but essentially repeats the same information as the previous publication, just with more specific localities.

Keate (1996) includes it as one of the study plants used in her ecological analysis of hanging garden endemics. She identified certain environmental characteristics of hanging gardens that allow the endemic species to survive there, such as low light levels, no flooding, course soil textures and large

seeplines. She did not, however, look at life history traits or investigate evolutionary histories.

Fowler et al. (2007) assessed vegetation associations in hanging gardens along the Colorado River, in Zion National Park, and in Dinosaur National Park. *Anticlea vaginata* was included in this study, since it was a component of the floras. This added a basic understanding of its floristic associations and localities.

Hanging garden studies conducted by Spence (2004) and regional floras (e.g. Harrison et al. 1964; Spence 2005; Rink 2005) have increased knowledge about the locations and environment of this species, as well.

Despite all these studies in which *Anticlea vaginata* is mentioned, little is known about its life history traits and evolutionary history. Pollination and reproductive methods remain unknown. Population dynamics and trends, fecundity rates, and methods of dispersal are also not understood. How or if gene flow occurs between populations is uncertain. In order for management decisions to be made about this species, more knowledge must be gathered about whether or not it is a good species, how it became restricted to hanging gardens, and how these populations persist from year to year.

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CHAPTER 2 - PHYLOGENY AND EVOLUTIONARY HISTORY OF *ANTICLEA VAGINATA* RYDB. (MELANTHIACEAE): A HANGING GARDEN ENDEMIC

The genus *Zigadenus* s.l. (Melanthiaceae) and the species included within it have a long and complicated taxonomic history. Recently, genetic studies of the tribe Melanthieae (Zomlefer et al. 2001) resulted in the reinstatement of two of the segregate genera of *Zigadenus* s.l. , *Anticlea* Kunth. and *Toxicoscordion* Rydb. (Zomlefer & Judd 2002). *Anticlea* currently comprises approximately 11 species (Zomlefer et al. 2001). One of these is *Anticlea vaginata* Rydb., Sheathed Death Camas, a rare endemic that is only found in unique desert springs on the Colorado Plateau called, "hanging gardens." Hanging gardens are islands of mesic habitat that occur in the alcoves of deep, sandstone canyons, and are buffered from the sun, heat, and aridity of the surrounding desert (Welsh & Toft 1981; Keate 1996; Flanagan et al. 1997; Spence 2008). This species requires the microclimates of the alcoves for survival (Keate 1996; Spence 2008), which makes it vulnerable to changes in climate and disturbance of habitat. Due to its status as a narrow endemic, it is currently listed as a "G2-Imperiled" species by NatureServe (March 2010) and is labeled as being "of conservation concern" in the Flora of North America (Schwartz 2002).

Anticlea vaginata is the only hanging garden species in the genus. All other species of *Anticlea* are found in montane habitats. *Anticlea vaginata* is therefore presumed to be of boreal origin, but it lacks long distance dispersal mechanisms that could explain its current disjunct distribution (Spence 2008). In order to explain the disjunct nature of this and other hanging garden endemics,

Keate (1996) and Spence (2008) suggest that they were more widespread during the cooler, wetter Pleistocene, and have since found refuge in the buffered habitat of hanging gardens as the climate became more arid. Spring studies conducted by Spence (2008) show patterns indicative of vicariance, which supports this hypothesis. If these populations are remnants of a once more widely spread species, it is likely that high levels of diversity are still present in these gardens. This could also mean that the populations of *A. vaginata* may actually be more closely related to geographically close congeners rather than more distant populations of *A. vaginata*. It is also possible that the hanging garden plants are no longer connected by gene flow and are embarking on their own evolutionary trajectories.

Very little research has been conducted on *Anticlea vaginata,* and it has never been the focus of a phylogeographic study. It was originally described in 1912 from a collection near Natural Bridges National Park (Rydberg 1912), and since then different researchers have come to different conclusions regarding its validity as a species and its closest relatives. It is typically treated as a distinct species (e.g. Welsh et al. 1993; Schwartz 2002) or as a synonym of *Anticlea elegans* Pursh (Cronquist et al. 1977). *Anticlea vaginata* was originally described as sharing some morphological characters with *Anticlea elegans* and some with a large flowered form of *Anticlea virescens* (Kunth) J.F. Macbr. (Rydberg 1912; Gates 1918). Both of these species have since been suggested as *A. vaginata*'s closest relative (MacBride 1918; Hess & Sivinski 1995). The ambiguity in its morphology has clearly been enough to create doubts about its distinctiveness

and therefore rarity. *Anticlea vaginata*'s status as a species has implications for its management and the ongoing endeavor to resolve the complicated taxonomy of the genus.

Anticlea vaginata and *Anticlea elegans* are separated from the other species in the genus by having erect pedicels and rotate to rotate-campanulate corollas at anthesis. *Anticlea vaginata* is typically separated from *Anticlea elegans* by the presence of persistent leaf bases (Preece 1956; Hess & Sivinski 1995; Schwartz 2002). Other characters that have been used to distinguish *A. vaginata* from *A. elegans* include white tepals vs. cream to greenish tepals in *A. elegans* (Welsh et al. 1993; Schwartz 2002), tepals 3-6 mm vs. 7-12 mm in *A. elegans* (Welsh et al. 1993; Hess & Sivinski 1995; Schwartz 2002), numerous loose sheaths at the base of the stem vs. old leaf sheaths not retained in *A. elegans* (Rydberg 1912), and growing in big clumps vs. bulbs not clumped in *A. elegans* (Rydberg 1912).

Two other *Anticlea* species occur in the Southwest and might be close relatives, *Anticlea mogollonensis* W.J. Hess & Sivinski and *Anticlea virescens* (Kunth.) J.F. Macbr. *Anticlea mogollonensis* is a very narrow, montane endemic in western New Mexico (Hess & Sivinski 1995; Schwartz 2002). It exhibits the usually racemose inflorescence and large flowers of *A. elegans*, but shares campanulate, nodding flowers with *A. virescens* (Hess & Sivinski 1995). In addition to flower shape and position, *Anticlea mogollonensis* differs from *A. vaginata* by having larger tepals, green-purple tepals, and large bracts (Schwartz 2002).

Anticlea virescens is a highly variable, widespread species that occurs from the southern mountains of Arizona and New Mexico into the Sierra Madres of Mexico (Hess & Sivinski 1995; Schwartz 2002). In Rydberg's original description of *Anticlea vaginata* (1912), he mentions that *A. vaginata* is similar to a large flowered form of *A. virescens* in that they share branched inflorescences and few veins in the perianth segments. *Anticlea vaginata*, however, differs from *A. virescens* in many other ways. *Anticlea vaginata*, as stated above, has erect to ascending pedicels at anthesis and rotate to rotate-campanulate flowers. *Anticlea virescens* has nodding and campanulate flowers (Hess & Sivinski 1995; Schwartz 2002). *Anticlea vaginata* has white, wide tepals versus greenish, narrow tepals that are sometimes tinged with purple (Schwartz 2002).

Several authors suggest that *Anticlea volcanica* (Bentham) J. G. Baker is the closest relative of *A. vaginata* (Welsh & Toft 1981; Spence 2008). This is unlikely since *A. volcanica* is morphologically similar to the *Anticlea virescens* complex, rather than the *Anticlea elegans* complex (Hess & Sivinski 1995), and is endemic to the mountains of southern Mexico and Guatemala.

An important characteristic of *Anticlea vaginata* that is not emphasized in most descriptions is that it flowers significantly later than *Anticlea elegans*. Flowering times of *A. vaginata* vary across the landscape, ranging from mid- to late August in northern populations to early October in Glen Canyon National Recreation Area. *Anticlea elegans* typically flowers from late June to early August (Welsh et al. 1993; Schwartz 2002). In a given geographic region, *A. elegans* populations will be finished flowering, or nearly so, when *A. vaginata* begins

flowering. For example, by the time *A. vaginata* is flowering in the hanging gardens of Dinosaur National Monument, the populations of *A. elegans* in the nearby Uintah Mountains are fruiting. This difference in phenology is a substantial barrier for gene flow and could result in different evolutionary outcomes for these two groups.

Anticlea vaginata occurs in desert sandstone canyons below 6500ft in elevation, usually lower. Its range is primarily in southeastern Utah and northeastern Arizona, with other known populations in Dinosaur National Park and Zion National Park. It is found in seven National Park units, the Navajo Nation, and BLM land and is embedded within the distribution of *A. elegans*. *Anticlea elegans* Pursh is a widespread, variable species. It currently consists of two subspecies; subsp. *elegans* , found from Alaska to the southern mountains of Arizona with a few scattered populations in northern Mexico (Hess & Sivinski 1995; Schwartz 2002), and subsp. *glaucus*, found from Quebec to North Carolina, with a gap in the distribution from Pennsylvania to Virginia (Zomlefer 1997). The two subspecies apparently intergrade in the eastern Dakotas and western Minnesota (Zomlefer 1997). *Anticlea elegans* subsp. *elegans* occurs in montane springs, wet montane meadows, exposed rocky slopes, and dense mixed conifer forests, all typically on limestone and well above 7,000ft (Welsh et al. 1993; Schwartz 2002). In regions where *A. vaginata* and *A. elegans* occur relatively close to each other, they are separated by thousands of feet in elevation, completely different habitats, and phenology.

The purpose of this study is to determine: 1) Is *Anticlea vaginata* a distinct taxon? 2) Are there morphological differences between *Anticlea vaginata* and *Anticlea elegans* that can be used to separate the taxa? 3) Does there appear to be gene flow among populations of *A. vaginata* or between *A. elegans* subsp. *elegans* and *A. vaginata*, or is there continuing diversification? 4) What is the most probable evolutionary history for *A. vaginata*?

MATERIALS AND METHODS

***Morphometric Plant Material* -** Measurements were taken from 208 specimens from field collections and the following herbaria (Appendix 2): 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 (Index Herbariorum). Field collections were made between May 2008 and October 2009 and were based on consultation with land managers, published and unpublished literature, and recommendations from regional botanists.

A total of 79 specimens of *Anticlea vaginata* were examined, including new collections from ten previously unvouchered populations. These specimens span the entire known geographic and morphological range for *A. vaginata*. A selection of *A. elegans* var. *elegans* specimens were chosen to represent the geographic and morphological range of that species, resulting in a total of 107 herbarium and field collections. *Anticlea virescens* was included as an outgroup. A total of 20 collections of *A. virescens* were examined. *Anticlea mogollonensis*

was not included in the morphological analysis, since only three specimens were available for study.

Morphometric Analyses - Characters were chosen based on those used to delineate *Anticlea vaginata* and *Anticlea elegans* in previous treatments (Rydberg 1912; Preece 1956; Welsh 1993; Hess & Sivinski 1995; Schwartz 2002). Sixteen vegetative and floral characters were recorded, 13 quantitative and three categorical. The quantitative characters consisted of proximal leaf length, proximal leaf width, capsule length, number of flowers, pedicel length, bract length, bulb length, bulb width, plant height, inflorescence height, flower diameter, tepal length, and tepal width. Categorical data consisted of presence or absence of persistent leaves, presence or absence of sheaths at the base of the stem, and inflorescence structure. Proximal leaf length was measured on the lowest complete leaf from where it diverges from the stem. Leaf width was measured on the lowest, flattened leaf. Capsule length was measured from where the persistent tepals connect to the capsule to the furthest point on the tip. The number of flowers included buds, flowers, pedicels, and capsules, and was approximate since not all floral parts had remained intact on all specimens. Pedicel length was the average of the lowest two to three pedicels of flowers on the main inflorescence axis. Bract length was the average of the lowest two or three subtending bracts and measured from where the bract diverged from the stem. Bulb length was measured from the bottom of the bulb, not including roots, to where the bulb became the same width as the stem. Bulb width was measured at the widest point of the bulb. Height of the plant was measured from the top of

the bulb to the bottom of the first flower pedicel or first branch. This was only done for individuals that had reached anthesis. Inflorescence height was measured from the bottom of the first flower pedicel or first branch to the bottom of the pedicel of the top most flower. This was only done for inflorescences that had open flowers or capsules, rather than buds, at the tip of the inflorescence. Inflorescence structure ranged from zero to six and indicated the number of branches of the inflorescence. Zero indicated a racemose inflorescence.

Multivariate analysis of morphological data was conducted in PC-ORD 5.10 (McCune & Mefford 2006). Principal Components Analysis (PCA) was used to assess structure in the morphological data. The twelve characters with less than five missing values were used for this analysis: proximal leaf length, proximal leaf width, number of flowers, pedicel length, bract length, flower shape, flower diameter, tepal length, tepal width, apparent persistent leaves, apparent sheaths at base of stem, and inflorescence structure. Missing values were approximated with the average value for that character. The PCA was followed by a non-parametric MANOVA, PERMANOVA (M. Anderson 2001), using 9999 permuations and Bray-Curtis dissimilarities. This evaluated whether or not *Anticlea vaginata* and *Anticlea elegans* were significantly morphologically different. Pairwise a-posteriori comparisons in PERMANOVA were made with 9999 permutations to determine which groups were significantly different.

One-way analysis of variance (ANOVA) was assessed for all traits. In addition, box plots were created for six traits reputed to differ between the two

species (Cronquist et al. 1977; Welsh et al. 1993; Schwartz 2002) in order to illustrate the range of variation: flower diameter, tepal length, capsule length, pedicel length, bract length, and leaf length.

AFLPs - Preliminary work showed 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 Melanthieae does not provide enough variation to determine relationships among species for this genus. Numerous studies have found the combination of morphometric evaluation and the AFLP technique (Vos et al. 1995) to be a successful method for determining species and subspecies in flowering plants (e.g. Saarela et al. 2003; Lihova et al. 2004; Ellis et al. 2009). AFLPs have also been useful for the assessment of population genetics and gene flow (e.g. Schmidt & Jensen 2000; Tremetsberger et al. 2003; Huft & Richardson 2006; Coppi et al. 2008). Though there are still some doubts about homology and asymetrical gain and loss of fragments for AFLPs, studies have shown that data generated using AFLP's are consistent with sequence data and are useful for determining fine-scale relationships (Koopman 2005; Ellis et al. 2009; Worley et al. 2009).

AFLPs Plant Material - AFLP profiles were generated for *Anticlea vaginata*, *Anticlea elegans* subsp. *elegans*, *Anticlea virescens*, and *Anticlea mogollonensis*. Fifteen populations of *A. vaginata*, nine populations of *A. elegans*, two populations of *A. virescens,* and one population of *A. mogollonensis* were included (Table 1). Fifteen plants were analyzed from each population, except in

populations with fewer than 15 plants. In such populations, a leaf sample from each individual was taken. The location each leaf was taken from within each population was noted. In order to avoid potential clones, only leaf samples that came from different clumps of plants were used for analysis. A total of 398 plants were analyzed. The closest known populations of *A. elegans* to populations of *A. vaginata* were chosen for sampling. Sampled populations of these two species spanned seven degrees of latitude from Dinosaur National Monument and the Uintah Mountains in the north, to the Mogollon Rim south of Flagstaff, AZ (Fig. 1). Leaf tissue was collected, put in silica gel in the field, and stored in silica gel until processing.

Voucher specimens were collected for each population sampled, except when an acceptable voucher specimen existed in a herbarium (Appendix 1). Voucher specimens collected during this study are housed in the herbarium of the National Park Service unit or in Deaver Herbarium (ASC) at Northern Arizona University.

AFLPs Procedure and Analysis - Genomic DNA was extracted using the Qiagen DNeasy 96 Plant Kit and the associated protocol with minor adjustments (Qiagen, Valencia, California, USA). DNA quality and quantity was measured using gel electrophoresis on a 2% agarose gel, as well as with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific).

The AFLP protocol used was that of Hersch-Green & Cronn (2009), with few modifications. For each individual, 15ng of genomic DNA was digested by *Eco*RI and *Mse*I, and ligation of corresponding adapters to the fragments occurred

simultaneously. Primers complementary to the adaptor sequences plus one selective nucleotide (*Eco*RI+A and *Mse*I+C) were used for preselective amplification. For the preamplification process, 2.5uL of a 1:5 dilution of the restriction/ligation product were added to the preamplification master mix for a total volume of 25uL. The master mix contained 1x Mg-free PCR buffer, 0.1 mg/mL BSA, 1.5mM $MgCl_2$, 0.2 mM each of dNTP's, 0.8 uM each of *Eco*RI+A and *Mse*I+C, and 1.25 U/uL of *Taq* DNA polymerase.

For selective amplification, eight fluorescently labeled primer pairs containing the complement to the adapter 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: *Eco*RI-ACT-(FAM), *Mse*I-CAG; *Eco*RI-ACT-(FAM), *Mse*I-CAA; *Eco*RI-ACC-(NED), *Mse*I-CAG; *Eco*RI-ACC-(NED), *Mse*I-CAA; *Eco*RI-AAC-(NED), *Mse*I-CAG; *Eco*RI-AAC-(NED), *Mse*I-CAA. The selective amplification reaction consisted of 1.5uL of undiluted preamplification product and 8.5uL of selective amplification master mix for a reaction volume of 10uL. The master mix contained 1x $MgCl_2$ (15mM) PCR Buffer, 0.2 mM each dNTP's, 0.375uM *Eco*RI+3 primer, 1.0 uM *Mse*I+3 primer, and 0.5 U/rxn *Taq* DNA polymerase.

The AFLP products were analyzed using capillary electrophoresis on an ABI 3730XL (Applied Biosystems, Foster City, California). A 1:10 dilution of the AFLP product was mixed with formamide and GeneScan 600 LIZ size standard as per the instructions included with the size standard.

GeneMapper Software v.4.0 (Applied Biosystems, Foster City, California) was used to analyze the AFLP fragments. Initially, profiles were analyzed with automated scoring using a base pair range of 100-600bp and a peak height minimum of 1000 for all primer combinations. To minimize the scoring of noise, only larger peaks were used for bin generation. Bins were then hand-edited for consistency and usefulness. Peaks that were automatically scored for no template controls were removed from the analysis. Following bin editing, profiles were rescored using the edited bin set, a base pair range of 100-600bp, and a peak height minimum determined separately for each primer combination. *Eco*RI-ACC-(NED), *Mse*I-CAA and *Eco*RI-ACT-(FAM), *Mse*I-CAA resulted in higher amounts of noise, so had a minimum peak height of 750. The rest of the primer combinations were set to 500. Loci present less than twice were removed from the data set. Loci with highly variable peak heights across samples were also removed from the data set, since it was shown in Pineiro et al. (2009) that these bands are unreliable and increase error.

Error rate was calculated using replicate samples and the following equation:

$$\frac{N(0,1) + N(1,0)}{N(0,0) + N(1,0) + N(0,1) + N(1,1)},$$

where N(0,0) and N(1,1) are the number of matching calls in the replicates, either both "peak absent" or both "peak present," and N(0,1) and N(1,0) are the number of mismatched calls in the replicates (Holland 2008).

The AFLP data were examined with four approaches. Non-metric Multidimensional Scaling (NMS) was used to visualize the relationships among samples using the program PCORD v. 5.10 (McCune & Mefford 2006). Jaccard's distance measure was used to calculate genetic similarity between individuals, since it reduces the risk of homoplasy by comparing only present bands (Bonin et al. 2007). The Slow and Thorough method on Autopilot mode was used to reach the final ordination.

To further compare the relationships between individuals and populations, phenetic cluster analyses were run on individuals and populations. A Neighbor-Joining (NJ) tree of all individuals rooted with *Anticlea virescens* and *Anticlea mogollonensis* was created in MEGA4 (Dudley et al. 2007) using a Nei's genetic distance matrix created in GenAlEx 6.4 (Peakall & Smouse 2006). This NJ tree was generated to evaluate whether individuals clustered as populations. An unrooted NJ tree of populations was also created and viewed in MEGA4 (Dudley et al. 2007) using a Nei's genetic distance matrix created in GenAlEx 6.4 (Peakall & Smouse 2006). This cluster analysis was used to determine if populations clustered as species, geographic regions, or neither.

To examine the degree of genetic structure within and between populations, species, and geographic regions, Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) was performed in GenAlEx 6.4 (Peakall & Smouse 2006). This analysis was run on a Nei's genetic distance matrix and included 9999 permutations to calculate a p-value. Two AMOVAs were run to assess whether presumed species boundaries or geographic location better explained

genetic variation. The first attributed genetic variation to species, populations, and within populations. The second partitioned genetic variance among geographic regions, populations, and within populations. In addition, G_{ST} values were calculated for *Anticlea vaginata* and *Anticlea elegans* and Nei's Gene Diversity estimates were calculated for each species and population in PopGene 1.32 (Yeh et al. 1997).

Finally, Structure 2.2 using $\triangle$K as described by Evanno (2005) was used to analyze the AFLP profiles of *A. elegans* and *A. vaginata* to determine the true number of interacting populations (Falush et al. 2007; Pritchard et al. 2000; 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 & Rosenberg 2007) and graphics were generated using Distruct (Rosenberg 2004). This analysis was used to assess whether or not the two species form distinct genetic groupings or if, instead, there is admixture between the two.

Pollinators – In order to determine if pollinators of *Anticlea vaginata* would be able to travel among hanging gardens, facilitating gene flow, potential pollinators were collected. Collections were only made if insect activity was high while I was at a population, resulting in collections from five locations: the hanging garden near Delicate Arch in Arches National Park, the hanging garden in the Labyrinths area of Dinosaur National Monument, the hanging gardens in Ribbon Canyon and Cottonwood Gulch of Glen Canyon National Recreation Area,

and the hanging garden at Inscription House Spring on the Navajo Reservation. Flowering inflorescences were watched for one hour by two people. Any insect that landed on a flower was collected, unless many of the same type of insect were landing on flowers. If the same type of insect was seen many times, only the first few were collected. Collections were taken to the Colorado Plateau Museum of Arthropod Diversity, where they were pinned, labeled, and identified to family. Family determinations were conducted by Neil Cobb, Ph. D. Pollen from each specimen was transferred onto a microscope slide and compared to reference slides of *Anticlea* pollen. All Tachinid flies were sent to James E. O'Hara, Ph.D. of the Canadian National Collection of Insects, Arthropods, and Nematodes for species determination. All Syrphid flies were sent to Jeff Skevington, Ph.D. of the Canadian National Collection of Insects, Arthropods, and Nematodes for species determination. The only Sarcophagid fly was sent to Gregory Dahlem, Ph.D. of Northern Kentucky University for determination. All other specimens were either identified by Neil Cobb, Ph.D. or were of such poor quality that they were not sent out for further identification.

Scanning Electron Microscope images – Tepals from three individuals, one from *Anticlea elegans* and two from *Anticlea vaginata*, were preserved in a Formalin-Aceto-Alcohol solution, then transferred to 70% ethanol solution. Scanning electron microscope images were taken of the nectar glands on each tepal to look for micro-morphological differences.

RESULTS

Morphometrics - The first two components from 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 (Table 2). The first component largely represents gradients in flower diameter (0.5091), tepal length (0.4949), and tepal width (0.4637) (Table 2). The second component largely represents gradients in inflorescence structure (0.5214), flower number (0.5084), and leaf length (0.5040) (Table 2). Thus, the first component is composed mostly of floral characters, while the second represents vegetative characters.

The morphological ordination (Fig. 2) shows *Anticlea virescens* separating out, but *Anticlea elegans* and *Anticlea vaginata* mostly overlapping. *Anticlea vaginata* is shifted slightly toward *A. virescens* on Axis 1 and does not overlap the entire spread of *A. elegans* points. There was a significant difference among *A. elegans*, *A. vaginata*, and *A. virescens* (PERMANOVA; $p < 0.001$; Table 3), as well as between all pairs of species. In particular, *A. vaginata* and *A. elegans* were significantly different (PERMANOVA; $p<0.001$; Table 4).

The one-way ANOVA's of the six characters previously thought to differ between *Anticlea vaginata* and *Anticlea elegans* indicate significant differences in means for each of these characters except capsule length (Table 5; Fig. 3). However, they all show mostly overlapping ranges (Fig. 3). For these characters, except leaf length, the range of variation in *A. vaginata* falls within the range for

A. elegans. *Anticlea vaginata* exhibits a greater range of variation than *A. elegans* for leaf length.

Capsule length, height of plant, flower number, and bulb length and width did not significantly differ between the two species (Table 5). Inflorescence height, leaf width, and tepal width did differ significantly ($p<0.0068$), but showed the same degree of overlap as the other characters (Table 5).

AFLPs **-** From the 398 samples of *Anticlea*, 341 polymorphic, reliable fragments were analyzed. The replicate error rate was 5.73%. This is likely due the large number of fragments analyzed and the use of automated scoring techniques in addition to hand editing, rather than entirely scoring by hand (Holland et al. 2008). Compared to fully automated scoring, this error rate is quite low (Holland et al. 2008).

An outlier analysis indicated 16 individuals that varied more than two standard deviations from the mean. These included individuals from each of the species (not shown). Those outliers were removed for analysis, since they obscured all other associations. The final NMS 3-dimensional solution had a stress of 19.24 and final instability of 0.00069. The scatterplot of Axes 1 and 3 gives the best representation of the results (Fig. 4 & 5). An overlay of species shows individuals of *Anticlea elegans* and *Anticlea vaginata* grouping together and apart from the other two species (Fig. 4). *Anticlea elegans* shows less variation than *A. vaginata*, but most *A. elegans* overlap those of *A. vaginata*. It also shows a distinction that separates the *A. vaginata/A. elegans* grouping into

an upper group and a lower group. These two clusters do not line up with either species.

A geographic region overlay shows that individuals within regions tend to group together loosely, but largely overlap (Fig. 5). Some regions, particularly Central, Moab, and East, are more dispersed and are only weakly grouped. The Glen Canyon region forms the tightest group, and when displayed in 3D (not shown) is separate. Individuals from the Mogollon Rim region are also more distinct than the other regions and overlap very little with other individuals. The separation within the *Anticlea vaginata*/*Anticlea elegans* group, mentioned above, weakly correlates with regions. Individuals from West, Glen Canyon, Mogollon Rim, and North are found in only the lower cluster, and Natural Bridges is mostly in the upper cluster. Individuals from the remaining regions occur in both.

Cluster analysis of individuals shows that although some individuals cluster with other groups, populations largely group together (Fig. 6). Twenty-two of 27 populations form distinct, individual groups. Courthouse and Delicate are the least cohesive populations. They do generally cluster together, but contain samples from other populations. Individuals from Kachina and Sipapu show a similar pattern, but they formed one cluster representing only their shared region. Labyrinth is also fairly scattered, though it tends to group with either Uintah or Bull, which are in its region.

The NJ tree of populations (Fig. 7) shows that *Anticlea elegans* and *Anticlea vaginata* are often comingled. The Mogollon Rim region, interestingly, forms a

group with *Anticlea virescens* and *Anticlea mogollonensis*. Two other regions appear as groups, Glen Canyon and West. All other regions show varying levels of cohesion. Some hanging garden populations that are close geographic neighbors group together, but do not group with others in their region: e.g. Sipapu/Kachina, Courthouse/Delicate. On the other hand, Junction and Refuge occur in the same canyon only a few miles apart, yet are placed very far apart in the cluster analysis. The two main branches that divide the bulk of the populations show the same pattern as the minor separation within the *Anticlea elegans/Anticlea vaginata* group in the NMS (Fig. 5; Fig. 7). The Glen Canyon region, North region, and West region all occur in only one branch, while the rest of the regions are split between the two. The populations, when overlain on the NMS (not shown), end up in the same groupings as shown in the NJ tree.

Both AMOVA's indicated that most of the variation is due to within population differences (64% and 65%), though a fairly large component of variation (29% and 22%) is due to among population differences (Table 6). Both AMOVAs show that the smallest amount of variation is explained by species or regional differences, 8% and 12% respectively. Grouping populations by geographic region, however, explained more variation than grouping them by species.

Among population differentiation (G_{ST}) was 0.299 for *Anticlea vaginata* and 0.251 for *Anticlea elegans* (Table 7). Overall G_{ST} for combined *A. vaginata* and *A. elegans* was 0.296. Percentage of polymorphic loci was 87.1% for *A. vaginata*

and 81.5% for *A. elegans.* Nei's gene diversity (1973) estimates for each population can be seen in Table 7.

The Structure analysis indicated that the most appropriate number of genetic groupings represented by the *Anticlea vaginata* and *Anticlea elegans* samples was k=2 (Fig. 8). These two genetic groups, however, do not correspond with either of the two species (Fig. 9). Individuals from the same population, however, seem to have similar membership probabilities (Fig. 10), particularly Ribbon, Reflection, Delicate, Dixie, and West Fork.

Pollinators – Most pollinators found on *Anticlea vaginata* belong to the family Tachinidae, a group of flies. These carried the largest amounts of *Anticlea* pollen and often very little of other types of pollen. Species determinations for these specimens are in progress, though it is unlikely that a species determination will lead to knowledge about its ecology. Bee flies, *Chrysotoxum integrum*, were collected in Dinosaur National Monument and nowhere else. These were the primary visitors at the time of collection in that area. Nothing is known about the ecology of this species. Other pollinators include a fly from the genus *Sarcophaga*, a wasp from the genus *Polistes*, a fly from the family Dolichopodidae, and flies from the Acalyptratae group. Again, very little is known about the ecology of these groups, and it is difficult to know if they could or would fly among these hanging gardens.

Scanning Electron Microscope images – The nectar glands of *Anticlea vaginata* individuals show a larger, more distinct ridge around the entire edge of the gland (Figs. 12 & 13), when compared to that of *Anticlea elegans* (Fig.13).

This trait is not readily seen in all specimens of *A. vaginata* and can be difficult to view in the field. Morphological analyses were not conducted on this trait due to the small sample size and the small likelihood of this trait being useful for field identification.

DISCUSSION

Delimitation of Species - Despite occurring in widely separate, distinct habitats and having different phenology, all analyses indicate that *Anticlea vaginata* and *Anticlea elegans* do not exhibit clear-cut differences. All morphological characters exhibit significant variation within species and widely overlap between species. The PCA of morphology and the NMS of AFLP data separate out the other two species, *Anticlea virescens* and *Anticlea mogollonensis*, but do not separate *A. elegans* and *A. vaginata*. Cluster analyses indicate the same pattern; the two species are genetically entwined.

However, the morphometric and genetic analyses do indicate that there are some differences. *Anticlea elegans* and *A. vaginata* do not overlap completely on the PCA of morphological characters or the NMS scatterplot of AFLP data (Fig. 2 & 4). *Anticlea vaginata* shows greater genetic variation, but less morphological variation. The points representing *A. elegans* in the NMS scatterplot are shifted toward *Anticlea virescens* and *Anticlea mogollonensis*. The points representing *A. vaginata* on the PCA scatterplot are shifted on Axis 1 toward *A. virescens*. The non-parametric MANOVA showed that the two species are different in regards to multivariate morphological data (Table 4). The one-way ANOVAs also indicated

significant differences of the means of many characters between the two species (Table 5). There appear to be some morphological and genetic differences, although none of them can be used to clearly differentiate between *A. vaginata* and *A. elegans*.

Anticlea vaginata should be combined with *Anticlea elegans* to reflect their current morphological and genetic similarity, but should be a distinct subspecies. The two species inhabit very different and widely separate habitats. There are indications that the morphology and genetics of the hanging garden populations are shifting away from *A. elegans*. In addition, it is unlikely that most of the hanging gardens are currently admixing with montane populations, or will in the future, considering the elevational and horizontal distances between populations and the difference in phenology. This level of distinction is consistent with the general definition of subspecies as a coherent evolutionary subset of a species. (Hamilton & Reichard 1992).

Shifting *Anticlea vaginata* to the taxonomic status of subspecies is also fairly consistent with the current treatment of *Anticlea elegans*. Currently, two subspecies of *A. elegans* are recognized; *Anticlea elegans* subsp. *elegans* occurs in the west and *A. elegans* subsp. *glaucus* occurs in the east. The morphological differences between these subspecies show a great deal of overlap and are mostly useful for geographic, and thus morphological, extremes (Zomlefer 1997). There are also indications that there is interbreeding where the two ranges come in contact (Schwartz 2002). Considering morphological, genetic, and ecological factors, *A. vaginata* shows at least as much distinction as the other two

subspecies, if not more. This taxonomic placement is also consistent with other studies evaluating morphologically similar species (e.g. Martinez-Ortega et al 2004; Perny et al. 2004). Thus, *A. vaginata* should be at least a subspecies of *A. elegans* to reflect the potential for differing evolutionary pressures.

Morphological Characters - Morphological characters that have been used to distinguish the two species do not hold up under examination. None of them can be used to distinguish *Anticlea vaginata* from *Anticlea elegans*. However, many characters show significant differences in their means and the ranges of many do not entirely coincide (Table 5).

It seems, after intensive field work, that the character of "persistent leaf bases" for *Anticlea vaginata* is the result of preservation due to the desert climate and protective alcove rather than an innate, physical characteristic. Although individuals in the alcoves retained many leaves from past seasons, those found in sheets of *A. elegans*, suggesting that if the climate allowed it, *A. elegans* would build up dead leaves at the base, as well. Also, Zomlefer (1997) indicated that having "an outer coat of membranaceous leaf bases" is a key distinction of the tribe Melanthieae, suggesting that this character is commonly seen in many of the species.

"Many loose sheaths" can be seen on herbarium sheets of both species, though are more common on *Anticlea vaginata* collections. This seems to be more a character of very large individuals rather than a particular species. As is indicated by the leaf length, plant height, and inflorescence height measurements (Fig. 3; Table 5), *A. vaginata* has the potential to get much larger than *A. elegans*.

Thus, *A. vaginata* tends to have more individuals with "loose sheaths" than *A. elegans*. The large habit seems to be a result of environmental variables, too. Large *A. elegans* are often found in very wet areas, and small ones in dry, exposed areas. This is of course, a generalization, and exceptions to this can be found. It also seems that plants of both species become larger with age, since a whole range of sizes occur in the same area, including very tiny individuals with only 2 narrow leaves to massive individuals with many, thick leaves.

Flower color also does not appear to be a good character. First, flower color is not preserved well on herbarium sheets, so determination of original color typically requires a note from the collector. In the field, white to cream to greenish tepals are exhibited by both species. The "green tepals" are a result of a green stripe of varying width that is sometimes present on the abaxial side of the tepals, rather than the presence of an entirely green tepal.

As for Rydberg's original description of *Anticlea vaginata* differing by "its habit of growing in big clumps," both species have a clumped habit in the right conditions. MacBride (1918) and Walsh (1940) also noted that this is not an uncommon condition. This bunched growth form is, however, much more prevalent and obvious in *A. vaginata* than in *A. elegans*. It became clear in the field and then when revisiting herbarium sheets that this clumped habit is due to these species being clonal. It was not uncommon to observe multiple plants connected by the bulbs. *Anticlea vaginata* seems to be far more prone to cloning than *A. elegans*. Large clumps of plants hanging from the walls of hanging gardens are the norm, whereas clumps of *A. elegans* rarely get larger than a few

stems and is more typically seen growing singly. If collections were made to show this habit, a propensity to be clonal vs. non-clonal would probably be the best characteristic to separate these taxa.

Other studies have shown that the propensity for cloning is affected by environmental conditions, particularly water availability, age of populations, size of population, and limited abilities to sexually reproduce (Xie et al 2001; Silvertown 2008). Silvertown (2008) determined that older populations, rare species, populations on the edge of the range, aquatic plants, and alien plants are the most likely to utilize clonal reproduction. Xie et al. (2001) noted that continual water availability and less disturbance increased clone size and decreased the number of genotypes. It is not surprising, then, that *A. vaginata* exhibits this characteristic, since these populations are likely old (see discussion below), it's a rare species, hanging gardens may represent the physiological limits of an otherwise montane species (*A. elegans*), and it has a steady water supply. The genetic diversity of *Anticlea vaginata* remains high, despite clonality, which could be due to any number of mechanisms that have the potential to maintain or increase diversity. Examples of these are disturbance (Xie et al. 2001) from sloughing of rock in hanging gardens that keep clones small, self-incompatability which is positively correlated with cloning (Vallejo-Marin & O'Brien 2006), and low levels of seedling recruitment (Soane & Watkinson 1979).

Ongoing Diversification - Although the data indicates that these two species are not currently distinct, there is some evidence that at least some populations are diverging from the others.

There are four groups of populations that appear more distinct than the rest. The Glen Canyon NRA populations, Ribbon and Reflection, are not only unusual for have the latest flowering time by far (early October), but also separate out in the genetic analyses (Fig. 5; Fig. 7; Fig. 10). The Natural Bridges populations, Kachina and Sipapu, are genetically mixed (Fig. 6), form a relatively tight cluster in the NMS (Fig. 5) and show the highest levels of genetic diversity (Table 7). Delicate and Courthouse, the Arches National Park populations, stand out in the Structure Analysis as being the only populations that have affiliations primarily with the gray group on the graph (Fig. 10). These two are also genetically mixed and are apparently very similar (Fig. 6; Fig. 7). The fourth group, the Mogollon Rim region, is discussed in greater detail below. These groups warrant particular consideration in conservation efforts, due to their unique genetic identities.

The level of population differentiation (G_{ST}) is higher for hanging garden populations than it is for montane populations, 0.299 vs. 0.251 (Table 7). Compared to the mean G_{ST} values of other long-lived perennials, 0.19 (Nybom 2004), both of these values are quite high. Comparing *A. vaginata's* G_{ST} value to that of other endemic (0.18) and narrow (0.21) species, that value is also very high (Nybom 2004). If we compare *A. elegan*'s G_{ST} value to that of other widespread species (0.31), it has a fairly low level of structuring. This suggests that a structuring mechanism such as low gene flow or genetic drift is occurring in the hanging gardens.

The AMOVAs indicated that geographic regions differed more than species differed. Both species and regions differed less than populations. This indicates that the populations are fairly differentiated, but at least some are more similar to their geographic neighbors than to their conspecifics. This suggests two things; retaining *Anticlea vaginata* as a species is unwarranted as the populations are in the process of drifting apart.

The Neighbor-Joining trees (Fig. 6; Fig. 7), the NMS scatterplots (Fig. 4; Fig. 5), and the Structure Analysis show a similar pattern of differentiation (Fig. 9; Fig. 10). A minor separation cuts through the scatterplots, but does not completely correspond with either species or regions. The NJ tree of populations shows this same pattern of separation (Fig. 7). The Structure Analysis determined that the most likely number of genetic groups is two, but they do not correlate with regions or species (Fig. 9; Fig. 10). Individuals seem to have similar assignment probabilities as the other individuals in their population, indicating that they are more similar to each other than individuals from other populations. All of these analyses show substantial amounts of structuring at the population level, but not as much at the species or regional level. The populations are not grouping by readily apparent categories, but instead seem to be randomly differentiated.

The Mogollon Rim region, composed of two populations of *Anticlea elegans*, show an interesting genetic pattern. These populations always cluster together and away from other populations (Fig. 5; Fig. 6; Fig. 7;). This suggests some genetic structuring along that geologic feature. They are also always closer the *Anticlea virescens* and *Anticlea mogollonensis* (Fig. 5; Fig. 7). The *A. elegans*

populations along the Mogollon Rim are geographically connected to *A. virescens* and *A. mogollonensis* of the Mogollon Mountains by the White Mountains. *Anticlea virescens* and *A. elegans* co-occur in the White Mountains, and there is some evidence of intergradation of those species there (Hess & Sivinski 1995, personal observation). The relationships among the Mogollon Rim, White Mountains, and Mogollon Mountain populations of *Anticlea* species warrants further study considering the close relationship mentioned above and the apparent lack of genetic differentiation between *A. virescens* and *A. mogollonensis* (Fig. 5; Fig. 7).

Evolutionary History - The current morphological similarity, population structuring, and high levels of within population diversity are consistent with other studies investigating historical vicariance (e.g. Tribsch et al. 2002; Saarela et al. 2003; Schonswetter & Tribsch 2005; Michalczyk et al. 2010). Some Eastern North America and eastern Asia disjunct species show little morphological differentiation, yet exhibit clear genetic structure (e.g. Saarela et al. 2003). *Anticlea vaginata* and *Anticlea elegans* show this same pattern, but with less genetic structure. Clearly, disjuncts across continents would exhibit higher levels of structuring, since their divergence times have been much longer. Due to the apparent differences in phenology and distance, it is not unreasonable to think that given more time, the genetic structuring will increase.

Studies assessing the number and locations of glacial refugia identify refugia based on high levels of genetic diversity and use genetic structuring to elucidate the number of refugia (e.g. Tribsch et al. 2002, Martinez-Ortega et al.

2004). Populations stemming from paleorefugia should exhibit higher levels of genetic diversity than populations formed by long-distance dispersal, since the former should harbor the genetic variation of the once wide-spread species, and the latter is expected to only contain the genetic variation of the founding individuals (Martinez-Ortega et al. 2004, Schonswetter & Tribsch 2005). Since *Anticlea vaginata* shows similar to greater levels of genetic diversity as its widespread congener and not reduced levels of genetic variation, this supports the idea that hanging gardens contain relictual populations of once –widespread species as suggested by Spence (2008), rather than the result of multiple independent dispersal events.

Conservation Implications - As a subspecies, *Anticlea vaginata* still warrants management separate from *A. elegans* subsp. *elegans* (Frankham et al. 2002). Following the methodology for defining management units of Crandall et al. (2000), *A. vaginata* falls into category 5: recent ecological distinction. It exhibits probable historic genetic and ecological exchangeability with *A. elegans*, but is currently showing ecological isolation and population-level genetic isolation. The management recommendation for this level is to treat *A. vaginata* and *A. elegans* as distinct evolutionarily significant units (ESU), meaning do not encourage artificial crosses between them. Within *A. vaginata*, the methodology of Crandall et al. (2000) would mean that crosses between hanging garden populations would be acceptable, despite the genetic separation. Considering the importance of genetic adaption to microhabitats (Huenneke 1991), the most conservative management plan would mean avoiding transplants between

regions of hanging gardens and not supplementing gene pools with genetic material from dissimilar groups. The results of this study suggest that populations in the Natural Bridges National Monument area can be managed as one unit, as can the populations in the area of Arches National Park. The Glen Canyon National Recreation Area populations in the area around the Reflection and Ribbon populations can also be treated as one grouping. The rest of the populations should be assessed individually concerning the degree of genetic differentiation and the similarity of ecology. Since the populations of *A. vaginata* appear to be structured and seem to be differentiating in somewhat unpredictable ways, the best method for conserving genetic diversity is to protect as many distinct groupings as possible.

Currently, the primary threats to these hanging garden populations are the potential impact of climate change on the water supply to the hanging gardens (Spence 2008) and in some places, grazing and trampling by livestock (personal observation). *Anticlea vaginata* is not at present threatened by low levels of genetic diversity, harvesting, or habitat destruction. It is unknown if it is declining in numbers or if it is sexually reproducing. For naturally rare, endemic species that are not immediately threatened by human impacts, the best management policy is to assess reproductive success and monitor population sizes (Holsinger & Gottlieb 1991). Human impacts on these populations, including the use of spring water for livestock, should be minimized in order to avoid overuse of the water supply.

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Table 1. Four *Anticlea* taxa sampled from 27 populations for AFLP analysis, with voucher, population locality, number of samples included, population code (Population), and geographic region code (Region). Herbarium codes follow Index Herbariorum, except GLCA – herbarium at Glen Canyon National Recreation Area, SEUG – herbarium for the Southeast Utah Group, ZION – herbarium at Zion National Park.

Taxon	Voucher	Herbarium	Locality	# Samples	Population	Region
A. elegans ssp. *elegans*	L. Hannon Williams 6	ASC	Abajo Mts, UT	13	Abajo	NaturalBridges
A. elegans ssp. *elegans*	M.Sommer 2	ASC	Mogollon Rim - Barbershop Canyon, AZ	15	Blue Ridge	Mogollon
A. elegans ssp. *elegans*	E. Palmquist 40	NAVA	Chuska Mts, Navajo Nation, AZ	15	Chuskas	East
A. elegans ssp. *elegans*	E. Palmquist 33	ASC	Markaguant Plateau, UT	15	Dixie	West
A. elegans ssp. *elegans*	G.Rink 7676	ASC	Grand Canyon National Park, AZ	15	Hades	GrandCanyon
A. elegans ssp. *elegans*	L. Hannon Williams 10	ASC	La Sal Mts, UT	15	LaSal	Moab
A. elegans ssp. *elegans*	E. Palmquist 45	ASC	Uintah Mts, UT	15	Uintah	North
A. elegans ssp. *elegans*	G.Rink 4877	ASC	Grand Canyon National Park, AZ	12	Modred	GrandCanyon
A. elegans ssp. *elegans*	M. Sommer 1	ASC	Oak Creek, AZ	15	WestFork	Mogollon
A. mogollonensis	C.Huff 693	UNM	Mogollon Mts, NM	15	ANMO	
A. vaginata	E. Palmquist 46	SEUG	Arches National Park, UT	15	Courthouse	Moab
A. vaginata	Welsh, Harrison, Moore 2335	SEUG	Arches National Park, UT	15	Delicate	Moab
A. vaginata	G.Rink 1366	ASC	Canyon de Chelly National Monument, AZ	15	Junction	East
A. vaginata	G.Rink 1371	ASC	Canyon de Chelly National Monument, AZ	15	Refuge	East
A. vaginata	N. Boschen S1-84	SEUG	Canyonlands National Park, UT	14	Canyon	Moab
A. vaginata	E. Palmquist 44	ASC	Dinosaur National Monument, UT	15	Bull	North
A. vaginata	E. Palmquist 43	ASC	Dinosaur National Monument, UT	15	Labyrinth	North
A. vaginata	E. Palmquist 46	GLCA	Glen Canyon National Recreation Area, UT	15	Reflection	GlenCanyon
A. vaginata	E. Palmquist 36	GLCA	Glen Canyon National Recreation Area, UT	15	Ribbon	GlenCanyon
A. vaginata	D.Roth 830	NAVA	INHO, Navajo Nation, AZ	15	Inscription	Central
A. vaginata	E. Palmquist 39	ASC	Johns Canyon, UT	15	Johns	Central
A. vaginata	E. Palmquist 41	ZION	Zion National Park, UT	14	Kolob	West
A. vaginata	E. Palmquist 38	SEUG	Natural Bridges National Monument, UT	15	Kachina	NaturalBridges
A. vaginata	E. Palmquist 37	SEUG	Natural Bridges National Monument, UT	15	Sipapu	NaturalBridges
A. vaginata	D.Roth 822	NAVA	Near Navajo Mountain, Navajo Nation, AZ	15	Surprise	Central
A. virescens	E. Palmquist 42	ASC	Mogollon Mts, NM	15	ANVI	
A. virescens	L. Hannon Williams 16	ASC	White Mts, AZ	15	ANVI	

Table 2. First two Eigenvectors, scaled to unit length, for all morphological characters of PCA. Percent variation is for each axis. P-value from randomization test.

	Axis	
	1	2
bract length	-0.1613	0.1082
flower diameter	-0.5091	-0.0593
flower number	0.1388	-0.5084
flower shape	0.3836	0.0227
inflor structure	0.1611	-0.5214
leaf length	-0.0291	-0.504
leaf width	-0.172	-0.4142
pedicel length	-0.1146	-0.1182
persistent leaves	-0.1308	0.0652
present sheaths	-0.042	-0.0769
tepal length	-0.4949	-0.0219
tepal width	-0.4637	-0.0606
Eigenvalue	3.165	2.586
% variation	26.376	21.554
p	0.0001	0.0001

Table 3. PERMANOVA results for 12 morphological characters for *A. elegans*, *A. vaginata*, and *A. virescens.*

Source	df	SS	F	P-value
species	2	8153.6070	15.2856	0.0001
residual	152	40539.7321		
total	154	48693.3391		

Table 4. Pairwaise a-posteriori comparisons of species using 12 morphological characters.

Groups	t	P-value
A. elegans, A. vaginata	5.2310	0.0001
A. elegans, A. virescens	2.2604	0.0126
A. vaginata, A. virescens	2.2223	0.0138

Table 5. Descriptive statistics of 16 morphological characteristics. Flower structure, persistent leaves, and present 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*.

	A.elegans					A.vaginata					
Character	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD	p-value
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
flwr_struct	84					56					
height	44	180	535	364.2	95.1	31	135	580	332.6	107.7	0.184
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*
persistent lvs	84					56					
present sheaths	84					56					
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*

Table 6. Analysis of molecular variance summary tables, showing the partition of genetic variance among either species or geographic region, among populations, and within populations.

Summary AMOVA Table - Species

Source	df	SS	MS	Est. Var.	%	P-value
Among Species	3	887.732	295.911	2.107	8%	0.000
Among Pops	23	3098.808	134.731	7.945	29%	0.000
Within Pops	371	6593.207	17.771	17.771	64%	0.000
Total	397	10579.746		27.823	100%	

Summary AMOVA Table - Regions

Source	df	SS	MS	Est. Var.	%	P-value
Among Regions	10	2282.185	228.218	3.394	12%	0.000
Among Pops	16	1704.355	106.522	6.026	22%	0.000
Within Pops	371	6593.207	17.771	17.771	65%	0.000
Total	397	10579.746		27.191	100%	

Table 7. Intra-population genetic variability for *A. elegans* and *A. vaginata*. GD=Nei's Gene Diversity, Std. Dev. = Standard deviation of gene diversity, % poly=percent polymorphic loci.

Group	GD	Std. Dev.	% poly	G_{ST}
All *A. vaginata* and *A. elegans*	0.119	0.1532	97.4	0.296
All *A. vaginata*	0.114	0.151	87.1	0.299
Bull	0.067	0.139	24.6	
Canyonlands	0.073	0.142	29.3	
Courthouse	0.067	0.135	27.0	
Delicate	0.058	0.120	28.7	
Inscription	0.074	0.153	25.8	
Johns	0.075	0.155	24.3	
Junction	0.072	0.131	33.4	
Kachina	0.114	0.155	48.1	
Kolob	0.064	0.139	22.9	
Labyrinths	0.083	0.153	29.6	
Reflection	0.067	0.142	23.2	
Refuge	0.087	0.159	31.1	
Ribbon	0.087	0.159	30.2	
Sipapu	0.135	0.183	50.2	
Surprise	0.078	0.148	31.1	
All *A. elegans*	0.121	0.160	81.5	0.251
Abajo	0.076	0.143	32.0	
BlueRidge	0.093	0.164	31.7	
Chuska	0.086	0.153	35.8	
Dixie	0.085	0.153	31.7	
Hades	0.100	0.167	34.0	
La Sal	0.126	0.17	52.8	
Modred	0.072	0.147	26.7	
Uintah	0.083	0.142	37.5	
WestFork	0.091	0.165	29.9	

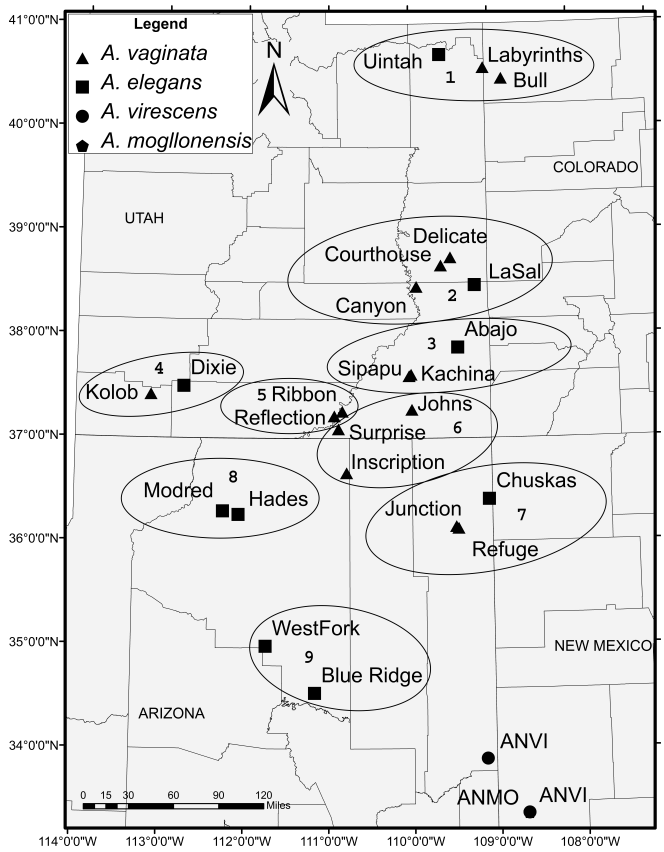

Fig. 1. Geographic locations of 27 *Anticlea* populations sampled. Geographic regions are circled and are numbered as follows: 1- North, 2- Moab, 3- NaturalBridges, 4 – West, 5 – GlenCanyon, 6- Central, 7- East, 8- GrandCanyon, 9- Mogollon

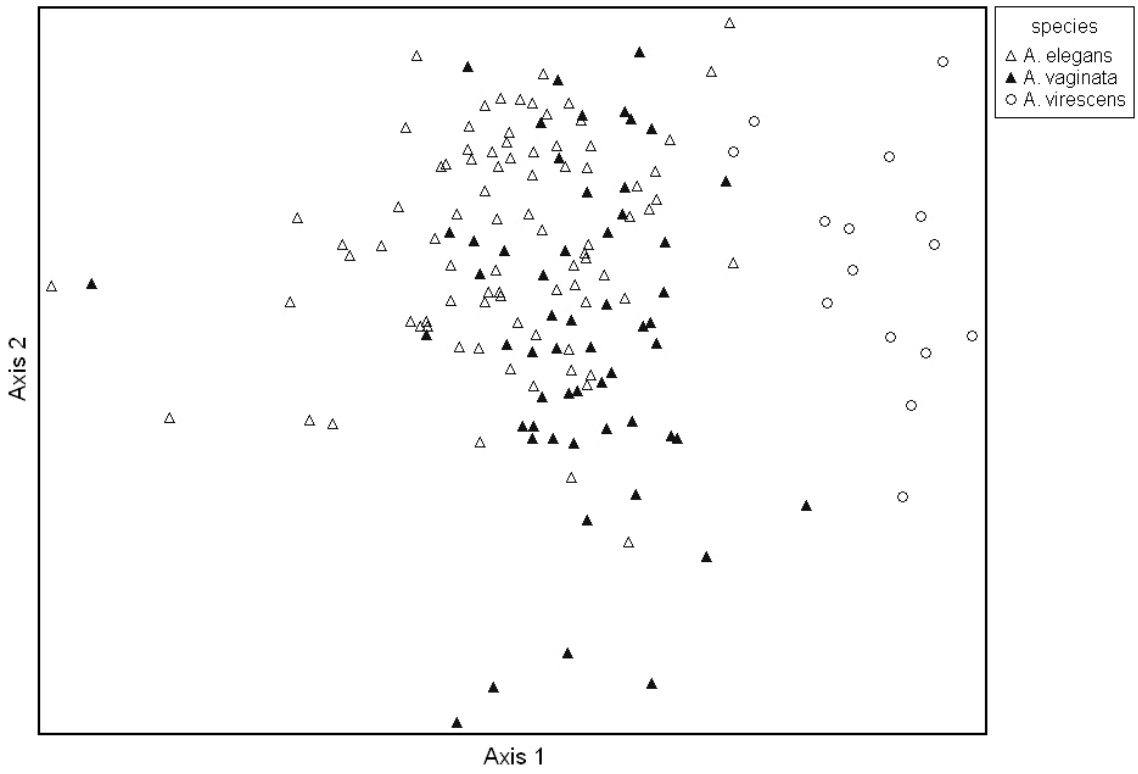

Fig. 2. Scatterplot of components 1 & 2 of the PCA of 12 floral and vegetative characters on 84 specimens of *A. elegans*, 56 specimens of *A. vaginata*, and 15 specimens of *A. virescens*. The first two axes explained 34.6% and 20% of the variation, respectively.

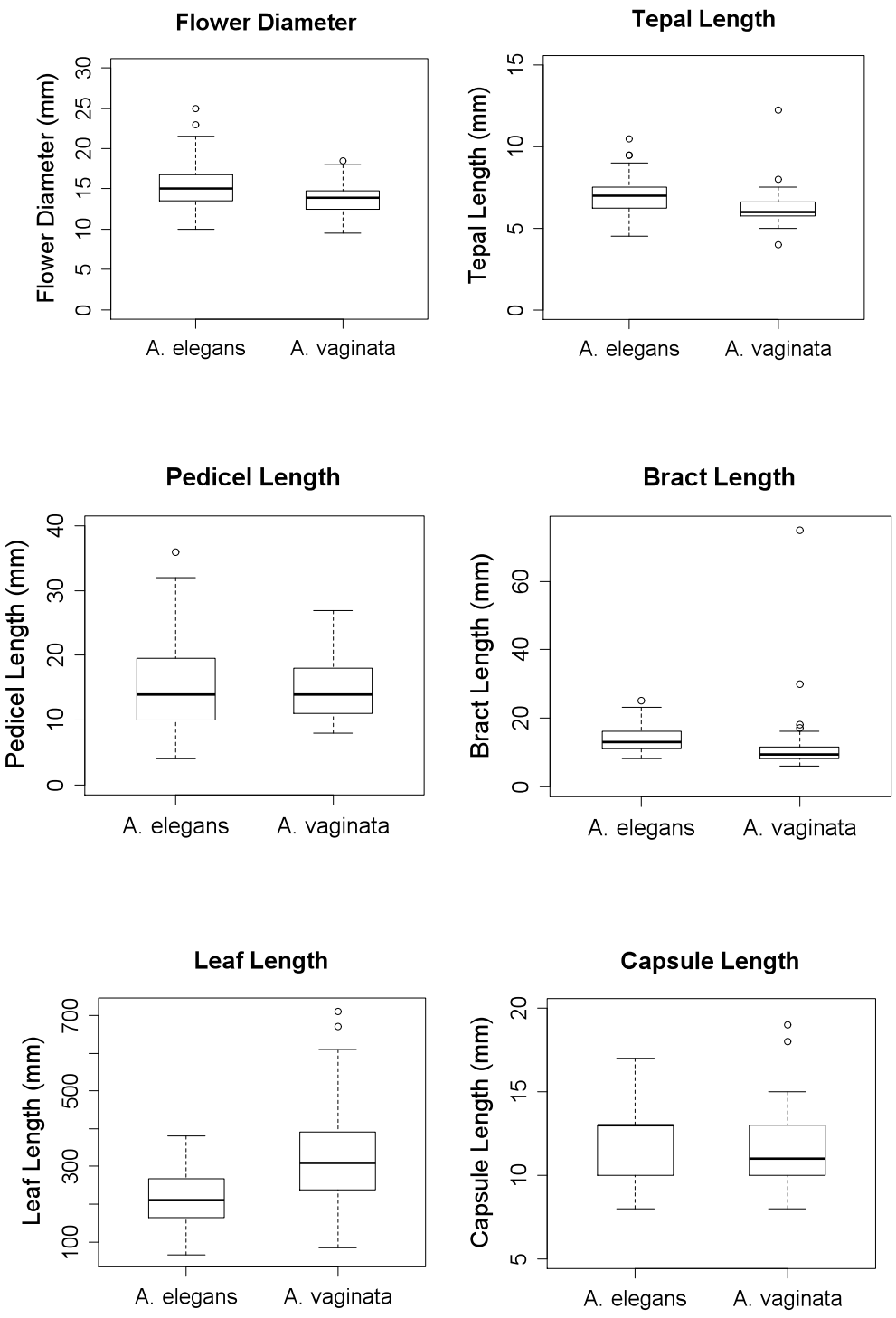

Fig. 3. Box plots showing variation of six morphological characters previously used to separate *A. vaginata* and *A. elegans*. Horizontal bars represent the median, boxes indicate the interquartile range, and whiskers extend to the most extreme data point that is not more than 1.5 times the interquartile range. Dots indicate outliers. Flower diameter, tepal length, pedicel length, bract length, and leaf length are all significant at $p<0.05$. Capsule length p-value = 0.483.

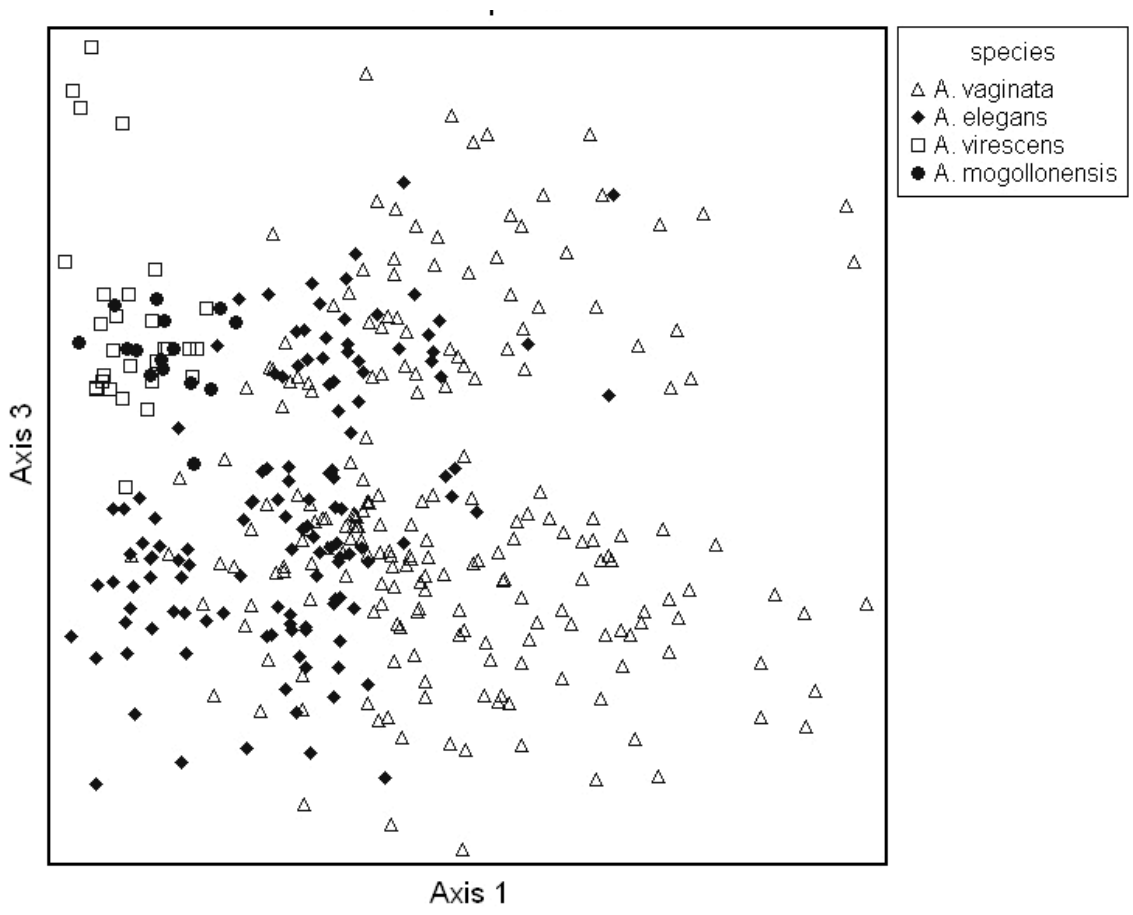

Fig. 4. NMS ordination of species using Jaccard's distance measure comparing samples of *A. vaginata, A. elegans, A. virescens, and A. mogollonensis* based on AFLP markers.

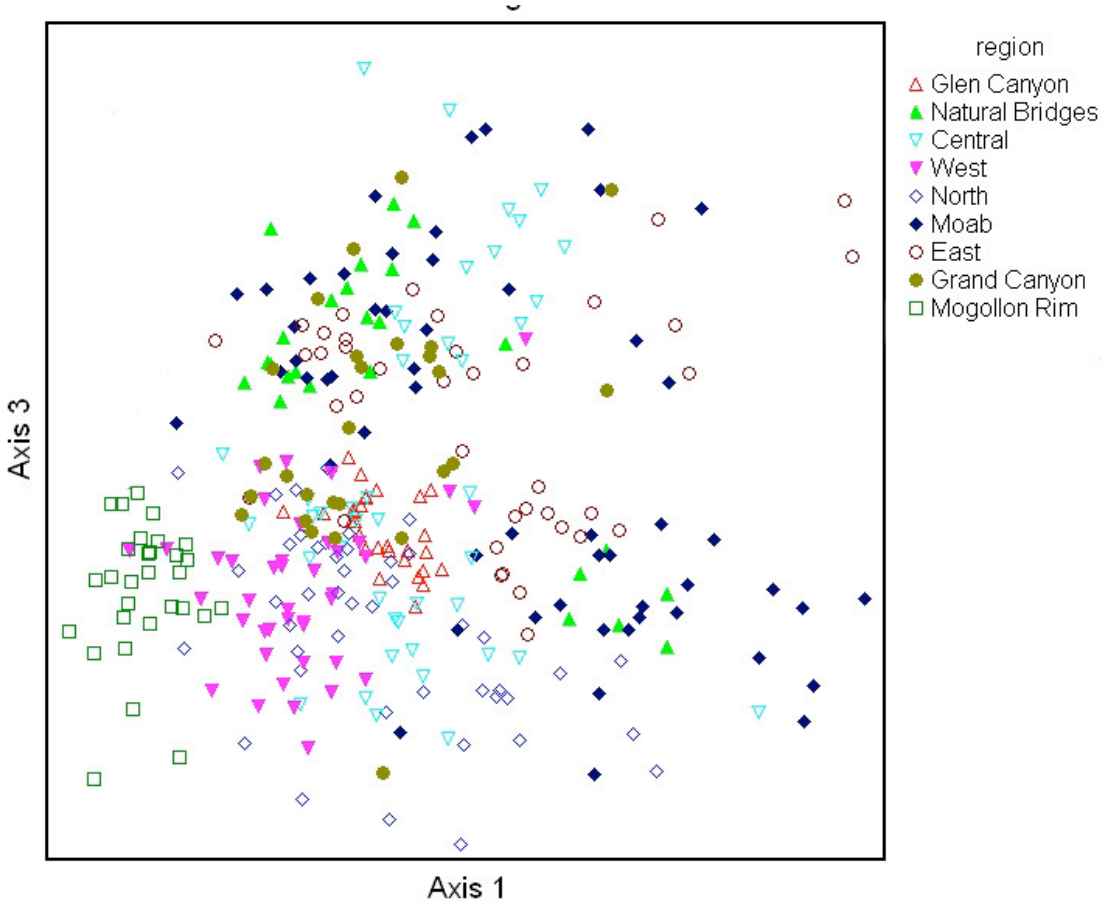

Fig. 5. NMS ordination of regions using Jaccard's distance measure based on AFLP markers, includes *A. vaginata and A. elegans*. Color and shape overlay are of geographic regions rather than species. See Fig. 1 or Table 1 for geographc groupings.

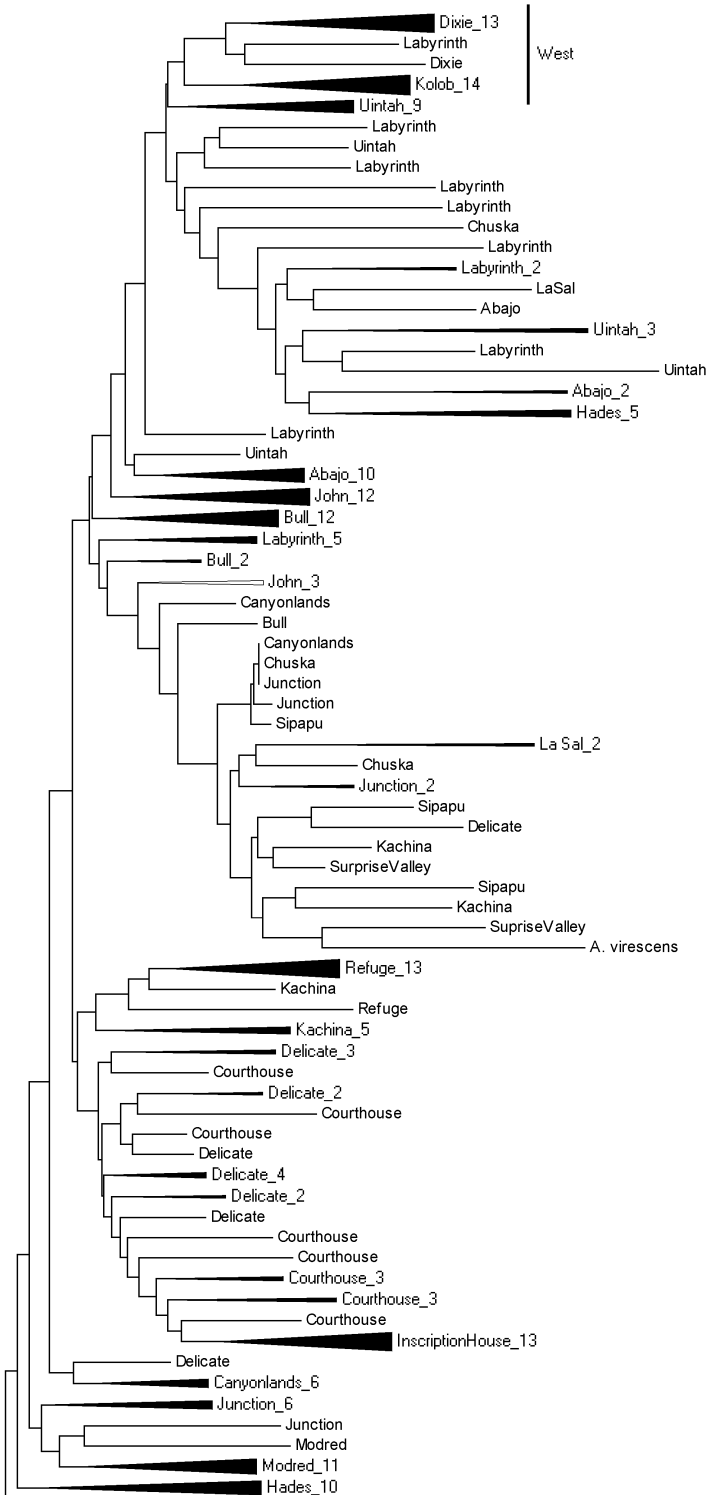

Fig. 6 Part 1 – tree continued on next page. Neighbor-Joining tree based on Nei's genetic distance measure of all 398 individuals. Tree is rooted with *A. virescens* and *A. mogollonensis* samples. Collapsed branches contained only individuals from that population, label indicates the population name and number of samples. Regions that largely clustered together are indicated by bars on right.

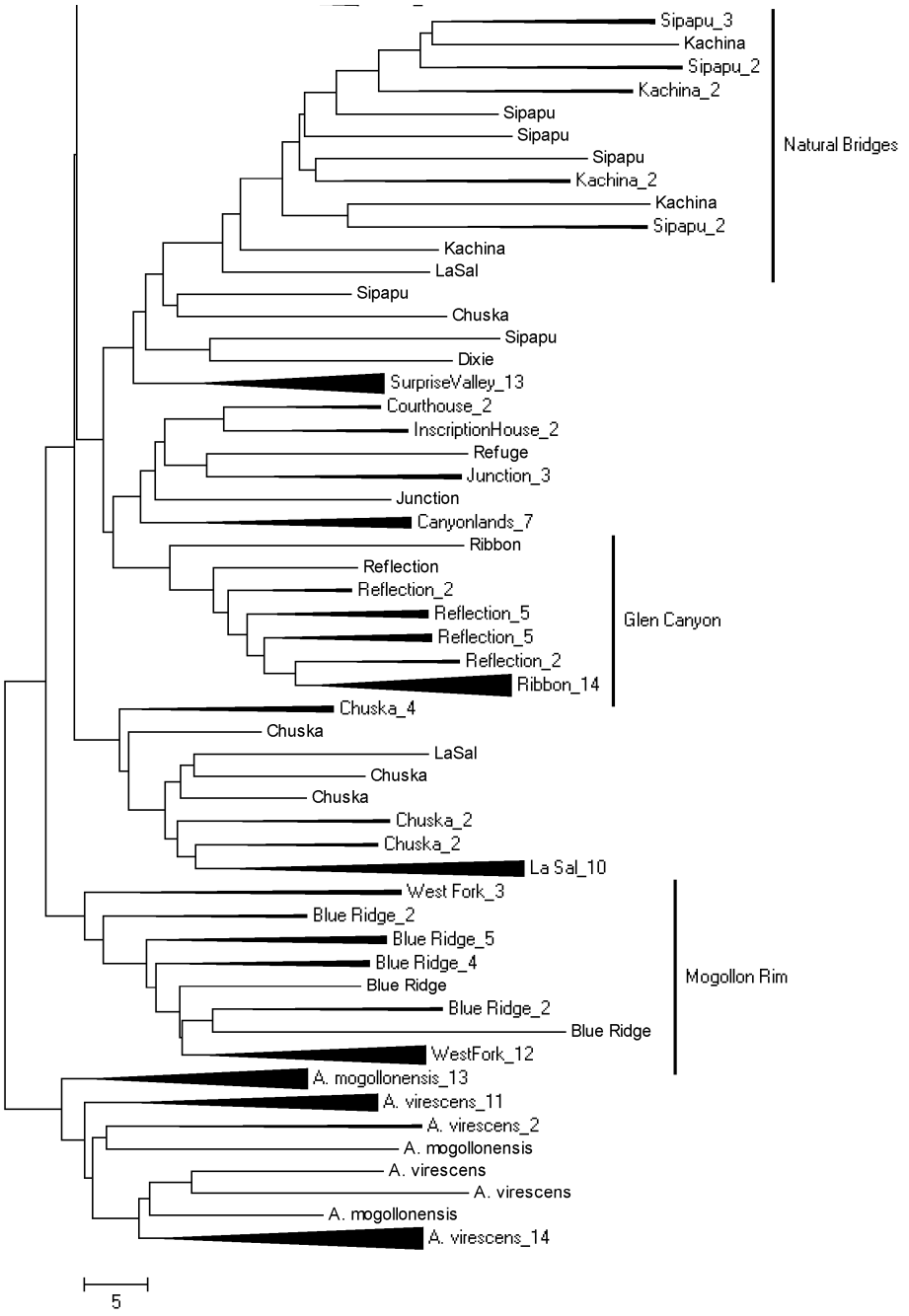

Fig. 6 Part 2 – tree continued from previous page. Neighbor-Joining tree based on Nei's genetic distance measure of all 398 individuals. Tree is rooted with *A. virescens* and *A. mogollonensis* samples. Collapsed branches contained only individuals from that population, label indicates the population name and number of samples. Regions that largely clustered together are indicated by bars on right.

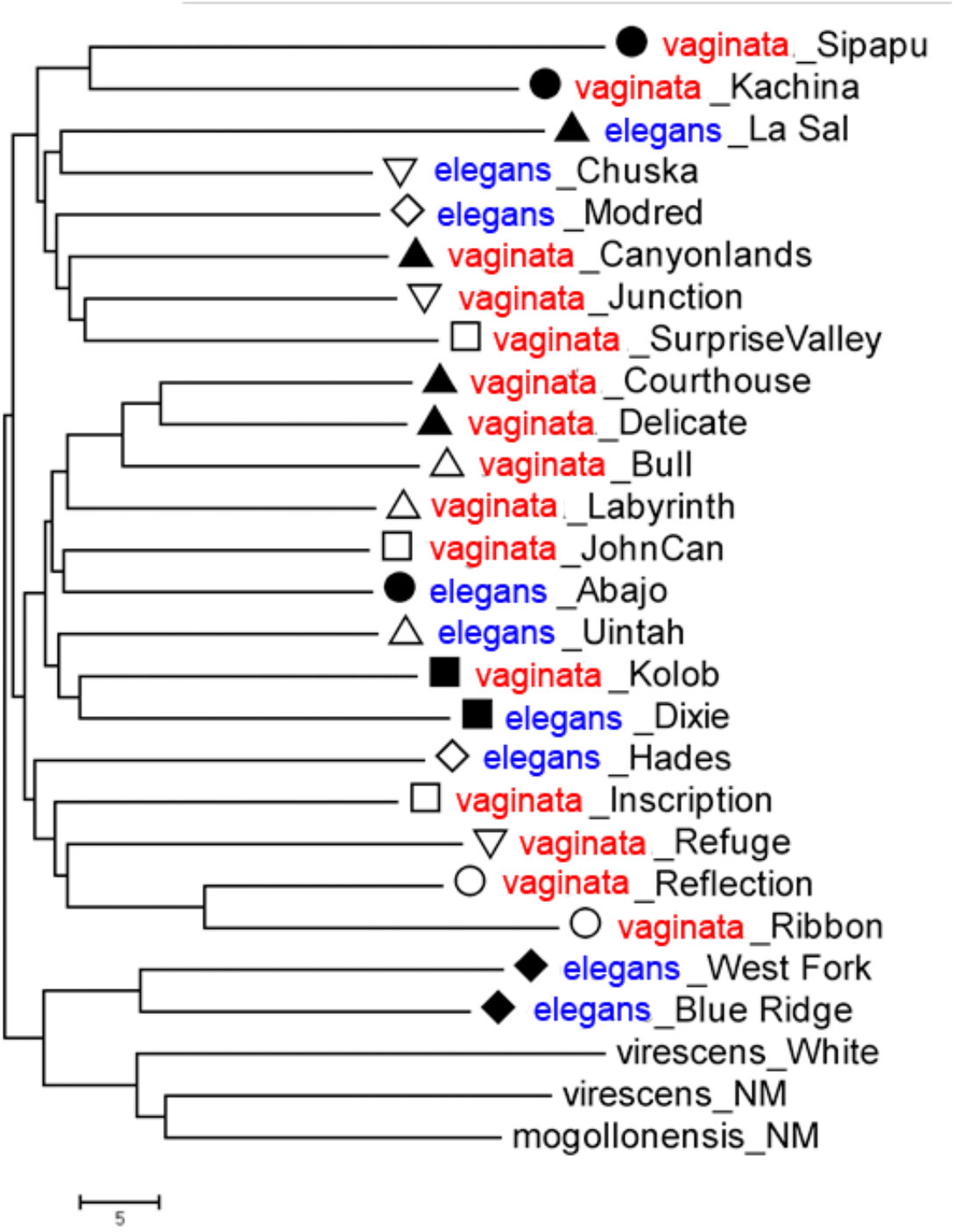

Fig. 7. Neighbor-Joining tree of all 27 populations using Nei's genetic distance of AFLP markers. Each population is labeled with region, species, populations code. Regions: ◆- Mogollon, ◇- GrandCanyon, ○-GlenCanyon, ●- NaturalBridges, □-Central, ■- West, ▲- Moab, △-North, ▽-East. Species: Red-*A. vaginata*, Blue – *A. elegans*.

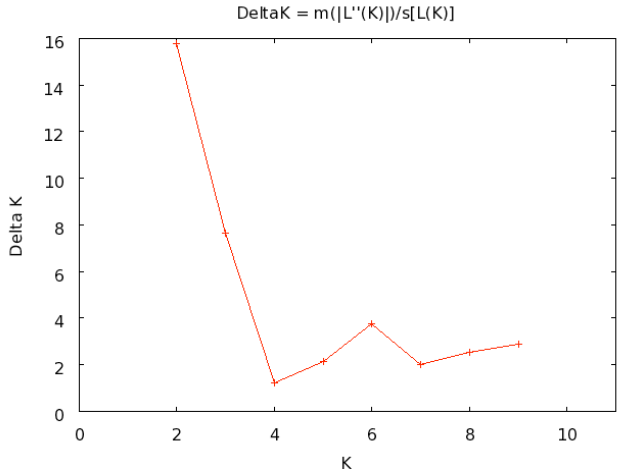

Fig. 8 Delka K graph from Structure analysis showing K=2.

Figure 9: Distruct graph of Structure analysis of *A. vaginata* and *A. elegans*. Each vertical bar represents one individual. The colors represent the proportion of shared genetic profiles from the 2 clusters identified by Structure.

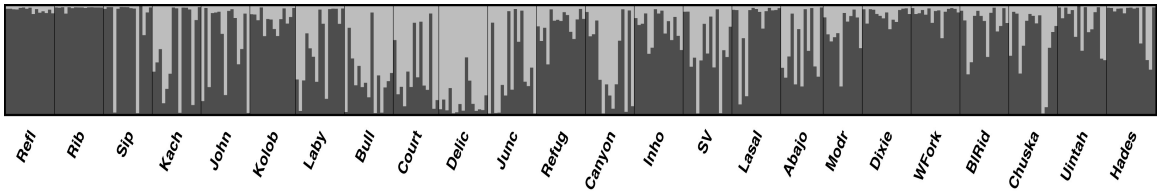

Fig. 10 Distruct graph of Structure results grouped by population. Each vertical bar represents one individual. The colors represent the proportion of shared genetic profiles from the 2 clusters identified by Structure.

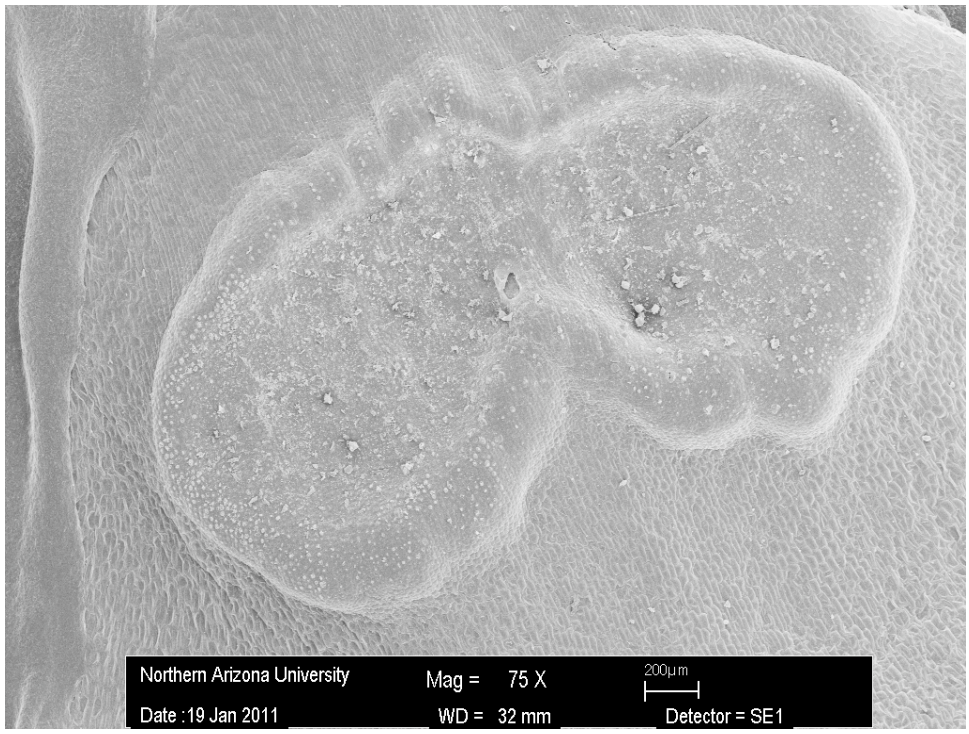

Figure 11. SEM image of *Anticlea vaginata* nectar gland from Ribbon Canyon.

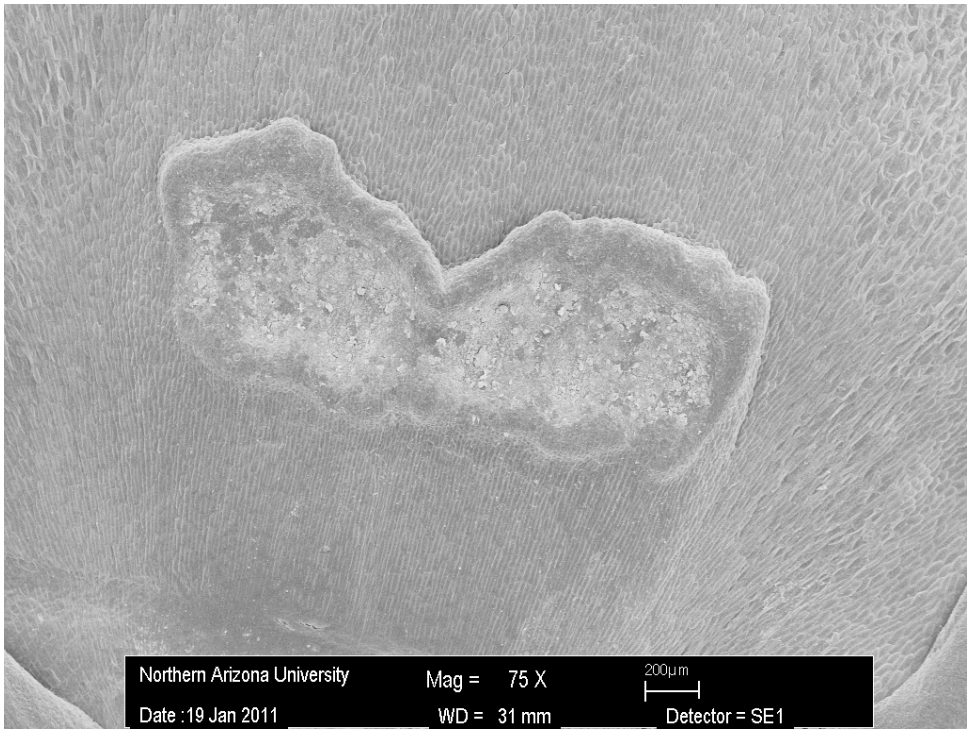

Figure 12. SEM image of *Anticlea vaginata* nectar gland from Labyrinths.

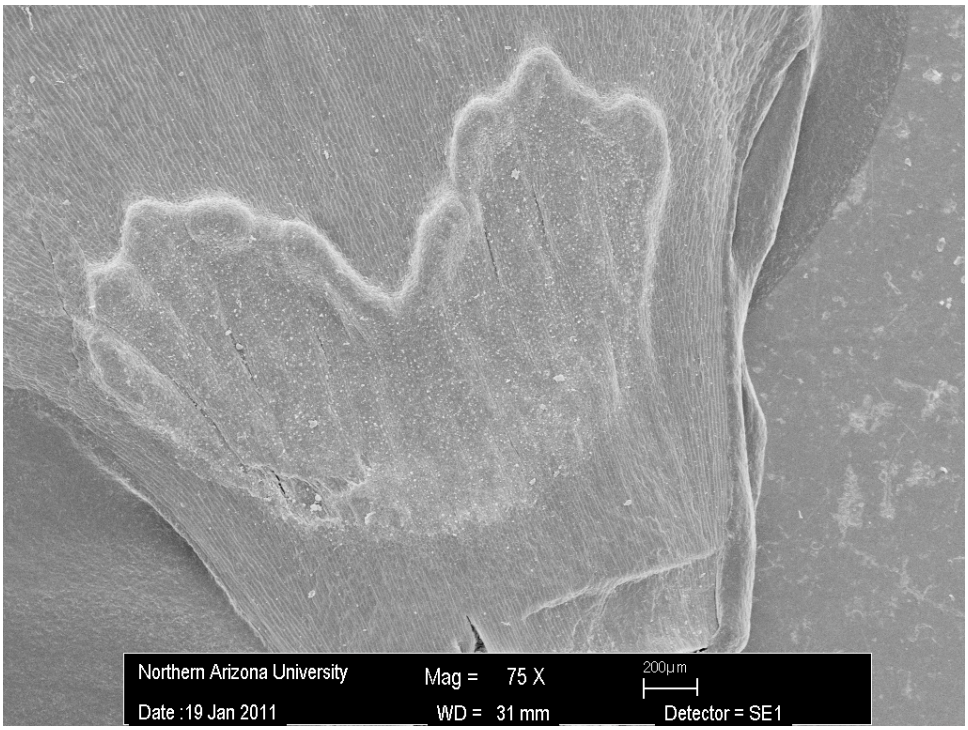

Figure 13. SEM image of *Anticlea elegans* nectar gland.

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). Taxon names follow Zomlefer and Judd (2002). Herbarium abbreviations follow Index Herbariorum 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). ***Anticlea elegans* subsp. *elegans***. BlueRidge, U.S.A. Arizona: Barbershop Canyon, Coconino National Forest, 15, *M. Sommer 2* (ASC). ***Anticlea elegans* subsp. *elegans***. Chuskas, U.S.A. Arizona: Chuska Mountains, Navajo Nation, 15, *E. Palmquist 40* (NAVA). ***Anticlea elegans* subsp. *elegans***., Dixie, U.S.A. Utah: Markaguant Plateau, Dixie National Forest, 15, *E. Palmquist 33* (ASC). ***Anticlea elegans* subsp. *elegans***. Hades, U.S.A. Arizona: Hades Lake, Grand Canyon National Park, 15, *G. Rink 7676* (ASC). ***Anticlea elegans* subsp. *elegans***. La Sal, U.S.A. Utah: La Sal Mountains, Manti-La Sal National Forest, 15, *L. Hannon Williams 10* (ASC). ***Anticlea elegans* subsp. *elegans***. Uinta, U.S.A. Utah: Uinta Mountains, Ashley National Forest, 15, *E. Palmquist 45* (ASC). ***Anticlea elegans* subsp. *elegans***. Modred, U.S.A. Arizona: Modred's Abyss, Grand Canyon National Park, 12, *G. Rink 4877* (ASC). ***Anticlea elegans* subsp. *elegans***. WestFork, U.S.A. Arizona: West Fork of Oak Creek Canyon, Coconino National Forest, 15, *M. Sommer 1* (ASC). ***Anticlea mogollonensis***. ANMO, U.S.A. New Mexico: Mogollon Mountains, 15, *C. Huff 693* (UNM). ***Anticlea vaginata***. Courthouse, U.S.A. Utah: Courthouse Wash, Arches National Park, 15, *E. Palmquist 46* (SEUG). ***Anticlea vaginata***. Delicate, U.S.A. Utah: Near Delicate Arch, Arches National Park, 15, *Welsh, Harrison, Moore 2335* (SEUG). ***Anticlea vaginata***. Junction, U.S.A. Utah: Junction of Canyon del Muerto and Canyon de Chelly, Canyon de Chelly National Monument, 15, *G. Rink 1366* (ASC). ***Anticlea vaginata***. Refuge, U.S.A. Utah: near Refuge Rock, Canyon de Chelly National Monument, 15, *G. Rink 1371* (ASC). ***Anticlea vaginata***. Canyon, U.S.A. Utah: Canyonlands, 14, *N. Boschen S1-84* (SEUG). ***Anticlea vaginata***. Bull, U.S.A. Utah: Bull Canyon, Dinosaur National Monument, 15, *E. Palmquist 44* (ASC). ***Anticlea vaginata***. Labyrinths, U.S.A. Utah: Labyrinths, Dinosaur National Monument, 15, *E. Palmquist 43* (ASC). ***Anticlea vaginata***. Reflection, U.S.A. Utah: Reflection Canyon, Glen Canyon National Recreation Area, 15, *E. Palmquist 46* (GLCA). ***Anticlea vaginata***. Ribbon, U.S.A. Utah: Ribbon Canyon, Glen Canyon National Recreation Area, 15, *E. Palmquist 36* (GLCA). ***Anticlea vaginata***. Inscription, U.S.A. Arizona: Inscription House Spring, Navajo Nation, 15, *D. Roth 830* (NAVA). ***Anticlea vaginata***. Johns, U.S.A. Utah: Johns Canyon, 15, *E. Palmquist 39* (ASC). ***Anticlea vaginata***. Kolob, U.S.A. Utah: Kolob Canyons, Zion National Park, 14, *E. Palmquist 41* (ZION). ***Anticlea vaginata***. Kachina U.S.A. Utah: Near Kachina Bridge, Natural Bridges National Monument, 15, *E. Palmquist 38* (SEUG). ***Anticlea vaginata***. Sipapu, U.S.A. Utah: Near Sipapu Bridge, Natural

Bridges National Monument, 15, *E. Palmquist 37* (SEUG). ***Anticlea vaginata***. Surprise, U.S.A. Arizona: Surprise Valley, Navajo Nation, 15, *D. Roth 822* (NAVA). ***Anticlea virescens***. ANVI1, U.S.A. New Mexico: Mogollon Mountains, Gila National Forest, 15, *E. Palmquist 42* (ASC). ***Anticlea virescens***. ANVI2, U.S.A. Arizona: White Mountains, Apache-Sitgreaves National Forest, 15, *L. Hannon Williams 16* (ASC).

APPENDIX 2: SPECIMENS EXAMINED FOR MORPHOLOGICAL ANALYSES

Taxon name assignments follow Zomlefer and Judd (2002). Herbarium abbreviations follow Index Herbariorum, 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 & J.H.Soper 9445* (RM); N.W.T.: vicinity of Aubry Lake, *R. Riewe & J. Marsh 181* (ASU); vicinity of Aubry Lake, *R. Riewe & J. Marsh 448* (ASU). U.S.A. Alaska: Denali Co., Denali National Park, Wonder Lake, *A. Nelson & 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. 19mi 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 Carnero Lake turnoff, *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, Pinkava, Kiel s.n.* (ASC); Coconino Co., SW slope of Agassiz, *L. Paulik SA-70* (ASC); Coconino Co., Kaibab National Forest, 100m below Kendrick Peak, trail side, *L.T. Greene III G175* (ASC); Coconino Co., Willow Valley, 9mi 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, 500m SW of fork, 6km 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, 9mi 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 & Pinkava 4563* (ASU); Coconino Co., West Fork Oak Creek, along stream, *Pinkava, Lane & Lehto L18780* (ASU); Coconino Co., Inner Basin, San Francisco Peaks, *T. Reeves & 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 & E. Lehto 26* (ASU); Coconino Co., San Francisco Peak, Inner Basin, 3-4km above water facility buildings, *R. Hevly, D. Pinkava, D. Keil, T. Reeves, 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, 6mi 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, Hodgson, Lehto L20208* (ASU); Coconino Co., Inner Basin, San Francisco Peaks, *Keil, Reeves, Hevly, Pinkava P13762* (ASU); Coconino Co., Hole-In-Ground campground, 3mi W of Woods Canyon Lake turnoff, rim rd, *T. Mason & 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 & 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, 4mi from State Route 288 junction, *M. Mittleman & 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, 1.5mi 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., 1.2mi 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 1/4mi 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., 4-6mi 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 & 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, 1/4mi below, *G. Goodwin 2008* (ASC). Idaho: Bonneville Co., Caribou Mt, *E.B.Payson & G.M.Armstrong 3590* (RM); Custer Co., Bear Canyon, *A. Nelson & J.F.MacBride 1492* (ASC); Elmore Co., 1mi east of Atlanta, Sawtooth Primitive Area, headwaters of Middle Fk. Boise River above Atlanta, *C.L.Hitchcock & C.V.Muhlick 10195* (RM); Idaho Co., 12mi 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, 10mi N of Blue Dome, just south of FS rd to Meadow Canyon and Coal Kiln Canyon, *D. & M. Hendersons 1045* (ASU); East Fork , Wood River, *C.N.Woods & I. Tidestrom 2786* (RM). Montana: Gallatin Co., Flathead Creek, *B.J. Jones s.n.* (ARIZ); Gallatin Co., Snowflake springs, 31mi N of west Yellowstone, *D. Patten & 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. & G. Davidse 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 hotsprings NW of McGill, *A. Atwood, S. Welsh, K. Harper 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. Sivinksii, B. Hevron, D. Bleakley 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 Todalena 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 8mi S of Enterprise, *C. Feddema 3599* (RM); Wallowa Co., Jewett Lake, Wallowa Mts, about 10mi S of Wallowa Lake, *G. Mason 7978* (ASU). Utah: Duchesne Co., Uinta Mts, south fork of Rock Canyon, ca 3 mi NW of turnoff 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 1.6km 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., 0.3mi 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 & E. Nelson 6486* (RM); Yellowstone National Park, Mammoth Hot Springs, *A. Nelson & E. Nelson 6055* (RM).

Anticlea elegans subsp. *glaucus*. 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, *Dewey 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); Mahnomen Co., along Hwy 200 west of Zerkel, *S.E. Hamilton 70* (ASU). North Dakota: Benson Co., prairies, Leed, *J. Lunnel s.n.* (RM); McHenry Co., 4mi 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., 1/4mi south of Custer, *O. Degener & L. Peiler 16325* (RM). Wisconsin: Green Lake Co., Boyscout Camp Tichora on Green Lake, *R. Peters 40* (ASU).

Anticlea vaginata. U.S.A. Arizona: Apache Co., hanging garden on north side of Coyote Creek about 2km 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 2km 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 2km 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 4mi 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, Harrison, Moore 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 & S.L. Welsh 11878* (BRY); Kane Co., Cottonwood Canyon off Reflection Canyon, Glen Canyon National Recreation Area, *E. 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 & Fleming 5669* (SEUG); San Juan Co., Navajo Nation, Cliff Canyon, west of Navajo Mountain, *D. Roth & 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 & K. McCoy 793* (BRY); San Juan Co., Armstrong Canyon between Kachina Bridge and Owachomo Bridge, Natural Bridges National Monument, *S.L. Welsh & 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 & R. Allen 3180* (BRY); San Juan Co., vicinity of Kachina Bridge, Natural Bridges National Monument, *S.L. Welsh & G. Moore 2409* (BRY); San Juan Co., John's Canyon drainage, 3.0mi N of Muhley Point, *A. Clifford & K. Heil 03-1080* (BRY); San Juan Co., Natural Bridges National Monument, alcove near Sipapu Bridge, *Heil & Fleming 5669* (BRY); San Juan Co., Navajo Nation, John's Canyon, 14mi WNW of Rd 316, *K. Heil & A. Clifford 22897* (BRY); San Juan Co., Ribbon Canyon, Grandaddy 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, 15mi 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 & 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 S1-84* (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 & C.M. Barber 184* (RM); 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 1/8mi 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. & B. Schaack 1264* (ASC); Apache Co., Reservation Ranch on Apache Indian Reservation, *L. N. Goodding & 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, ½ mi up from Forest Service rd 275, *J. Cordts & W. Hodgson 3138* (DES); Greenlee Co., n. of Cliffton, *O.M. Clark 12937* (UNM). Colorado: Gunnison

Co., West Elk Mts, Summit of McClure Pass, 0.8mi from main hwy along dirt rd following the ridge eastward, *W.A. Weber & 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, V. Gass, T. Daniel, s.n.* (DES); Catron Co., Gila National Forest, along drainage flowing NW into BS canyon on N slope of Bearwallow Mt, *T. F. Daniel & 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).