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Intraspecific Phylogeography of Cycladenia humilis (Apocynaceae)

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INTRASPECIFIC PHYLOGEOGRAPHY OF *CYCLADENIA HUMILIS* (APOCYNACEAE)

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A thesis submitted to the faculty of
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Master of Science

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ABSTRACT

INTRASPECIFIC PHYLOGEOGRAPHY OF *CYCLADENIA HUMILIS*

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Cycladenia humilis (Apocynaceae) is a rare perennial herb native to western North America and has a fragmented distribution in California, Utah, and Arizona. Populations in Utah and Arizona are federally listed as threatened, while there is no conservation status applied to California populations. Using genetic (three chloroplast and two nuclear DNA loci) and morphological characters, intraspecific variation between populations of *C. humilis* and current taxonomic conventions were assessed. Nested Clade Phylogeographic Analysis and Bayesian phylogenies were used to assess patterns within *C. humilis* and supported three main population groupings: a northern California, southern California, and Colorado Plateau group. The northern California populations represent a distinct group and include populations from the Santa Lucia Mountains contrary to current classifications. The southern California group consistently includes populations in the San Gabriel and Inyo Mountains and was unique from any other region. The Colorado Plateau represents a group distinct from all other groups. The resilience of *C. humilis* on the Colorado Plateau to human threats remains unknown, but based on its frequency being comparable to California and our findings that considerable genetic variation exists within the species and within populations on the Colorado Plateau, we recommend that the threatened status of *C. humilis* be lifted.

Keywords: nested clade phylogeographic analysis (NCPA), plant conservation, rare plants.

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INTRODUCTION

Intraspecific phylogeography examines the history of populations over space and time. Such understanding is essential for making informed conservation decisions because it is at the population-metapopulation level that evolutionary forces operate that lead to genetic divergence, morphological differentiation, and, ultimately, speciation (Crandall et al. 2000). Knowledge of population history can aid the design of conservation strategies by providing insights into the processes that have contributed to patterns of contemporary variation. It also provides insight into observed patterns of population subdivision with respect to both space and time.

Cycladenia humilis Benth. (Apocynaceae) is a small rhizomatous perennial of uncertain longevity. The species is native to western North America and has a fragmented distribution, with scattered populations in southern Utah, northern Arizona, and California. Whereas the distribution of this species is well known, nothing is known about the historical phylogeography of this species across its range. Filling this void is important given current efforts to develop recovery plans for populations from Utah and Arizona that are federally listed as threatened, and given the recent proposal that populations from the Inyo Mountains of Inyo County, California are the same taxonomically as the populations from Utah and Arizona (Rosatti 2008; http://ucjeps.berkeley.edu/tjm2/review/treatments/apocynaceae.html#21617)

Taxonomic Background—*Cycladenia* is a unispecific genus with three varieties currently recognized within the species. These varieties are distinguished by a few floral characteristics but are largely distinguished by their geographic distributions. C. *humilis* var. *humilis* and *C. humilis* var. *venusta* (Eastw.) Woodson ex. Munz are endemic to northern and southern California, respectively. They are typically found at elevations between 1200–2800 m on sandy flats, talus slopes, open pine forest, or chaparral ecosystems (Dempster 1993). *C.*

humilis var. *jonesii* (Eastw.) S.L. Welsh & N.D. Atwood is endemic to the Colorado Plateau in southern Utah and northern Arizona. It usually grows at elevations between 1300–1800 m on steep side slopes, the bases of mesas, and mixed desert shrub ecosystems (Welsh et. al 1987). A fourth variety, *C. humilis* var. *tomentosa* (A. Gray) A. Gray, was erected for plants that are densely pubescent throughout; however, these occur side by side with glaucus-leaved plants in northern California and are now synonymized with var. *humilis*. Sipes et al. (1994) suggested that the differences between these two varieties are due to a single gene trait.

Early classifications recognized var. *jonesii* as a distinct species (Eastwood 1942), but it was later reduced to a variety because of the close morphological similarity with the two California varieties (Welsh et. al 2003). Most published research concerning *C. humilis* has focused on var. *jonesii* because it is federally listed as threatened, whereas there is no federal conservation status applied to the varieties in California. When var. *jonesii* was first listed, it was only know from four disjunct sites (Sipes et al. 1994), but since then, more sites have been discovered. An allozyme survey of var. *jonesii,* including a single population of var. *humilis* for comparison, revealed considerable divergence between the two varieties (Sipes and Wolf 1997). The extent of genetic variation throughout the species' entire distribution, within and between all varieties, and between geographic regions remains unstudied.

All current classifications of *C. humilis* are based entirely on morphology and geographic distribution. The objective of our research is to investigate the intraspcific variation between populations of *Cycladenia* and assess whether genetic groupings correspond with current taxnomic conventions or morphological traits that have been used as taxonomic characters.

MATERIALS AND METHODS

Sampling and DNA isolation—To assess within-species variation a broad geographic sampling of *C. humilis* was gathered from 26 sites in California, Utah, and Arizona (Fig. 1; Appendix 1). Ten sites representing var. *jonesii* in southern Utah and northern Arizona, seven sites representing var. *venusta* in southern California, and nine sites representing var. *humilis* in northern California were sampled. At each site, individuals were sampled by taking one or two leaves from each plant. Due to the clonal nature of this species, individuals were sampled approximately 8–10 m apart to avoid redundant sampling of genets, as a previously determined for this species (Sipes and Wolf 1997). Leaf samples were desiccated with silica gel for long-term storage and further processing in the lab. Six to eight arbitrarily selected individuals from each geographical area were included in this study, resulting in a total of 204 individuals sampled.

DNA was isolated from leaf samples using a modified CTAB protocol (Doyle and Doyle 1987; Cullings 1992). Three chloroplast regions, *trnS–trnG* (~571 bp; Hamilton 1999), *psbM–trnD* (~954 bp; Shaw et al. 2005) , and *trnQ–rpS16x1* (~1210 bp; Shaw et al. 2007), and two regions from the nuclear rDNA cistron (ITS-1 through ITS-2; ~625 bp; Baldwin 1995) and the ETS region; ~648bp; Baldwin and Markos 1998) were analyzed. The chloroplast regions were selected after initial screening of highly variable regions in the chloroplast genome (Shaw et al. 2005, 2007). DNA regions were amplified using locus specific primers published elsewhere (White et al. 1990; Porter 1996; Hamilton 1999; Shaw et al. 2005, 2007) or, for ETS, using the 18S-ETS primer of Baldwin and Markos (1998) paired with a primer we designed, CycETS1i: 5'-TCGTGAAATCGCAACCTCGT-3'. The PCR profile consisted of 30 cycles of 1 min at 95°C, 1 min at 52°C (55°C for ETS) and 1 min at 72°. Amplified fragments were cleaned using

Millipore plates and both strands cycle sequenced (BigDye v.3, Applied Biosystems, Foster City, CA) and electrophoresed on an AB 3730xl automated sequencer in the DNA Sequencing Center at Brigham Young University.

Sequence chromatograms were edited using Sequencer 4.6 (Gene Codes Corp., Ann Arbor, MI), and sequences aligned by eye with Se-Al (Rambaut 2002). Indels were coded with simple indel coding (Simmons and Ochoterena 2000) using SeqState 1.40 (Müller 2005); gaps resulting from length variable poly-A and poly-T regions were not included in analyses, because of uncertainty in the exact length of poly-N regions and because these regions appear to be hypervariable and prone to homoplasy in our data.

Heterozygous sites in the nuclear data were resolved using Bayesian approaches as implemented in Phase 2.1(Stephens et al. 2001; Stephens and Donnelly 2003). Each data set was run multiple times with a different random number seed in each analysis with 1000 iterations and 100 burn-in iterations. Sites with less than 95% posterior probability were excluded from the data set because the analytical programs employed are unable to support missing data. Less than 1.5% of nuclear data was excluded from the matrices.

Phylogenetic Analysis—Three independent phylogenetic analyses were performed with MrBayes 3.1(Huelsenbeck and Ronquist 2001), one for each nuclear locus and one for the combined chloroplast data. Appropriate models for each locus were evaluated using the Akaike Information Criterion (AIC, Akaike 1974) implemented in MrModeltest (Nylander 2004); for the chloroplast data, this was repeated for each gene separately. All 3 data sets also included indel data from simple indel coding partitioned as standard data in the analyses. ITS and ETS data sets were simplified by choosing one representative from each unique haplotype. In the chloroplast analysis, one representative for each haplotype at each collection locality was selected.

Trachelospermum difforme (Walter) A. Gray was used as the outgroup in all analyses, based on its relationship near Cycladenia in a family level phylogenetic analysis (Livishultz 2007) All analyses were performed several times with two runs of 10 million generations, with a sampling frequency of 1000 and a burn-in of 2000.

Phylogeographic analysis—Presence of recombination was tested for in the nuclear loci with RDP3 beta 34 (Martin et al. 2005) using the default settings.

Haplotype networks were constructed using TCS 1.21(Clement et al. 2000; 2002). In these analyses, all three chloroplast regions were combined into a single data set. The nuclear regions were analyzed separately, because some individuals were polymorphic at several residues and it was not possible to determine which haplotype phases in ITS and ETS corresponded. Information from coded gaps was included by scoring indels as C's (absence) and T's (presence), and appending these to the end of the sequences. Gaps were treated as missing for all data sets and the connection limit was set at 95% for ITS and the combined chloroplast data, and 94% for the ETS data.

Nested Clade Phylogeographic Analysis (NCPA; Templeton 1998; Templeton 2004) was then used to analyze the sequence data and understand population histories. The haplotype networks were manually nested into clades and input into GEODIS 2.6 (Posada et al. 2000). The GEODIS analysis was based on 10,000 random permutations and was used to test for significant relationships between geographic locations and genetic distances. The 2008 inference key (http://darwin.uvigo. es/software/geodis.html) was used to determine which historical processes might have lead to the current patterns of genetic diversity.

Morphology—Morphological characters were evaluated using specimens from BRY and new collections now deposited in the BRY collection. Whole flowers from 11 collection sites

were preserved in 70% ETOH. Preserved flowers were dissected in Pohl's solution (Pohl 1965) for examination under a light microscope. In keys, the presents or absents of pubescences on the inflorescence has been used as a characters to define taxonomic boundaries within Cycladenia. For our study we focused specifically on the length of hairs (trichomes) on the inside and outside of the corolla. Trichomes were measured in µm using MicroSuite™ v. 1.20 (Olympus, Center Valley, PA). Petioles measurements were taken from the first two rows of leaves starting at the base, the above ground portion of the plants, and were measured in mm. Significant differences between the means of each character from different geographic regions were assessed with a single-factor Between-Subjects ANOVA and pair-wise t-tests with a Bonferroni-Dunn correction applied for multiple comparisons in Aabel 3 (Gigawiz, Tulsa, OK).

RESULTS

Phylogenetic Analysis—The following models of sequence evolution were chosen for each locus by MrModeltest: GTR + G for ITS, HKY for ETS, HKY + G for *trnS–trnG*, and GTR for *psbM–trnD* and *trnQ–rpS16x1*. The Bayesian phylogeny for the partitioned chloroplast loci (Fig. 2) indicates three significant geographic clades corresponding to Utah, northern California and southern California. The Utah clade was supported with a posterior probability of 1.00 and included all haplotypes from Utah and Arizona, except for the haplotype from site 26 (Joe Hutch Canyon area). This haplotype weakly grouped with the two California clades with a posterior probability of 0.76. The northern California clade was supported with a posterior probability of 1.00 and included all northern California haplotypes and the sites from the Santa Lucia Mountains in Monterey County. The southern California clade was supported with a posterior probability of 1.00 and included all haplotypes from the San Gabriel Mountains and Inyo

Mountains. The Bayesian phylogeny for the ITS data set (Fig. 3) weakly supported three geographic clades corresponding to Utah, northern California, and southern California regions. The ETS phylogeny (Fig.4) strongly supported a northern California clade, which included haplotypes from the Santa Lucia Mountains with a posterior probability of 0.99. The haplotype from the San Gabriel Mountains was strongly supported as sister to the northern California group and the Inyo Mountain haplotype was also strongly supported as sister to the rest of the California haplotypes forming a California clade with a posterior probability of 0.98. The haplotypes from the Colorado plateau were weakly supported as a series of successive sister groups to the California clade.

Phylogeographic analysis—All analyses performed in RDP3 detected no recombination in either of the nuclear data sets. The TCS analysis for the combined chloroplast data resulted in a single network (Fig.5) and consisted of 20 haplotypes (Appendix 2). The connection limit was set at 95%. The network contains two main haplotype groupings separated by 20 mutational steps. One group corresponds to populations from California (clade 4.1) and the other to populations from Utah (clade 3.6), with the exception of haplotypes 11 (site 25, Arizona) and 12 (site 26, Joe Hutch Canyon area), which are located on intermediate branches between the California and Utah groups. The inference key showed (Table 1) that allopatric fragmentation was observed in clade 1.33, 1.34, 1.35, 3.1, and 4.1, restricted gene flow with some long distance dispersal in clade 2.17 and 4.2, restricted gene flow with some long distance dispersal or past gene flow followed by extinction of intermediate populations in the total cladgram, and inadequate geographic sampling in clade 1.7, 2.2, 3.2, and 3.6.

ITS1-ITS2 resulted in a single network (Fig.6) with 26 haplotypes (Appendix 3) at a connection limit of 95%. The network contains two main centers correlating with northern

California and Utah and is separated by seven mutational steps. The haplotypes representing northern Arizona and southern California are each on intermediate branches between the two main centers. The inference key (Table 2) showed that allopatric fragmentation was observed in clade 2.1, 3.3, 3.5 and the total cladogram, restricted gene flow/ dispersal but with some long distance dispersal in clade 4.2, restricted gene flow with isolation by distance in clade 2.10, past fragmentation and/or long distance dispersal in clade 4.1, restricted gene flow with some long distance dispersal or past gene flow followed by extinction of intermediate populations in clade 1.19, and inadequate geographic sampling or inconclusive outcome in clade 1.1, 1.13, 2.2, and 3.1.

The TCS analysis for ETS resulted in 2 networks at a connection limit of 95%. One network consisted of a single haplotype found at localities 1, 2, and 13 (San Gabriel Mountains) and the other included 16 haplotypes. Lowering the connection limit to 94% produced a single network (Fig.7) with 17 haplotypes (Appendix 4). This network consists of two main centers separated by eight mutational steps. These centers correlate to northern California and Utah /Arizona populations while the southern California populations are intermediate on long divergent branches between the two centers. The inference key (Table 2) showed that allopatric fragmentation was observed in clade 3.1, 3.3 and the total cladogram, restricted gene flow with some long distance dispersal or past gene flow followed by extinction of intermediate populations in clade 1.1, 1.16 and 2.7, and inconclusive outcome in clade 1.14.

Morphology—Outcome of the Single-factor Between-Subjects ANOVA for petiole length and inner and outer trichomes on the corolla showed significance with a $p < 0.001$. Figures 8–10 show the box and whisker plots of sample measurement ranges for the inner corolla

hair, outer corolla hair, and petioles. The outcomes of pair-wise tests of differences between population means with Bonferroni-Dunn correction are in Table 3–5.

DISCUSSION

Intraspecific phylogeography examines the history of populations over space and time. NCPA (Templeton et al 1998; Templeton 2004) was one of the first statistical methods developed to assess population histories and is especially useful in intraspecific phylogeography. Recently, criticisms have been made of single-locus NCPA. One of the biggest criticisms of single-locus NCPA was that it had a high rate of both type I and type II errors (Knowles 2008). Improvements have been made to NCPA by the development of multilocus cross-validation, which has reduced the amount of both type I and type II errors (Templeton 2002, 2004). In addition to the use of mulitlocus NCPA, simulation techniques and other forms of data, such as test of gene flow and recombination, can also be used in order to provide strength to a phylogeographic study (Temepleton 2009).

Understanding intraspecific evolutionary history is essential to making informed conservation decisions because it is at the population-metapopulation level that evolutionary forces operate that lead to genetic divergence, morphological differentiation, and, ultimately speciation. Knowledge of a population history can aid the design of conservation strategies by providing insights into the processes that have contributed to patterns of contemporary variation. It also provides insight into observed patterns of population subdivision with respect to both space and time.

Taxonomic Implications—Our study included the reevaluation of the status of populations of var. *jonesii* as a separate species. We used the Unified Species Concept as a

guideline to aid in identifying species boundaries. The Unified Species Concept defines a species as a metapopulation-level lineage that is evolving separately from other closely related lineages (de Queiroz 2005). Criteria such as breeding barriers, ecological or morphological differentiation, or genetic differentiation may be used to determine if a group under study consists of just one, or more than one, separately evolving metapopulation lineages. Understanding population histories on spatial and temporal levels aid in this determination. NCPA is designed to measures and make inferences about the distinctness of population lineages, making this type of analysis an appropriate tool for our study.

Previously, geography and a few morphological differences were the only observed variation used to distinguish taxonomic units within *C. humilis.* Sipes and Wolf (1997) were the first to investigate genetic variation of *C.humilis* with their allozyme analysis. Their study focused on populations of var. *jonesii* and showed that variation existed between these populations. They also showed that considerable variation existed between var. *jonesii* and *C. humilis* from California, based on the incorporation of a single population from var. *humilis*. However, the genetic variation that exists throughout the entire distribution of *Cycladenia* remained unknown. Our study is the most comprehensive genetic sampling of the entire distribution of *Cycladenia humilis* to date. Our expanded sampling supports the findings of Sipes and Wolf (1997) and confirms that there is notable genetic variation between populations of *C. humilis* from the Colorado Plateau and California. We further infer the relationships and patterns that lead to the current distribution of populations within *C. humilis*.

Populations of var. *humilis* from northern California represent a group that is distinct from populations from southern California and the Colorado Plateau. Our findings further support the synonymy of tomentose plants found in northern California with var. *humilis*,

because tomentose and glabrous plants share identical haplotypes. The populations from the Santa Lucia Mountains, traditionally considered var. *venusta*, in every locus sampled and in every analysis performed showed a consistent association with the haplotypes from northern California (var. *humilis*). This contradicts the traditional classification of the Santa Lucia Mountain populations, which was originally based on the shared character of inflorescence pubescence. The statistical analysis of corolla hairs showed that the mean trichome length of populations in the Santa Lucia Mountains were not significantly different from those of var. *venusta* in southern California. However, our review of morphology also found indications that presence of hairs on the corolla is not fixed in the Santa Lucia Mountains populations and, in a few instances, it is hardly present. In addition, we also found that the density of pubescence within these populations was noticeably lower than those of other populations of var. *venusta*. It is possible that this characteristic has varied independently several times in *C. humilis*, or that the Santa Lucia Mountains maintain ancestral polymorphism in this feature.

In the nuclear data, haplotypes from the Santa Lucia Mountains were more closely related to var. *humilis* than they were to var. *venusta*, and in the chloroplast data, the Santa Lucia Mountain populations are identical to the var. *humilis* populations from northern California at all three loci. NCPA suggested that the separation between populations in northern California and the Santa Lucia Mountains was the result of allopatric fragmentation, suggesting that these two groups have had more recent gene flow with each other than either have with var. *venusta*; therefore, our data suggests that populations from the Santa Lucia Mountains should be grouped with var. *humilis*.

TCS indicated that the ancestral haplotype for all three networks was from northern California, although this finding can be biased because it is based on the premise that the

ancestral haplotype is one that is most abundant. This assumption makes it susceptible to sampling biases, if one area is over-sampled or has fewer mutations within the geographic area compared to other groups. We attempted to sample evenly among the three varieties, but the unexpected placement of the two sites of var. *venusta* from the Santa Lucia Mountains might have biased the assumption of the ancestral haplotype to northern California.

Populations of var. *venusta* from the San Gabriel and Inyo Mountains consistently represented a group distinct from any other region. We did not find compelling genetic evidence supporting the placement of populations from the Inyo Mountains within var. *jonesii*. Within var. *venusta*, the haplotypes for populations in the Inyo Mountains were unique from those from in the San Gabriel Mountains in all five loci. NCPA inferred in both ITS and the chloroplast data that the separation between these two mountain ranges was due to allopatric fragmentation. This suggests that populations from the Inyo Mountains have had more recent gene flow with populations from the San Gabriel Mountains than it has with any other group of populations.

In taxonomic descriptions, it has been observed that populations in the Inyo Mountains have morphological differences from the other var. *venusta* populations (Rosatti 2008; http://ucjeps.berkeley.edu/tjm2/review/treatments/apocynaceae.html#21617). Our review of morphology confirms that the mean length of inner and outer corolla hair in individuals from the Inyo Mountains was significantly different from southern California var. *venusta*. Compared to the populations on the Colorado Plateau, the mean length of inner and outer corolla hairs between the Inyo Mountains and Arizona was not significantly different. Between the Inyo Mountains and Utah, the mean length of the inner hair was significantly different but was not for the outer hair. Generally speaking, populations in the Inyo Mountains share more similarities in length of corolla hair with Utah and Arizona populations, although the range of variation within

groups makes these characters difficult to use quantitatively or taxonomically. Length of the petioles has been the key morphological character used in indentification keys to distinguish between var. *venusta* and var. *jonesii* (Cronquist 1984). Our analysis of morphology showed that the mean length of the petioles among most groups was significantly different, but as with corolla hair length, the range of variation of petiole length within groups makes this character difficult to use quantitatively or taxonomically. In both morphological and genetic data, populations from the Inyo Mountains tended to be intermediate between southern California and the Colorado Plateau populations, but the genetic data indicates that the Inyo Mountains have had more recent interaction with var. *venusta* of southern California. Therefore, our data suggests that populations from the Inyo Mountains should be maintained within var. *venusta*.

Populations of var. *jonesii*, from the Colorado Plateau, consistently represented a group distinct from other groups. The pattern leading to the current distribution among populations of var. *jonesii* seems to be the result of restricted gene flow with some long distance dispersal. The single population from northern Arizona (site 25) grouped with the other var. *jonesii* but was always represented by its own unique haplotype that was usually intermediate between the northern California and Colorado Plateau groupings. All plants examined in this population have a tuft of approximately 10– 20 hairs on the apex of each leaf, a character not observed in any other population throughout the species range. Although of questionable adaptive significance, this morphologic character further supports the genetic distinctness of this population from other population of var. *jonesii*.

When first discovered, populations of var. *jonesii*, were classified as a species and later were reduced to a variety. Our results support this subspecific ranking in that the populations from the Colorado Plateau were generally only separated by California *Cycladenia* by a few

mutational steps. Additionally, the overlap of variation in the morphological data did not give strong indications that this group is significantly distinct from other California *C. humilis* populations. Therefore, our data supports the circumscription of var. *jonesii* encompassing all populations of *C. humilis* on the Colorado Plateau, retaining *Cycladenia* as a unispecific genus.

Relationships among the three varieties remain somewhat uncertain in the chloroplast and nuclear data sets. Within a species, this pattern is common. Varieties represent an early stage of divergence and it is not unexpected that character differences, such as the morphological features we quantified, have not yet become completely fixed. Some of the uncertainties among relationships with *C. humilis* might also be due to outgroup rooting artifacts. Livshultz et al.'s (2007) analysis placed *Cycladenia* in the "New World Clade" of Apocynoideae and suggested *Trachelospermum difforme* (Walter) A. Gray from the southeastern United States and *Pinochia* (M. E. Endress & B. F. Hansen), a small genus from Central America and the Caribbean, were the most likely sister taxa of *C. humilis*. Geographically, genetically, and morphologically, these two genera are very different from *Cycladenia* despite the evidence for shared recent common ancestory exclusive of other genera. The placement of the root on any of the population trees (Figs. 2–4) are thus suspect in that the outgroup has attached along the longest internal branch in all cases, which may reflect long branch attraction rather than an indication of ancestral condition.

Distribution and Habitat Indicators—*Cycladenia* is an uncommon plant throughout its entire range. However, it is possible that it is somewhat more common than our present understanding suggests due to the extreme and isolated terrain it typically inhabits accompanied by its tendency to occur in small, localized populations. At present, nearly all the known sites of *Cycladenia* are within short hiking distance of dirt roads, trails, in National Parks, State Parks, or

around areas with human activity, making our knowledge of its distribution somewhat restricted to circumstantial discovery. Additional populations may well exist in suitable, but less accessible and as yet unexplored areas.

Illustrating the elusive nature of *C. humilis*, a single authenticated collection has been made in the southern coastal range of Venture Co., California. The specimen was collected from a gravel bank of a river, and the collector speculated that it was possible waif. Botanists have subsequently searched the collection area with no success in finding *C. humilis*, and this occurrence is speculated to have possibly originated from a secluded site upstream that remains undiscovered (D. Wilken, Santa Barbara Botanic Gardens, pers. comm.). It is highly probable that more sites, most likely secluded, small populations, and extremely fragmented, exist in the southern Coastal range as well as in other areas of *Cycladenia* distribution. For example, it would not be surprising to find that *Cycladenia* extends into southern Oregon in the High Cascades and the Klamath Mountains in locations that are rarely accessed by humans because of the harsh terrain with fewer roads and trails.

Some plants with unique distributions similar to *Cycladenia* are known to be exclusive to certain soil types derived from specific geologic formations. For example, several species are known to be obligates of soil derived from serpentinite. The distribution of *Cycladenia* within California seems to be as diverse as California's geologic past. On one extreme, *Cycladenia* can be found growing on relatively recent volcanic rock in the Modoc Plateau and High Cascades Range, which contrast sharply with the uplifted Paleozoic Marine sedimentary rocks of the Inyo Mountains. We found that soil consistency, not necessarily soil composition, was a more reliable indicator of suitable growth habitat for *Cycladenia* in California. In all sites we visited, *Cycladenia* grows in well drained mediums such as talus, sandy or gravely soils that are

generally more favorable to drought tolerant species. They also prefer areas where competition for light is low, such as summits, sparse Chaparral, and very steep slopes. Because *Cycladenia* also grows at high elevations throughout its entire distribution, snowfall is common during the winter months.

The habitat that *Cycladenia* inhabits in the Colorado Plateau is strikingly different from those in California. Based on the typical habitat patterns found in California, it would seem more intuitive to find *Cycladenia* inhabiting the talus slopes of the Wasatch Mountains, than it would the mesa slopes of the Colorado Plateau. It has been suggested that var. *jonesii* is an obligate gypsophile and restricted to soils derived from Cutler, Summerville, and Chinle formations (Sipes et al. 1994; Welsh et al. 2003); however, this claim was based on a very few observed sites and perhaps a very general review of geology. In our review of geology, we additionally found that var. *jonesii* can be found in soils derived from the Wasatch Formation, Glenn Canyon group, and intrusive rock from the La Sal Mountains. It is also important to note that var. *jonesii* grows on steep slopes on the bases of mesas, where the soils are generally composites of the several formations. Taking into consideration that *Cycladenia* in California are geologic generalist, and the high probability that more undiscovered sites of var. *jonesii,* exist and it is unknown what formations they might be found, we are uncertain that var. *jonesii* is restricted to specific geologic formations. No studies have formally examined *Cycladenia*'s relationships to soil chemistry, but we again observed that soil consistency, usually sandy soils often intermixed with colluvium, seemed to be informative indicators for suitable var. *jonesii* habitat. But note that 'suitable' does not equate with 'presence.' We explored several suitable sites where *Cycladenia* did not occur, which likely reflects dispersal ability and establishment factors for this species that remain poorly understood.

Conservation Implications—When var. *jonesii* was federally listed as a threatened species over 20 years ago, it was only know from three general areas on the Colorado Plateau. The small number of known sites coupled with concerns about the potential impacts of mineral, oil, and gas exploration, and off-road vehicles, were the main reasons for listing it as threatened. Since the time that var. *jonesii* was initially listed, several more populations of have been discovered, including from a single general location in Arizona. The majority of known var. *jonesii* populations inhabit extreme terrain inaccessible to motorists and inconvenient for industrial activities because they are only accessible by strenuous foot journeys. For the few sites that are at risk from off road vehicles and industrial exploration, it is unknown how resilient *Cycladenia* is to such disturbances. Claims have been made that *Cycladenia* in Utah survived disturbances causes by past seismic operations (Welsh et al. 2003), but empirical studies exploring the resilience of *Cycladenia* to these kinds of disturbances are lacking. Two sites we sampled in California were on mountain summits were radio towers had been constructed. One site had a relatively large population and seemed to be unaffected. The other site's population was relativity small. While it is impossible to gauge the impact that construction had at either site, it is interesting to note that a few plants at the site with the smaller population showed signs of resilience by growing adjacent to or out from under concrete slabs. Although it is not clear how resilient *Cycladenia* is to disturbances on the Colorado Plateau, it is likely that in areas where human disturbance is a concern, precautions such as designated off road trails and detour roads from existing *Cycladenia* populations will help reduce the possible impact on this rare plant. Because the known sites of *Cycladenia* on the Colorado Plateau have increased since its original listing and the genetic variation among these populations is as great or greater than that

observed within either of the California varieties, this new understanding should be taken into consideration when recovery plans are listing status are re-evaluated for this species.

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TABLES

TABLE 1. Outcome of geographic and genetic structure in Geodis 2.6 (Posada et al. 2000) for the combined chloroplast data set. Only clades with significant associations were included. The path and results of the NCPA inference key (Templeton, 2008) are shown along with list of associated localities with each clade. AF=allopatric fragmentation. GF=gene flow. LDD=long distance dispersal. RGF=restricted gene flow.

Locus	Clade	χ^2	P-value	Inference	Localities
Chloro	1.7	128.0000	<0.0001	1-19-20-NO, Inadequate Geographic Sampling	3, 4, 8, 12, 18, 19,–11–16
	1.33	16.0000	0.0001	1-19-NO, AF	21–24
	1.34	16.0000	0.0001	1-19-NO, AF	14–15
	1.35	24.0000	<0.0001	1-19-NO, AF	7, 22–6
	2.2	21.0000	<0.0001	1-19-20-NO, Inadequate Geographic Sampling	1, 2–13
	2.17	112.0000	<0.0001	1-19-20-2-3-5-6-7-YES, RGF some LDD	21, 24–14, 15–6, 7, 22
	3.1	37.0000	<0.0001	1-19-NO, AF	1, 2, 13–5, 17
	3.2	176.0000	<0.0001	1-19-20-NO, Inadequate Geographic Sampling	3, 4, 8, 12, 11, 16 18, 19,–20–9, 10
	3.6	64.0000	<0.0001	1-19-20-NO, Inadequate Geographic Sampling	6, 7,14,15, 21, 22, 24–23
	4.1	125.0000	<0.0001	1-19-NO, AF	1, 2, 5, 13, 17–3, 4, 8, 9, 10, 11, 12, 16, 18, 19,20
	4.2	15.0000	0.0001	1-19-20-2-3-5-6-7- YES, RGF some LDD	25–26
	Total	408.0000	<0.0001	1-19-20-2-3-5-6-7-8- YES, RGF/ dispersal some LDD over intermediate areas not occupied OR Past GF followed by extinction of intermediate haplotypes	1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19, 20–25, 26–6, 7,14,15, 21, 22, 23, 24

TABLE 2. Outcome of geographic and genetic structure in Geodis 2.6 (Posada et al. 2000) for the nuclear data sets. Only clades with significant associations were included. The path and results of the NCPA inference key (Templeton, 2008) are shown along with list of associated localities with each clade. AF=allopatric fragmentation. GF=gene flow. IBD=isolation by distance. LDC=long distance colonization. LDD=long distance dispersal. PF=past fragmentation. RGF=restricted gene flow.

Locus	Clade	χ^2	P-value	Inference	Localities
ITS	1.1	98.5367	<0.0001	1-2-11-17-NO, Inconclusive Outcome	8, 9, 10, 11, 12, 16, 18, 19, 20–9–8, 9, 16–10, 24–11–8
	1.13	7.2356	0.0378	1-2-11-17-NO, Inconclusive Outcome	1, 2, 13–1, 2
	1.19	12.0000	0.0025	1-2-3-5-6-7-8-YES, RGF/ dispersal some LDD over intermediate areas not occupied OR Past GF followed by extinction of intermediate haplotypes	21–21–26
	2.1	49.0000	<0.0001	1-19-NO, AF	3,4–8, 18, 19
	2.2	19.1381	0.0111	1-2, Inconclusive Outcome	8, 9, 10, 11, 12, 16, 18, 19, 20, 24–8, 9, 11, 12, 18
	2.10	5.7143	0.0396	1-2-3-4-NO, RGF with IBD	21, 26–26
	3.1	114.3082	<0.0001	1-2-3-5-6-7-8- Sampling Inadequate	3, 4, 8, 18, 19–8, 9, 10, 11, 12, 16, 18, 19, 20, 24
	3.3	74.0000	<0.0001	1-19-NO, AF	5, 17–1, 2, 13
	3.5	26.0000	<0.0001	1-19-NO, AF	23–21, 26
	4.1	182.0000	<0.0001	1-19-20-2-3-5-15-NO, PF or LDC	3, 4, 8, 9, 10, 11, 12, 16, 18, 19, 20, 24–25
	4.2	355.4393	<0.0001	1-2-3-6-7-YES, RGF some LDD	1, 2, 5, 13, 17–6, 7, 14, 15, 21, 22, 23, 24, 26
	Total	396.0000	<0.0001	1-19-NO, AF	3, 4, 8, 9, 10, 11, 12, 16, 18, 19, 20, 24, 25–1, 2, 5, 6, 7, 13, 14, 15, 17, 21, 22, 23, 24, 26
ETS	1.1	57.3798	<0.0001	1-2-3-5-6-7-8-YES, RGF/ dispersal some LDD over intermediate areas not occupied OR Past GF followed by extinction of intermediate haplotypes	8, 9, 10, 11, 12, 16, 18, 19, 20–8, 9, 18, 19–18
	1.14	82.6548	<0.0001	1-2, Inconclusive Outcome	25–23, 21–6, 7, 14, 21, 22, 23, 24
	1.16	65.6297	<0.0001	1-2-3-5-6-7-8-YES, RGF/ dispersal some LDD over intermediate areas not occupied OR Past GF followed by extinction of intermediate haplotypes	6, 7, 14, 15, 21, 22, 23, 24–6, 7, 22–15–7
	2.7	183.0229	<0.0001	1-2-3-5-6-7-8-YES, RGF/ dispersal some LDD over intermediate areas not occupied OR Past GF followed by extinction of intermediate haplotypes	6, 7, 14, 21, 22, 23, 24, 25–6, 7, 14, 15, 21, 22, 23, 24–21, 26
	3.1	176.0000	<0.0001	1-19-NO, AF	8, 9, 10, 11, 12, 16, 18, 19, 20–3, 4
	3.3	190.0000	<0.0001	1-19-NO, AF	6, 7, 14, 15, 21, 22, 23, 24, 25, 26–5, 17
	Total	816.0000	<0.0001	1-19-NO, AF	3, 4, 8, 9, 10, 11, 12, 16, 18, 19, 20–6, 5, 7, 14, 15, 17, 21, 22, 23, 24, 25, 26–1, 2, 13

TABLE 3. Pair-wise t-tests with Bonferroni-Dunn correction applied for multiple comparisons for the inter hairs (trichomes) by geographic region, where $\alpha = 0.05$ and the adjusted $\alpha = 0.0024$. Measurements from northern California distinguished as glabrous (g) or tomentose (t).

Groups	Difference	Statistic	P	Significant
Santa Lucia vs. S. California	-32.967	1.021	0.308	No
Santa Lucia vs. Inyo	-335.879	12.250	< 0.001	Yes
Santa Lucia vs. Utah	-228.483	6.879	< 0.001	Yes
Santa Lucia vs. Arizona	-311.136	12.686	< 0.001	Yes
Santa Lucia vs. N. California-g	-9.981	0.454	> 0.5	No
Santa Lucia vs. N. California-t	8.904	0.329	> 0.5	No
S. California vs. Inyo	-302.912	8.825	< 0.001	Yes
S. California vs. Utah	-195.516	4.999	< 0.001	Yes
S. California vs. Arizona	-278.169	8.676	< 0.001	Yes
S. California vs. N. California-g	22.986	0.762	0.446	No
S. California vs. N. California-g	41.870	1.230	0.220	No
Inyo vs. Utah	107.396	3.051	0.003	No
Inyo vs. Arizona	24.743	0.911	0.363	No
Inyo vs. N. California-g	325.898	13.098	< 0.001	Yes
Inyo vs. N. California-t	344.782	11.702	< 0.001	Yes
Utah vs. Arizona	-82.653	2.505	0.013	No
Utah vs. N. California-g	218.502	7.014	< 0.001	Yes
Utah vs. N. California-t	237.386	6.798	< 0.001	Yes
Arizona vs. N. California-g	301.155	13.909	< 0.001	Yes
Arizona vs. N. California-t	320.039	11.945	< 0.001	Yes
N. California-g vs. N. California-t	18.884	0.771	0.441	No

TABLE 4. Pair-wise t-tests with Bonferroni-Dunn correction applied for multiple comparisons for outer hairs (trichomes) by geographic region, where $\alpha = 0.05$ and the adjusted $\alpha = 0.0033$. All measurements from northern California are from tomentose (t) plants.

Groups	Difference	Statistic	P	Significant
Santa Lucia vs. S. California	35.604	0.954	0.341	No
Santa Lucia vs. Inyo	-127.693	2.486	0.014	No
Santa Lucia vs. Utah	82.109	1.494	0.136	No
Santa Lucia vs. Arizona	-154.008	4.127	< 0.001	Yes
Santa Lucia vs. N. California-t	180.836	5.572	< 0.001	Yes
S. California vs. Inyo	-163.298	3.074	0.002	Yes
S. California vs. Utah	46.505	0.822	0.412	No
S. California vs. Arizona	-189.612	4.774	< 0.001	Yes
S. California vs. N. California-t	145.232	4.128	< 0.001	Yes
Inyo vs. Utah	209.803	3.145	0.002	Yes
Inyo vs. Arizona	-26.315	0.495	> 0.5	No
Inyo vs. N. California-t	308.529	6.191	< 0.001	Yes
Utah vs. Arizona	-236.117	4.171	< 0.001	Yes
Utah vs. N. California-t	98.727	1.845	0.066	No
Arizona vs. N. California-t	334.844	9.517	< 0.001	Yes

TABLE 5. Pair-wise t-tests with Bonferroni-Dunn correction applied for multiple comparisons for petiole length by geographic region, where $\alpha = 0.05$ and the adjusted $\alpha = 0.0033$.

Groups	Difference	Statistic	P	Significant
N. California vs. Santa Lucia	-5.644	3.518	< 0.001	Yes
N. California vs. S. California	3.313	2.645	0.008	No
N. California vs. Inyo	8.495	7.879	< 0.001	Yes
N. California vs. Utah	12.321	12.374	< 0.001	Yes
N. California vs. Arizona	14.554	14.486	< 0.001	Yes
Santa Lucia vs. S. California	8.957	4.630	< 0.001	Yes
Santa Lucia vs. Inyo	14.139	7.742	< 0.001	Yes
Santa Lucia vs. Utah	17.965	10.100	< 0.001	Yes
Santa Lucia vs. Arizona	20.198	11.323	< 0.001	Yes
S. California vs. Inyo	5.182	3.394	< 0.001	Yes
S. California vs. Utah	9.008	6.129	< 0.001	Yes
S. California vs. Arizona	11.241	7.617	< 0.001	Yes
Inyo vs. Utah	3.826	2.890	0.004	No
Inyo vs. Arizona	6.059	4.553	< 0.001	Yes
Utah vs. Arizona	2.233	1.766	0.078	No

APPENDIX 1. Collection and Voucher information, presented in the following order: Site number (number of individuals sampled), locality, collection date, *collectors and collection number*. All vouchers deposited at BRY.

Site 1 (7), California, San Bernardino Co., Devils backbone near Mt. San Antonio, San Gabriel Mts., 09 June 2007, *M. Last & L. Chan ml-001*. **Site 2.** (6), California, Los Angeles Co., Mt. Disappointment, San Gabriel Mts., June 11, 2007, *M. Last & L. Chan ml-002*. **Site 3**. (8), California, Monterey Co., Junipero Serra, Santa Lucia Mts., June 12, 2007, Last, *M. Last & L. Chan ml-003*. **Site 4.** (8), California, Monterey Co., Near Cone Peak, Santa Lucia Mts., June 12, 2007, *M. Last & L. Chan ml-004*. **Site 5**. ml-005 (8), California, Inyo Co., Cerro Gordo Springs area, Inyo Mts., June 14, 2007, *M. Last & L. Chan ml-005*. **Site 6**. (8), Utah, Garfield Co., Purple hills, Glenn Canyon Recreation Area, June 20, 2007, *J. M. Spence, M. Last and L. Johnson. ml-006*. **Site 7**. (8), Utah, Garfield Co., Horse Pasture Mesa area, Glenn Canyon Recreation Area, June 21, 2007, *J. M. Spence, M. Last and L. Johnson. ml-007*. **Site 8**. (8), California, Glenn Co., Noel Springs, northern Coastal Range, July 13, 2007, *M. Last and A. Maas ml-009*. **Site 9**. (8), California, Butte Co., Bottle Hill, Sierra Nevada, July 16, 2007, *M. Last and A. Maas ml-010*. **Site 10**. (8), California, Tehama Co., Guernsey Camp, Sierra Nevada, July 16, 2007, *M. Last and A. Maas ml-011*. **Site 11**. (8), California, Siskiyou Co., Black Butte, High Cascades, July 17, 2007, *M. Last and A. Maas ml-012*. **Site 12**. (8), California, Siskiyou Co., Jot Dean Ice Cave, July 18, 2007, *M. Last and A. Maas ml-013*. **Site 13**. (8), California, San Bernardino Co., Mt. San Antonio, San Gabriel Mts., July 20, 2007, *M. Last and A. Maas ml-014*. **Site 14**. ml018 (8), Utah, Garfield Co., Moody Canyon, Glenn Canyon Recreation Area, June 17, 2008, *J. M. Spence, M. Last, N. Laitinen, and D. Kunakeva ml-018*. **Site 15**. (8), Utah, Garfield Co., Moody Canyon, Glenn Canyon Recreation Area, June 17, 2008, *J. M. Spence, M. Last, N. Laitinen, L and D. Kunakeva ml-019*. **Site 16.** (8), California, Siskiyou Co., Caldwell Butte, Modoc Plateau, June 24, 2008, *M. Last and T. Taylor ml-020*. **Site 17**. (8), California, Inyo Co., Seep Hole Spring, Inyo Mts., June 26, 2008, *M. Last and T. Taylor ml-021*. **Site 18**. (8), California, Humboldt Co., Devils backbone -Salmon Mts., Klamath Mountains, July 5, 2008, *M. Last, R. Last and T. Taylor ml-022*. **Site 19**. (8), California, Lake Co., Cobb Mt., Sierra Nevada, July 14, 2008, *M. Last and T. Taylor ml-023*. **Site 20**. (8), California, Sierra Co., Stanford Mt., Sierra Nevada, July 14, 2008, *M. Last and T. Taylor ml-024*. **Site 21**. Utah, Grand Co., Onion Creek, 28 June 2007, *L. Chan lmc-001*. **Site 22**. Utah, Garfield Co., Choprock Bench, 21 June 2007, *L. Johnson, M. Last, and J. Spence 07-023*. **Site 23**. Utah, Grand Co., Castle Valley, 21 May 2007, *L. Johnson & L. Chan 07-016*. **Site 24**. (8), Utah, Emery Co., San Rafael Reef, 15 May 2007, *L. Johnson & H. Barnes 07-011*. **Site 25**. (8) Arizona, Mohave Co., Vermillion Cliffs, 21 May 2008, *L. Johnson & C. Zanotti 08-018*. **Site 26**. (7), Utah, Grand Co., Joe Hutch Canyon area, 26 July 2008, *N. D. Atwood 32467*. **Out Group**: North Carolina, Jonston Co. 2008. *A. Krings 2227, 2232, 2233, 2255*.

APPENDIX 2. List of individuals in each haplotype for the combined Chloroplast data.

Haplotype 1: ch03.1, ch03.2, ch03.3, ch03.4, ch03.5, ch03.6, ch03.7, ch03.8, ch04.1, ch04.2, ch04.3, ch04.4, ch04.5, ch04.6, ch04.7, ch04.8, ch08.1, ch08.2, ch08.3, ch08.4, ch08.5, ch08.6, ch08.7, ch08.8, ch12.1, ch12.2, ch12.3, ch12.4, ch12.5, ch12.6, ch12.7, ch12.8, ch18.1, ch18.2, ch18.3, ch18.4, ch18.5, ch18.6, ch18.7, ch18.8, ch19.1, ch19.2, ch19.3, ch19.4, ch19.5, ch19.6, ch19.7, ch19.8 **Haplotype 2:** ch11.1, ch11.2, ch11.3, ch11.4, ch11.5, ch11.6, ch11.7, ch11.8 **Haplotype 3:** ch16.1, ch16.2, ch16.3, ch16.4, ch16.5, ch16.6, ch16.7, ch16.8 **Haplotype 4:** ch20.1, ch20.2, ch20.3, ch20.4, ch20.5, ch20.6, ch20.7, ch20.8 **Haplotype 5:** ch01.1, ch01.2, ch01.3, ch01.4, ch01.5, ch01.6, ch01.7, ch02.1, ch02.2, ch02.3, ch02.4, ch02.5, ch02.6 **Haplotype 6:** ch13.1, ch13.2, ch13.3, ch13.4, ch13.5, ch13.6, ch13.7, ch13.8 **Haplotype 7:** ch05.1, ch05.2, ch05.3, ch05.4, ch05.5, ch05.6, ch05.7, ch05.8, ch17.1, ch17.2, ch17.3, ch17.4, ch17.5, ch17.6, ch17.7, ch17.8 **Haplotype 8:** ch09.1, ch09.5, ch09.6, ch09.7, ch10.1, ch10.2, ch10.3, ch10.4, ch10.5, ch10.6, ch10.7, ch10.8 **Haplotype 9:** ch09.2 **Haplotype 10:** ch09.3, ch09.4, ch09.8 **Haplotype 11:** ch25.1, ch25.2, ch25.3, ch25.4, ch25.5, ch25.6, ch25.7, ch25.8 **Haplotype 12:** ch26.1, ch26.2, ch26.3, ch26.4, ch26.5, ch26.6, ch26.7 **Haplotype 13:** ch23.1 **Haplotype 14:** ch23.2, ch23.3, ch23.4, ch23.5, ch23.6, ch23.7, ch23.8 **Haplotype 15:** ch21.1, ch21.2, ch21.3, ch21.4, ch21.5, ch21.6, ch21.7, ch21.8 **Haplotype 16:** ch24.1, ch24.2, ch24.3, ch24.4, ch24.5, ch24.6, ch24.7, ch24.8 **Haplotype 17:** ch07.1, ch07.2, ch07.3, ch07.4, ch07.5, ch07.6, ch07.7, ch07.8, ch22.1, ch22.2, ch22.3, ch22.4, ch22.5, ch22.6, ch22.7, ch22.8 **Haplotype 18:** ch06.1, ch06.2, ch06.3, ch06.4, ch06.5, ch06.6, ch06.7, ch06.8 **Haplotype 19:** ch14.1, ch14.2, ch14.3, ch14.4, ch14.5, ch14.6, ch14.7, ch14.8 **Haplotype 20:** ch15.1, ch15.2, ch15.3, ch15.4, ch15.5, ch15.6, ch15.7, ch15.8

APPENDIX 3. List of individuals in each haplotype for ITS data set.

Haplotype 1: ch08.2, ch08.5, ch09.1, ch09.2, ch09.2, ch09.3, ch09.3, ch09.5, ch09.6, ch09.7, ch09.7, ch09.8, ch10.1, ch10.1, ch10.2, ch10.2, ch10.3, ch10.3, ch10.4, ch10.4, ch10.5, ch10.5, ch10.6,ch10.6, ch10.7, ch10.8, ch11.1, ch11.3, ch11.4, ch11.5, ch11.6, ch11.8, ch12.1, ch12.1, ch12.2, ch12.2, ch12.3, ch12.3, ch12.4, ch12.4, ch12.5, ch12.6, ch12.6, ch12.7, ch12.8, ch16.1, ch16.1, ch16.2, ch16.2, ch16.3,ch16.4, ch16.4, ch16.5, ch16.5, ch16.6, ch16.6, ch16.7, ch16.8, ch16.8, ch18.2, ch18.3, ch18.4, ch18.4, ch18.5, ch18.5, ch18.6, ch18.7, ch18.7, ch18.8, ch19.1, ch19.2, ch19.3, ch19.3, ch19.4, ch19.4, ch19.5, ch19.6, ch19.7, ch19.8, ch20.1, ch20.4, ch20.4, ch20.5, ch20.5, ch20.6, ch20.6 **Haplotype 2:** ch03.1, ch03.1, ch03.2, ch03.2, ch03.3, ch03.3, ch03.4, ch03.4, ch03.5, ch03.5, ch03.6, ch03.6, ch03.7, ch03.7, ch03.8, ch03.8, ch04.1, ch04.1, ch04.2, ch04.2, ch04.3, ch04.3, ch04.4, ch04.4, ch04.5, ch04.5, ch04.6, ch04.6, ch04.7, ch04.7, ch04.8, ch04.8 **Haplotype 3:** ch08.1, ch08.2, ch08.3, ch08.6, ch08.7, ch18.1, ch18.2, ch18.3, ch19.1, ch19.2, ch19.5, ch19.6, ch19.7 **Haplotype 4:** ch08.6 **Haplotype 5:** ch09.8 **Haplotype 6:** ch08.1, ch08.4, ch08.7, ch08.8, ch16.3, ch09.5, ch16.7 **Haplotype 7:** ch10.7, ch10.8, ch20.1 **Haplotype 8:** ch11.2, ch11.3, ch11.6 **Haplotype 9:** ch08.3 **Haplotype 10:** ch08.4, ch08.8, ch08.5, ch09.1, ch09.6, ch11.1, ch11.2, ch11.4, ch11.5, ch11.7, ch11.7, ch11.8, ch12.5, ch12.7, ch12.8, ch18.1, ch18.6, ch18.8, ch19.8 **Haplotype 11:** ch25.1, ch25.1, ch25.2, ch25.3, ch25.3, ch25.4, ch25.4, ch25.5, ch25.5, ch25.6, ch25.6, ch25.7, ch25.7, ch25.8, ch25.8 **Haplotype 12:** ch25.2 **Haplotype 13:** ch05.1, ch05.1, ch05.2, ch05.2, ch05.3, ch05.3, ch05.4, ch05.4, ch05.5, ch05.5, ch05.6,ch05.6, ch05.7, ch05.7, ch05.8, ch05.8, ch17.1, ch17.1, ch17.2, ch17.2, ch17.3, ch17.3, ch17.4, ch17.4, ch17.5, ch17.5, ch17.6, ch17.6, ch17.7, ch17.7, ch17.8, ch17.8 **Haplotype 14:** ch01.1, ch01.2, ch01.3, ch01.4, ch01.5, ch01.5, ch01.6, ch01.7, ch02.1, ch02.3, ch02.4, ch02.5, ch02.6, ch13.1, ch13.2, ch13.3, ch13.3, ch13.4, ch13.5, ch13.5, ch13.6, ch13.6, ch13.7, ch13.7, ch13.8, ch13.8 **Haplotype 15:** ch01.1, ch01.2, ch01.3, ch01.4, ch01.6, ch01.7, ch02.2, ch02.3, ch02.6 **Haplotype 16:** ch02.1, ch02.2, ch02.4, ch02.5, ch13.1, ch13.2, ch13.4 **Haplotype 17:** ch06.1, ch06.1, ch06.2, ch06.2, ch06.3, ch06.3, ch06.4, ch06.4, ch06.5, ch06.5, ch06.6, ch06.6, ch06.7, ch06.7, ch06.8, ch06.8, ch07.1, ch07.1, ch07.2, ch07.2, ch07.3, ch07.3, ch07.4, ch07.4, ch07.5, ch07.5, ch07.6, ch07.6, ch07.7, ch07.7, ch07.8, ch07.8, ch14.1, ch14.1, ch14.2, ch14.2, ch14.3, ch14.3, ch14.4, ch14.4, ch14.5, ch14.5, ch14.6, ch14.6, ch14.7, ch14.7, ch14.8, ch14.8,ch15.1, ch15.1, ch15.2, ch15.2, ch15.3, ch15.3, ch15.4, ch15.4, ch15.5, ch15.5, ch15.6, ch15.6, ch15.7, ch15.7, ch15.8, ch15.8, ch24.1, ch24.2, ch24.3, ch24.4, ch24.5, ch24.6, ch24.7, ch24.8, ch24.1, ch24.2, ch24.3, ch24.4, ch24.5, ch24.6, ch24.7, ch23.1, ch23.1, ch23.2, ch23.3, ch23.3, ch23.4, ch23.5, ch23.6, ch23.7, ch23.8, ch22.1, ch22.1, ch22.2, ch22.2, ch22.3, ch22.3, ch22.4, ch22.4, ch22.5, ch22.5, ch22.6, ch22.6, ch22.7, ch22.7, ch22.8, ch22.8, ch21.1, ch21.2, ch21.2, ch21.4, ch21.6, ch21.7, ch21.8 **Haplotype 18:** ch24.8 **Haplotype 19:** ch21.4 **Haplotype 20:** ch23.5, ch23.8 **Haplotype 21:** ch23.2, ch23.4, ch23.6, ch23.7 **Haplotype 22:** ch21.5, ch21.5, ch21.6, ch21.7, ch21.8 **Haplotype 23:** ch21.1 **Haplotype 24:** ch26.1, ch26.2, ch26.3, ch26.4, ch26.6, ch26.7 **Haplotype 25:** ch26.5, ch26.5 **Haplotype 26:** ch26.1, ch26.2, ch26.3, ch26.4, ch26.6, ch26.7

APPENDIX 4. List of individuals in each haplotype for ETS data set.

Haplotype 1: ch08.1, ch08.3, ch08.4, ch08.4, ch08.5, ch08.6, ch08.7, ch08.8, ch08.8, ch09.1, ch09.1, ch09.2, ch09.2, ch09.3, ch09.3, ch09.4, ch09.4, ch09.5, ch09.5, ch09.6, ch09.6, ch09.7, ch09.7, ch09.8, ch09.8, ch10.1, ch10.1, ch10.2, ch10.2, ch10.3, ch10.3, ch10.4, ch10.4, ch10.5, ch10.5, ch10.6, ch10.6, ch10.7, ch10.7, ch10.8, ch10.8, ch11.1, ch11.1, ch11.2, ch11.2, ch11.3, ch11.3, ch11.4, ch11.4, ch11.5, ch11.5, ch11.6, ch11.6, ch11.7, ch11.7, ch11.8, ch11.8, ch12.1, ch12.1, ch12.2, ch12.2, ch12.3, ch12.3, ch12.4, ch12.4, ch12.5, ch12.5, ch12.6, ch12.6, ch12.7, ch12.7, ch12.8, ch12.8, ch16.1, ch16.1, ch16.2, ch16.2, ch16.3, ch16.3, ch16.4, ch16.4, ch16.5, ch16.5, ch16.6, ch16.6, ch16.7, ch16.7, ch16.8, ch16.8, ch18.2, ch18.3, ch18.4, ch18.4, ch18.5, ch18.5, ch18.6, ch18.7, ch18.8, ch19.1, ch19.2, ch19.3, ch19.3, ch19.4, ch19.4, ch19.5, ch19.6, ch19.7, ch19.8, ch19.8, ch20.1, ch20.1, ch20.2, ch20.2, ch20.3, ch20.3, ch20.4, ch20.4, ch20.5, ch20.5, ch20.6, ch20.6, ch20.7, ch20.7, ch20.8, ch20.8 **Haplotype 2:** ch08.1, ch08.2, ch08.2, ch08.3, ch08.5, ch08.6, ch08.7, ch18.1, ch18.2, ch18.3, ch18.7, ch18.8, ch19.1, ch19.2, ch19.5, ch19.6, ch19.7 **Haplotype 3:** ch18.1, ch18.6 **Haplotype 4:** ch03.1, ch03.1, ch03.2, ch03.2, ch03.3, ch03.3, ch03.4, ch03.4, ch03.5, ch03.5, ch03.6, ch03.6, ch03.7, ch03.7, ch03.8, ch03.8, ch04.1, ch04.1, ch04.2, ch04.3, ch04.5, ch04.5, ch04.6, ch04.6, ch04.7, ch04.7, ch04.8, ch04.8 **Haplotype 5:** ch04.3, ch04.4 **Haplotype 6:** ch04.2, ch04.4 **Haplotype 7:** ch01.1, ch01.1, ch01.2, ch01.2, ch01.3, ch01.3, ch01.4, ch01.4, ch01.5, ch01.5, ch01.6, ch01.6, ch01.7, ch01.7, ch02.1, ch02.1, ch02.2, ch02.2, ch02.3, ch02.3, ch02.4, ch02.4, ch02.5, ch02.5, ch02.6, ch02.6, ch13.1, ch13.1, ch13.2, ch13.2, ch13.3, ch13.3, ch13.4, ch13.4, ch13.5, ch13.5, ch13.6, ch13.6, ch13.7, ch13.7, ch13.8, ch13.8 **Haplotype 8:** ch05.1, ch05.1, ch05.2, ch05.2, ch05.3, ch05.3, ch05.4, ch05.4, ch05.5, ch05.5, ch05.6, ch05.6, ch05.7, ch05.7, ch05.8, ch05.8, ch17.1, ch17.1, ch17.2, ch17.2, ch17.3, ch17.3, ch17.4, ch17.4, ch17.5, ch17.5, ch17.6, ch17.6, ch17.7, ch17.7, ch17.8, ch17.8 **Haplotype 9:** ch25.1, ch25.1, ch25.2, ch25.2, ch25.3, ch25.3, ch25.4, ch25.4, ch25.5, ch25.5, ch25.6, ch25.6, ch25.7, ch25.7, ch25.8, ch25.8 **Haplotype 10:** ch23.2, ch23.4, ch23.5, ch23.6, ch23.7, ch23.8, ch21.4 **Haplotype 11:** ch21.1, ch21.3, ch21.5, ch21.5, ch21.6, ch21.6, ch21.7, ch21.8, ch26.1, ch26.2, ch26.2, ch26.3, ch26.3, ch26.4, ch26.4, ch26.5, ch26.5, ch26.6, ch26.6, ch26.7, ch26.7 **Haplotype 12:** ch26.1 **Haplotype 13:** ch06.1, ch06.2, ch06.3, ch06.4, ch06.5, ch06.6, ch06.7, ch06.8, ch14.2, ch14.3, ch14.6, ch14.7, ch14.8, ch21.3, ch22.1, ch22.2, ch22.3, ch22.4, ch22.5, ch22.6, ch22.7, ch22.8, ch23.1, ch23.3, ch23.4, ch24.1, ch24.2, ch24.4, ch24.5, ch24.8 **Haplotype 14:** ch06.4, ch06.5, ch06.6, ch06.7, ch06.8, ch07.1, ch07.2, ch07.3, ch07.4, ch07.6, ch07.7, ch07.8, ch14.1, ch14.1, ch14.2, ch14.3, ch14.4, ch14.4, ch14.5, ch14.5, ch14.6, ch14.7, ch14.8, ch15.1, ch15.2, ch15.2, ch15.3, ch15.3, ch15.4, ch15.5, ch15.6, ch15.6, ch15.7, ch15.7, ch15.8, ch21.1, ch21.2, ch21.2, ch21.4, ch21.7, ch21.8, ch22.1, ch22.2, ch22.3, ch22.4, ch22.5, ch22.6, ch22.7, ch23.1, ch23.2, ch23.3, ch23.5, ch23.6, ch23.7, ch23.8, ch24.1, ch24.2, ch24.3, ch24.3, ch24.4, ch24.5, ch24.6, ch24.6, ch24.7, ch24.7, ch24.8 **Haplotype 15:** ch06.1, ch06.2, ch06.3, ch07.5, ch07.7, ch22.8 **Haplotype 16:** ch15.1, ch15.4, ch15.5, ch15.8 **Haplotype 17:** ch07.1, ch07.2, ch07.3, ch07.4, ch07.5, ch07.6, ch07.8

FIGURES

Fig. 1. Map of collection sites and general distribution for *Cycladenia humilis* Benth. *C. h.* var. *humilis* from northern California, Sites: 8, 9, 10, 11, 12, 16, 18, 19, 20. *C. h.* var. *venusta* from southern California, sites: 1, 2, 3, 4, 5, 13, 17. *C. h.* var. *jonesii* from the Colorado Plateau, sites: 6, 7, 14, 15, 21, 22, 23, 24, 25, 26.

Fig. 2. Bayesian consensus phylogram for the partitioned analysis of chloroplast sequences and coded indels. Unique haplotypes from each site used in analysis. The posterior probability for each clade is shown above branches and geographic information for each clade is provided.

Fig. 3. Bayesian consensus phylogram for unique haplotypes from the partitioned analysis of the ITS sequences and coded indels. The posterior probability for each clade is shown above branches and geographic information for each clade is provided.

Fig. 4. Bayesian consensus phylogram for unique haplotypes from the partitioned analysis of the ETS sequences and coded indels. The posterior probability for each clade is shown above branches and geographic information for each clade is provided.

Fig. 5. A) Combined chloroplast network (95% connection limit) for *Cycladenia humilis* Benth. Large circles represent observed haplotypes with haplotype-frequency represented by size. Square represents the ancestral haplotype. Small black circles represent un-sampled haplotypes. Each line represents a single mutation. First and second nesting levels shown. B) third level nesting. C) Fourth level nesting and total cladogram.

Fig. 6. A) ITS network (95% connection limit) for *Cycladenia humilis* Benth. Large circles represent observed haplotypes with haplotype-frequency represented by size. Square represents the ancestral haplotype. Small black circles represent un-sampled haplotypes. Each line represents a single

mutation. First and second nesting levels shown. B) third level nesting. C) Fourth level nesting and total cladogram.

Fig. 7. A) ETS network (94% connection limit) for *Cycladenia humilis* Benth. Large circles represent observed haplotypes with haplotype-frequency represented by size. Square represents the ancestral haplotype. Small black circles represent un-sampled haplotypes. Each line represents a single mutation. First and second nesting levels shown. B) third level nesting and total cladogram.

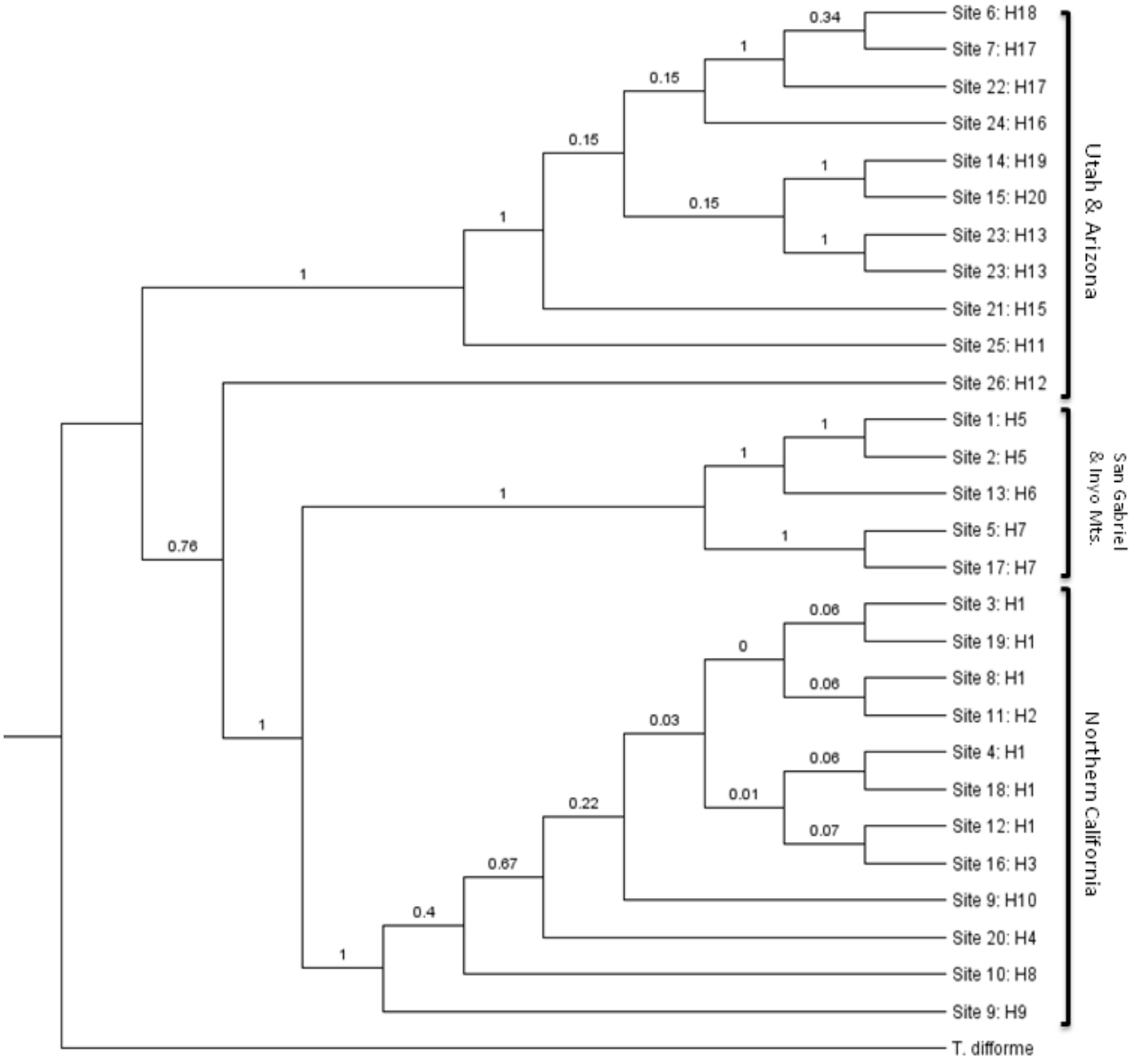
Fig. 8. Whisker Plots of inner hair (trichome) length. Circles indicate outliers in the data set, diamond indicate range of the mean, and bar represents the median.

Fig. 9. Whisker Plots of outer hair (trichome) length. Circles indicate outliers in the data set, diamond indicate range of the mean, and bar represents the median.

Fig. 10. Whisker Plots of petiole length. Circles indicate outliers in the data set, diamond indicate range of the mean, and bar represents the median.

Fig. 1

Fig. 2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Site 7: H17
Site 22: H17
Site 24: H16
Site 14: H19
Site 15: H20
Site 23: H13
Site 23: H13
Site 21: H15
Site 25: H11
Site 26: H12
Site 1: H5
Site 2: H5
Site 13: H6
Site 5: H7
Site 17: H7
Site 3: H1
Site 19: H1
Site 8: H1
Site 11: H2
Site 4: H1
Site 18: H1
Site 12: H1
Site 16: H3
Site 9: H10
Site 20: H4
Site 10: H8
Site 9: H9
T. difforme
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1
0.15
1
1
1
1
0.76
1
1
1
1
0.06
0
0.06
0.03
0.06
0.01
0.07
0.22
0.67
0.4
1
Utah & Arizona
San Gabriel & Inyo Mts.
Northern California

Fig. 3

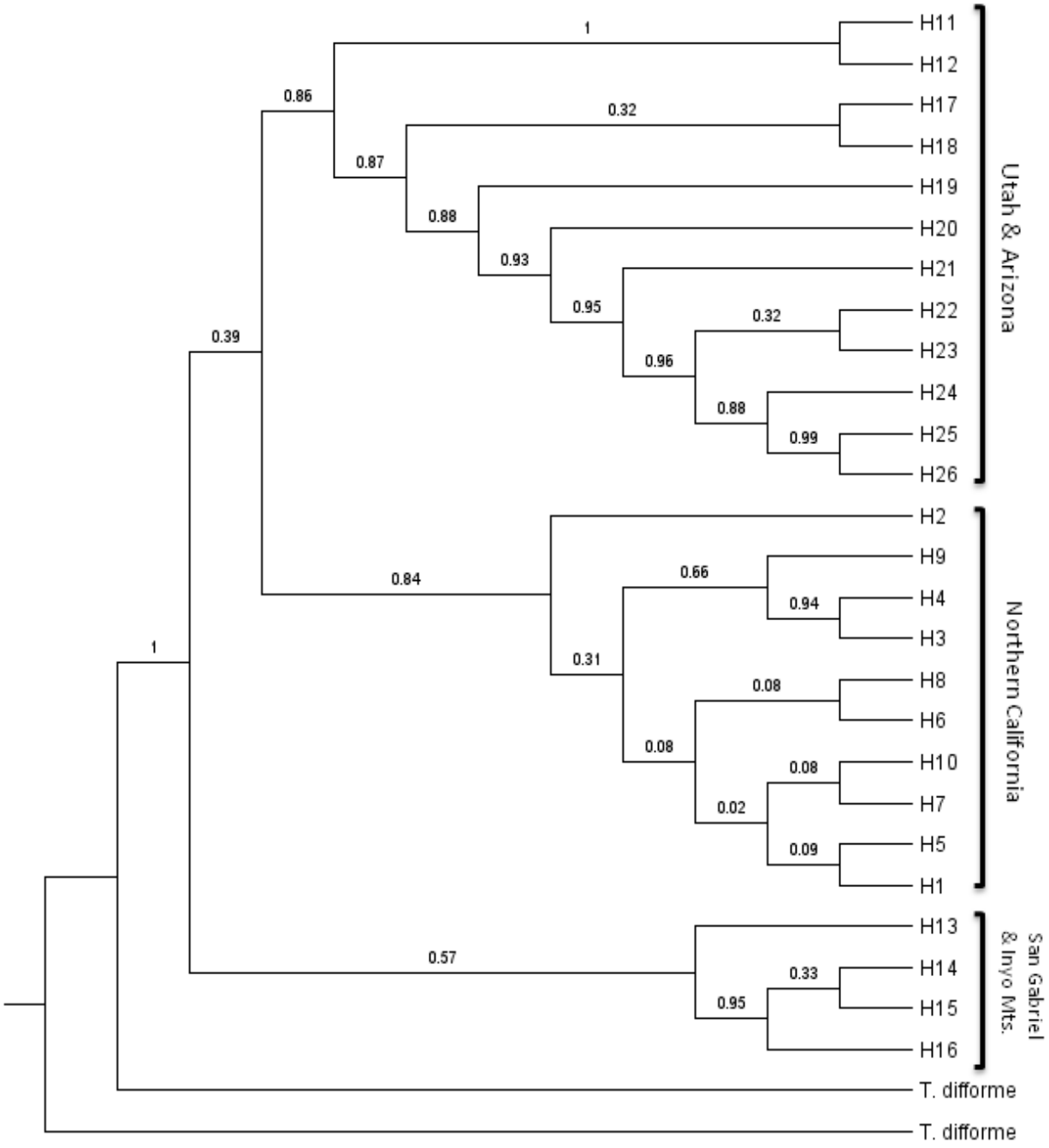

H11
H12
H17
H18
H19
H20
H21
H22
H23
H24
H25
H26
H2
H9
H4
H3
H8
H6
H10
H7
H5
H1
H13
H14
H15
H16
T. difforme
T. difforme
Utah & Arizona
Northern California
San Gabriel & Inyo Mts.
1
0.86
0.32
0.87
0.88
0.93
0.95
0.32
0.96
0.88
0.99
0.39
1
0.84
0.66
0.94
0.31
0.08
0.08
0.08
0.02
0.09
0.57
0.33
0.95

Fig. 4

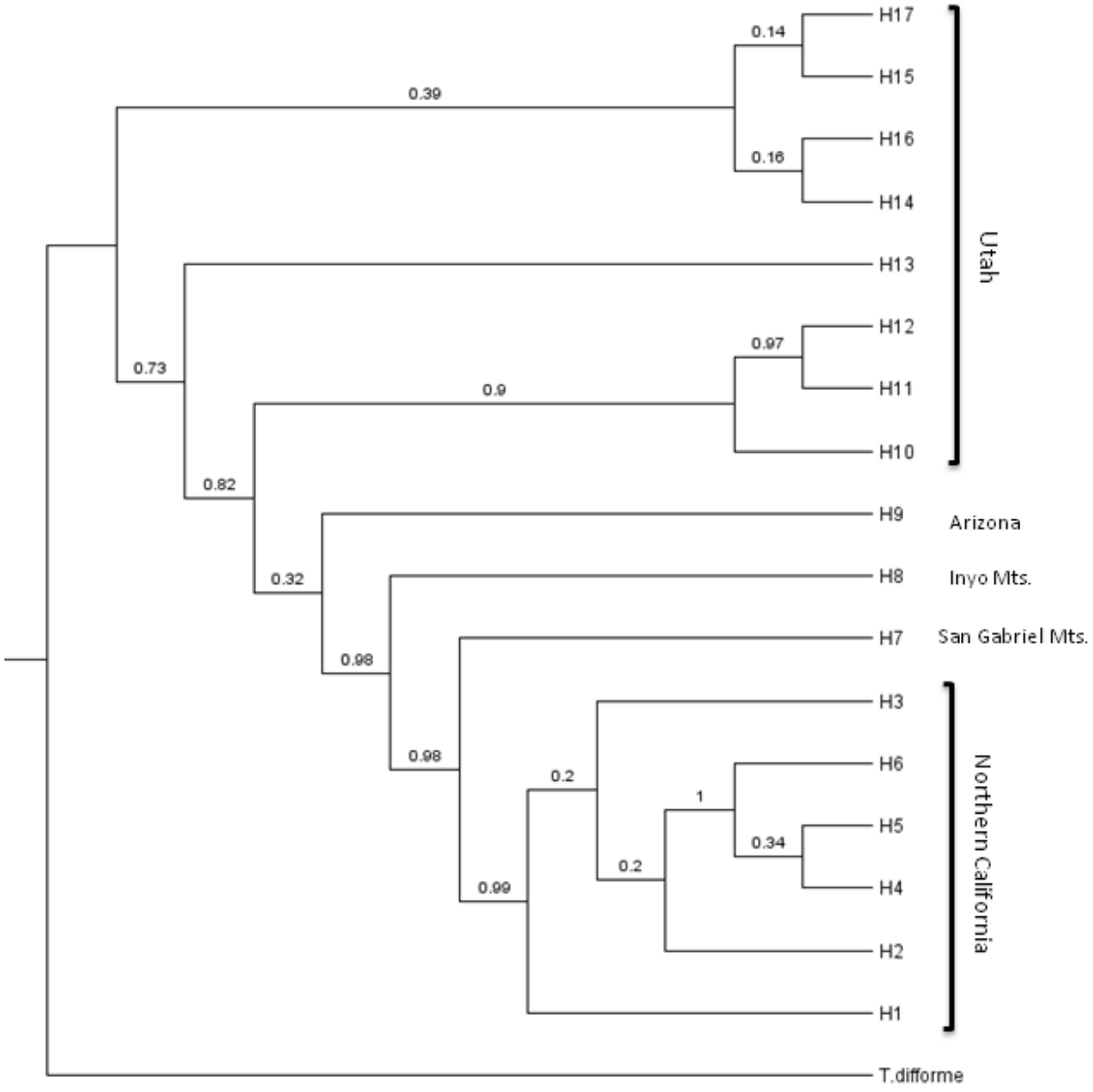

H17
H15
H16
H14
H13
H12
H11
H10
H9
H8
H7
H3
H6
H5
H4
H2
H1
T.difforme
0.14
0.39
0.16
0.97
0.73
0.9
0.82
0.32
0.98
0.98
0.2
1
0.34
0.2
0.99
Utah
Arizona
Inyo Mts.
San Gabriel Mts.
Northern California

Fig. 5

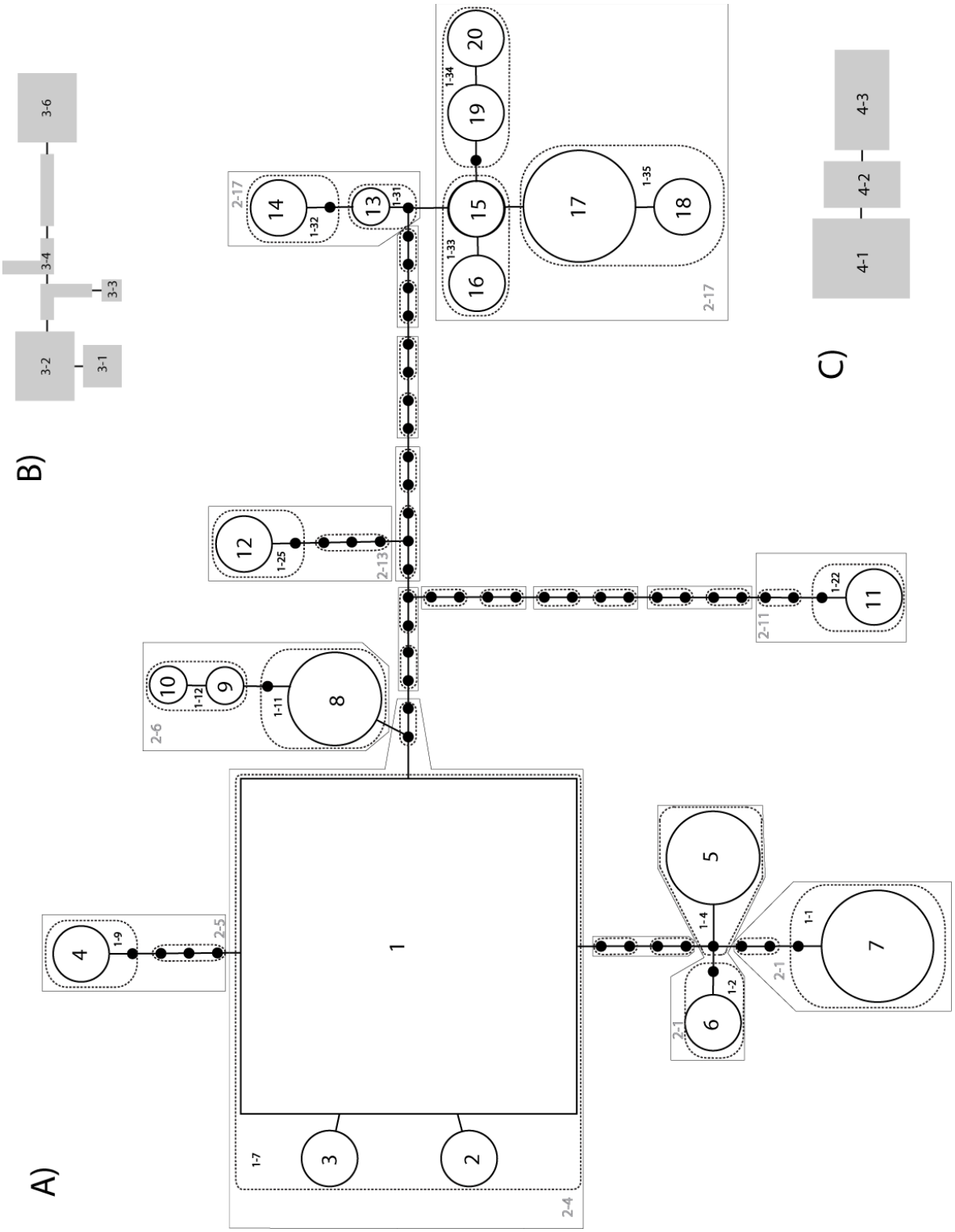

A)
B)
C)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
1-7
1-9
1-4
1-2
1-1
1-11
1-12
1-22
1-25
1-31
1-32
1-33
1-34
1-35
2-4
2-5
2-1
2-6
2-11
2-13
2-17
3-1
3-2
3-3
3-4
3-6
4-1
4-2
4-3

Fig. 6

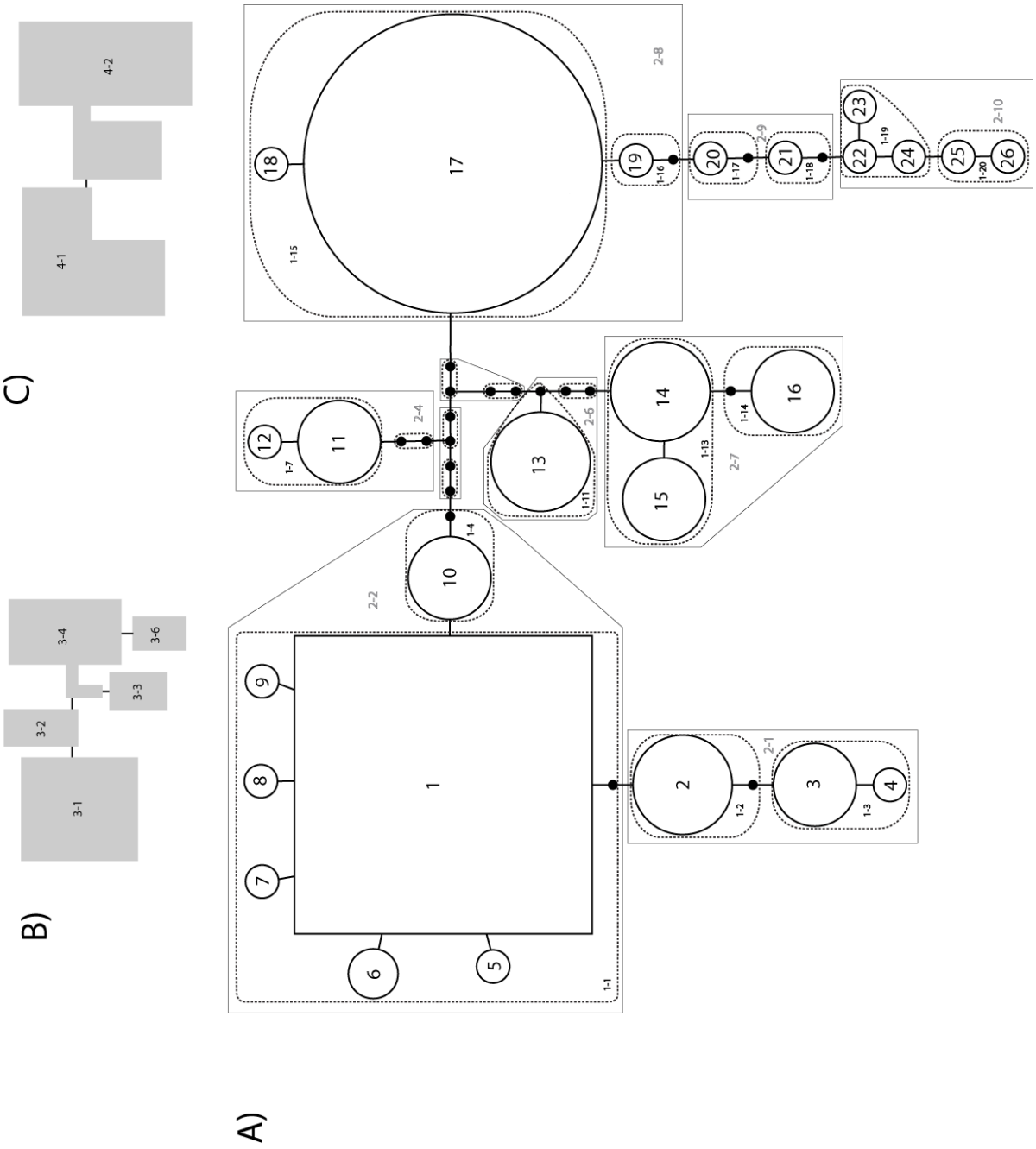

A)
B)
C)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
1-1
1-2
1-3
1-4
1-7
1-11
1-13
1-14
1-15
1-16
1-17
1-18
1-19
1-20
2-1
2-2
2-4
2-6
2-7
2-8
2-9
2-10
3-1
3-2
3-3
3-4
3-6
4-1
4-2

Fig. 7

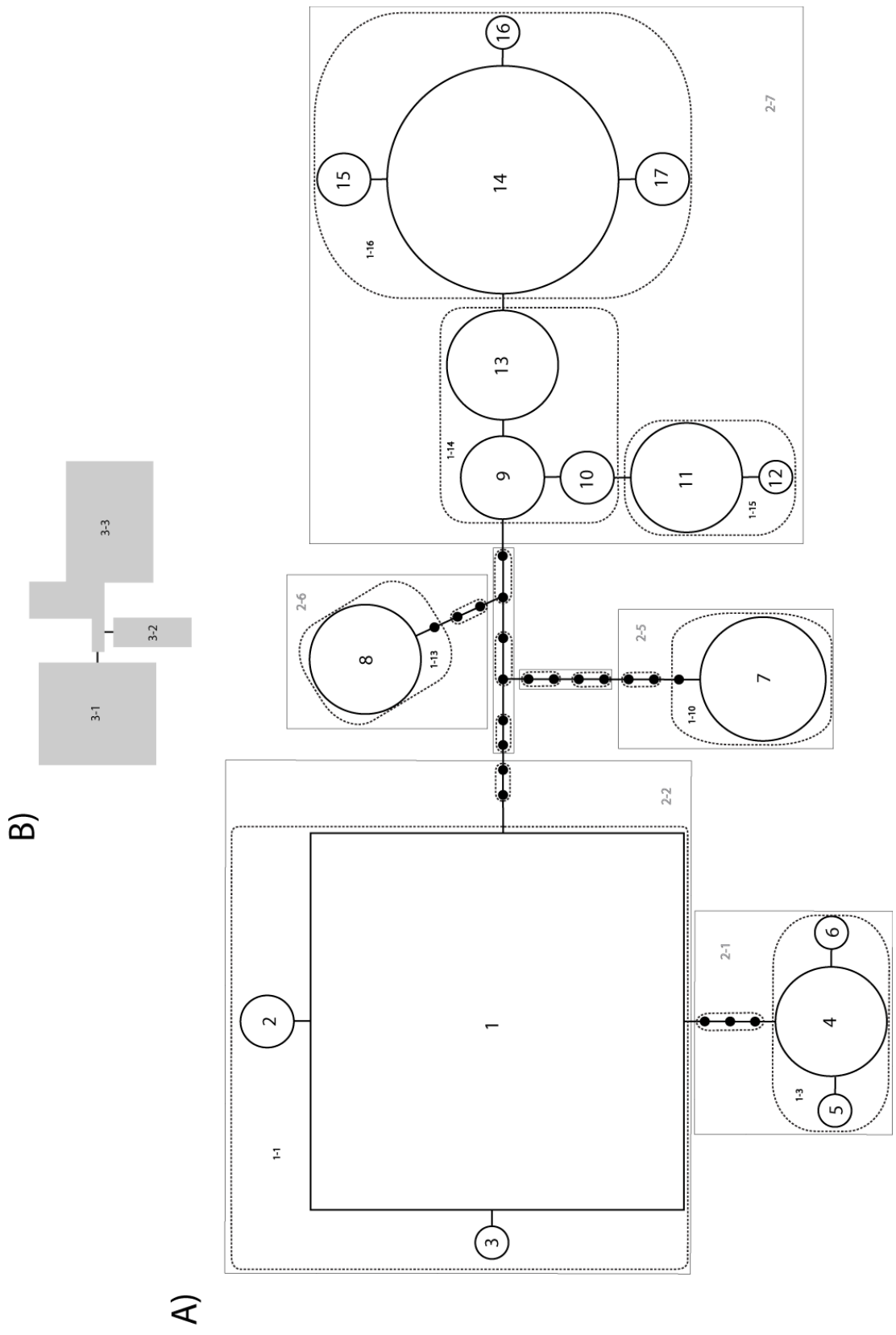

A)
B)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
1-1
1-3
1-10
1-13
1-14
1-15
1-16
2-1
2-2
2-5
2-6
2-7
3-1
3-2
3-3

Fig. 8

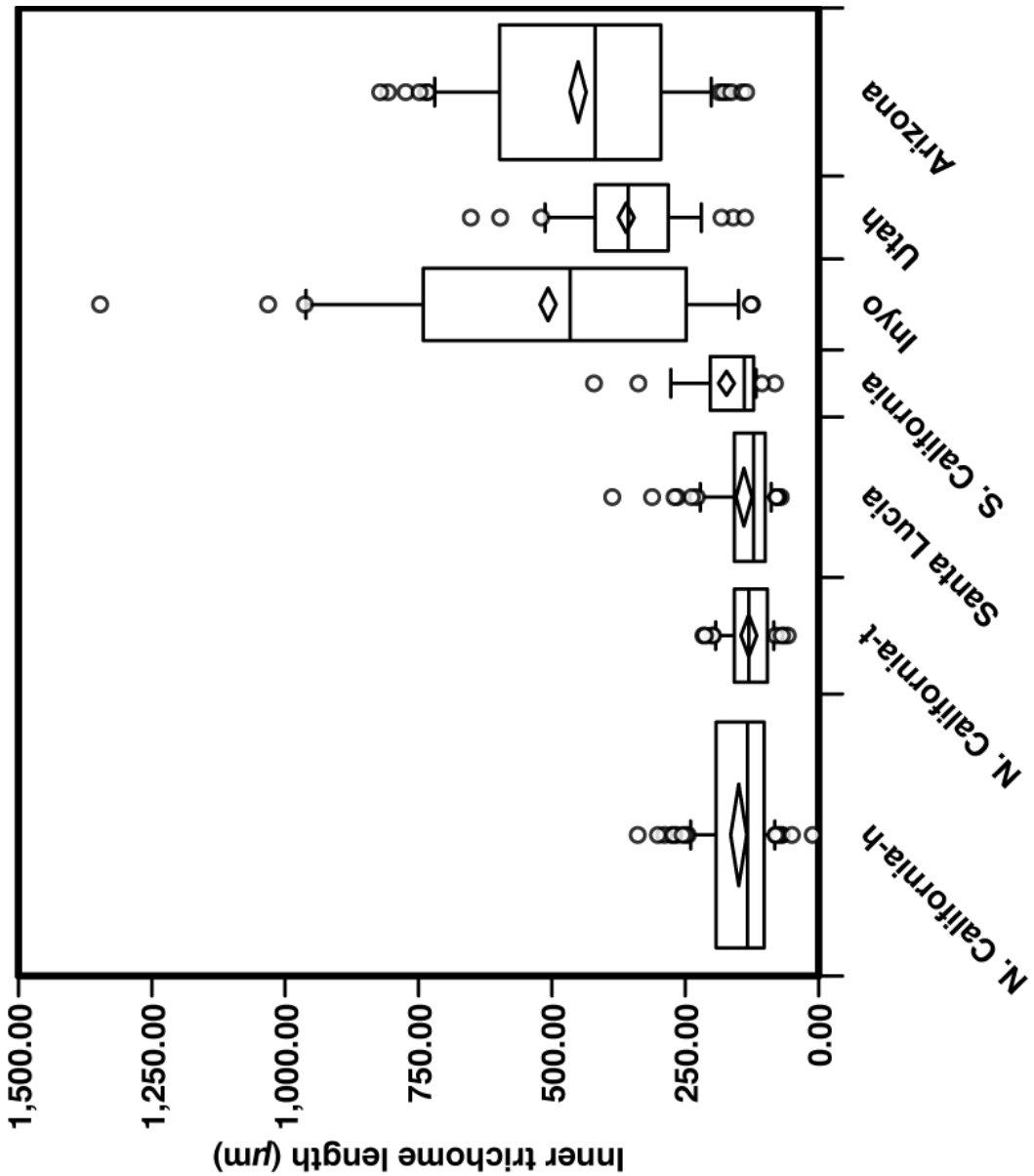

Inner trichome length (μm)
1,500.00
1,250.00
1,000.00
750.00
500.00
250.00
0.00
N. California-h
N. California-t
Santa Lucia
S. California
Inyo
Utah
Arizona

Fig. 9

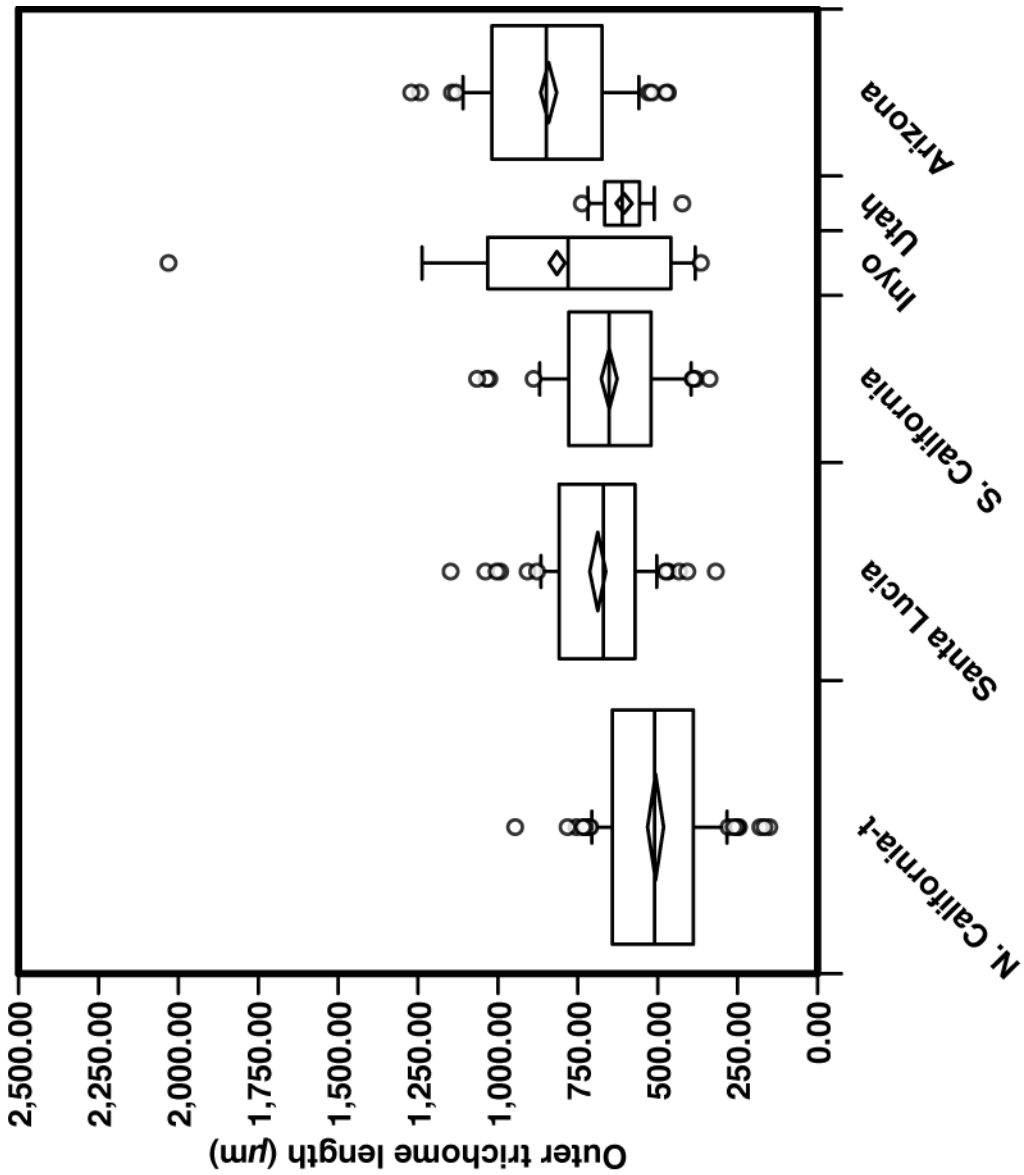
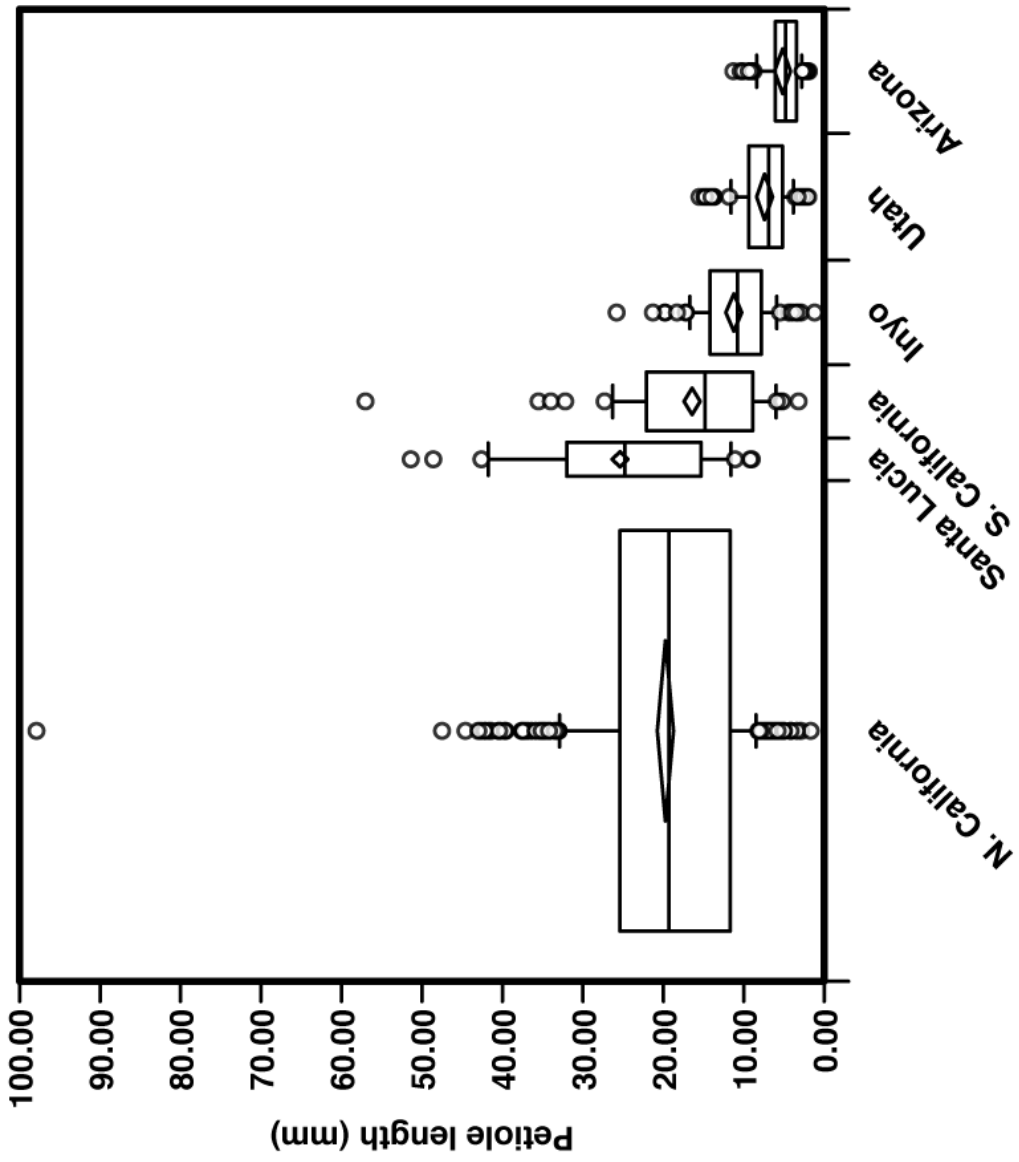

Outer trichome length (μm)
2,500.00
2,250.00
2,000.00
1,750.00
1,500.00
1,250.00
1,000.00
750.00
500.00
250.00
0.00
N. California-t
Santa Lucia
S. California
Inyo
Utah
Arizona

Fig.10


Petiole length (mm)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
N. California
Santa Lucia
S. California
Inyo
Utah
Arizona