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UNIVERSITY OF CALIFORNIA
RIVERSIDE

L'enfer de Michel Adanson: Adventures in Diverse, Rare, Endangered, Hybrid and
Sometimes Even Paraphyletic Manzanitas

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Tito Augusto Abbo

June 2026

Dissertation Committee:
Dr. Amy Litt, Chairperson
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University of California, Riverside

ABSTRACT OF THE DISSERTATION

L'enfer de Michel Adanson: Adventures in Diverse, Rare, Endangered, Hybrid and
Sometimes Even Paraphyletic Manzanitas

by

Tito Augusto Abbo

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, June 2026

Dr. Amy Litt, Chairperson

Conservation of natural species requires an understanding of organisms' geographic ranges and existential threats that occur within these ranges. A species' rarity or commonness is ultimately determined by taxonomy which defines boundaries between closely related species. As rare species tend to be at inherently greater extinction risk than widespread ones, taxonomy interacts heavily with conservation. Similarly, hybridization strongly interacts with taxonomy because it points to limitations in species boundaries.

Hybridization also interacts directly with conservation in that hybrid individuals are difficult to classify in terms of legal conservation status, and outbreeding may be beneficial or harmful to species' persistence depending on context. Thus, the topics of conservation, taxonomy, and hybridization are heavily intertwined, but they have not been expressly studied together as a conceptual framework. To develop this conceptual framework, I use the study system of manzanitas to investigate how these topics interact

at three scales of observation. At the population level, I show that detecting hybridization led to the discovery of a new species of manzanita and ongoing hybridization poses a threat to its persistence. At higher scales of observation, hybridization could only be inferred indirectly but remained a prominent consideration. At the subregion scale, I show that the genetic distinctiveness of federally endangered Del Mar manzanita could only be observed after broad sampling and considering all neighboring manzanita species because they appear to be linked by hybridization. The study defines the range of Del Mar manzanita which is limited to the Coast of San Diego County. At the bioregional scale, I evaluate the phylogenetic relationships of 88% of species in the genus of manzanitas and find at least partial support for the majority investigated species. I can identify which taxa seem well-understood and which taxa require further study such as several widespread tetraploid species. Overall, the study suggests that for diverse systems with frequent hybridization, higher scales of observation are useful for identifying general patterns and complications such as cryptic hybridization, but taxonomic revision is better reserved until cases can be evaluated at small or intermediate scales, especially at the population scale where it is easier to directly observe hybridization.

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INTRODUCTION

Taxonomy: the study of classification of natural organisms, hybridization: the product of geneflow between distinguished organisms, and conservation: the principal of maintaining the environment in its ideal state, are all fundamental topics in (or are subdisciplines of) the biological sciences. To my knowledge, however, the connections and interactions between these topics have not been summarized in the literature as a theoretical conceptual framework. Figure 0.1 provides a summary schema of how these three topics interact and outlines each one's relative importance within the chapters of this dissertation. The interaction between taxonomy and conservation is likely the most apparent, for without knowing how individuals separate into species, one can not know what species are rare or otherwise at risk. In this way, taxonomy directs how resources for conservation are allocated. Nonetheless, conservation enables taxonomy as the more environments are protected, the more opportunity there is to gather evidence about the relationships between the individuals that inhabitant of those environments and discover new ones. In contrast, the manner in which taxonomy determines hybridization is implicitly assumed but seldom articulated. Simply put, classification dictates whether a reproductive event is considered hybridization because the progeny of a giving mating would no longer be consider a hybrid if the parental taxa were reclassified as members of the same species. When Gärtner and Kölreuter carried out hybridization experiments in the late 18th and early 19th century, they assumed under the doctrine of special creation

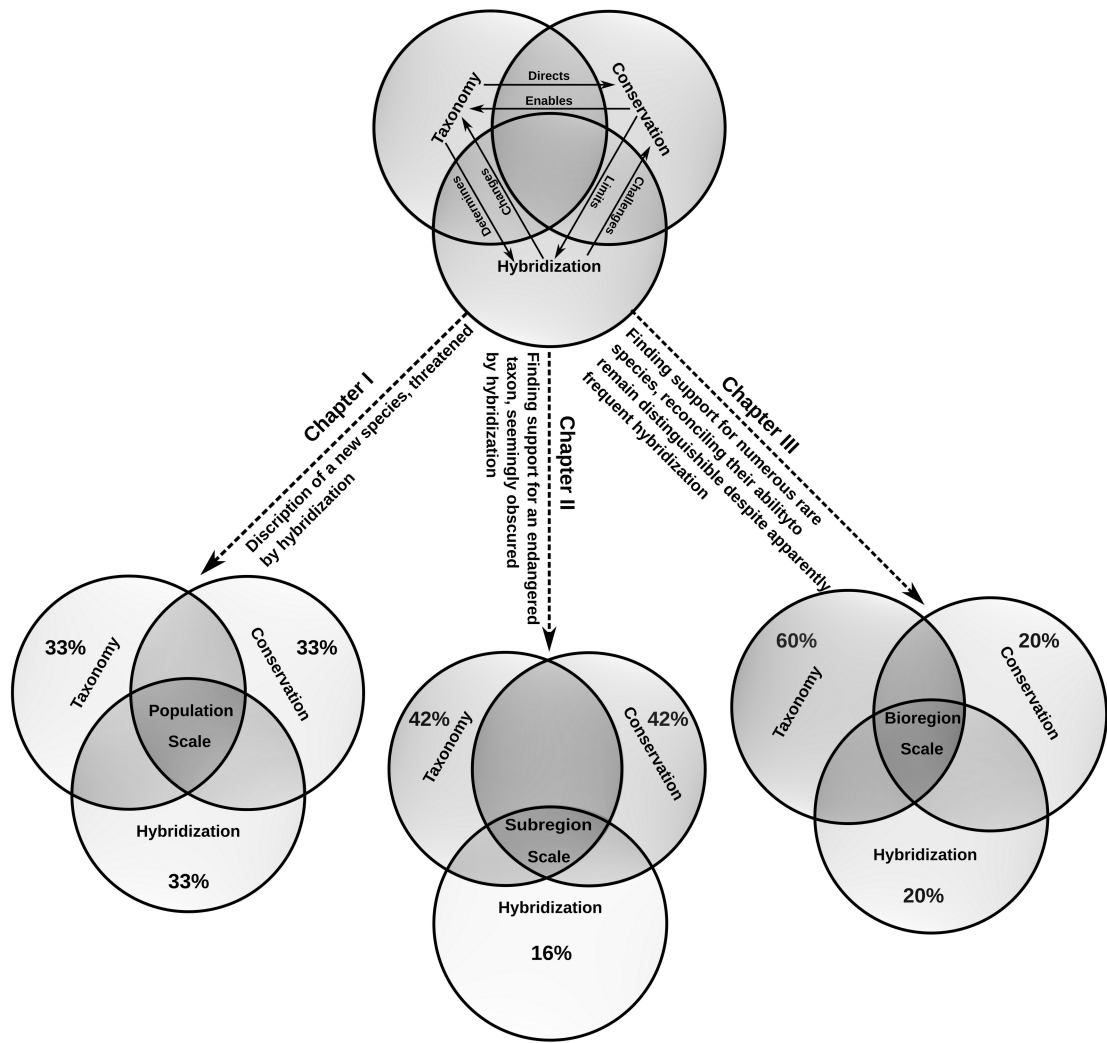


Figure 0.1: Conceptual framework diagram. Illustrates the connections between taxonomy, hybridization, and conservation and their respective prominence in the dissertation chapters. The percentages are meant to be illustrative of the relative importance each factor plays in the chapter.

that separate species must be independent and should not bare fertile offspring when crossed; if a hybrid cross produced fertile offspring, the taxonomy was simply changed to include the parents and the progeny in the same species (Mendel 1865; Darwin, 1872; Stebbins, 1959). Despite abandoning special creation for well over a century, the assumption of no hybridization is still a feature of the leading species concepts used in biology; albeit, the assumption is routinely relaxed given that hybridization is such a common phenomenon. The biological species concept enforces no hybridization by stipulating reproductive isolation, and the phylogenetic species concept enforces no hybridization by stipulating that species must be monophyletic, which hybrids by definition are not (Mayr, 1942; Donoghue, 1985). In this manner it can be said that hybridization changes, or at least leads to the reconsideration of, taxonomy.

Hybridization also challenges conservation because at-risk species may become so introgressed that they can no longer be considered the same biological entity, or hybridization may lower the fitness of at-risk species via demographic swamping (Todesco et al., 2016). This is particularly a concern following the introduction of invasive species which may not have evolved reproductive barriers to the local flora and fauna because they evolved in allopatry. As original shown by Anderson (1953), hybridization can increase in disturbed areas particularly when the disturbance was caused by anthropogenic means, so conservation practices that mitigate disturbed areas or remove invasive species have the potential to limit unwanted hybridization. Thus, taxonomy, hybridization, and conservation interact strongly. My research aims to develop

an integrative framework which synthesizes their combined effects at different scales in the field of systematics.

Key gaps in biological knowledge in this area center around hybridization because it has not been fully integrated into our species concepts and is not fully reconciled in conservation outside of focusing on its negative effects. A notable exception is gene rescue which has been implemented to increase genetic diversity in inbred populations by promoting hybridization but its risks and long term applicability are not fully understood (Todesco et al., 2016). My primary focus, however, is natural hybridization which is routinely detected in genomic studies and appears to be a normal part of evolution in natural communities (see eg: Green et al., 2010; McVay et al., 2017; Edelman et al., 2019; Todesco et al., 2020; Morales-Cruz et al. 2021; Crowl et al., 2022). A critical question both from a conceptual scientific perspective but also from a governmental policy perspective is how do we rank and prioritize the conservation of hybrids of rare species. At the organismal scale perhaps one could argue that the individual is a hybrid which is fundamentally a different entity and not the rare species. Still at the molecular scale one could say that half of its genome is from a rare species. Even if it would be absurd to say that the individual was half of a rare species, it is also invalid to assume that this genetic reservoir has no utility in the conservation of the rare species. Fundamentally, it is the context of the environment that determines the interaction of hybrids and rare

species. As such, studying actual populations is necessary to determine these contexts before the concept can be synthesized into predictive theory.

I approach this conceptual framework using manzanitas (a genus of woody plants) as a study system because they have a complex and debated taxonomy, are known to hybridize frequently, and have numerous species of conservation concern, meaning that they reveal how taxonomy, conservation and hybridization interact in natural environments. My first chapter demonstrates the interactions of these concepts at the population scale. The sand mesa manzanita (*Arctostaphylos rudis*) was originally described from a mixed population, in sympatry with two other members of the genus and was thought to occur in mixed populations throughout the south of its range and occur in isolation in the north of its range. I demonstrate that the south of its range is actually a hybrid zone and the northern population is a distinct species composed of a small number of extant individuals in a population that is heavily fragmented by urban development. My collaborators and I named this species *Arctostaphylos nipumu*, and now the threats to its existence, which include continued habitat fragmentation and genetic swamping from heterospecific plants used in restoration, are properly documented enabling its management and protection. Here the factors of taxonomy, hybridization, and conservation interact proportionally whereby the previous taxonomy did not accurately reflect evolutionary relationships due to undiscovered hybridization. This meant that the key threats to a rare species were unknown for the purposes of conservation. Subsequent

chapters approach the system at progressively higher levels of observation. At these scales, hybridization is not as readily confirmable but is inferred as a background process in the system which contextualizes evolutionary dynamics. In Chapter II I find evidence for the distinctiveness of the endangered Del Mar manzanita (DMM) which is endemic to a critically threatened ecosystem in San Diego County. Previous research provided conflicting evidence on whether DMM should be recognized as a taxon making it challenging for conservation. Were DMM not distinct, then limited conservation resources may be better used for the protection of other threatened taxa, but if distinct, which populations actually contain DMM and need protection as opposed to the ones that contain similar looking more widespread species? My study provided context for the conflicting evidence by expanding the scale of observation from beyond the local scale and comparing DMM to a broad sample of individual manzanitas in the Peninsular Ranges and South Coast subregions of California. This study showed that there was a distinct genetic signal for DMM that was only present around its type locality, and outside of this narrow range similar plants displayed complex intergrading. This ultimately suggested that the three narrow endemics in the subregion: DMM, *A. rainbowensis*, and *A. otayensis* were linked by hybridization but were distinct at the cores of their respective narrow ranges. My final chapter expands the scale observation further to the entire genus at the regional scale of the California Floristic Province, a biodiversity hotspot. At this scale, I was able to find molecular support for numerous rare taxa. The study also provides insight into the hyperdiversification of the genus which appeared to

have taken place in the presence of hybridization, and hybridization may have facilitated a rapid radiation in the genus. Hybridization appears to be very common in manzanitas but also frequently seems to be cryptic suggesting that the genus can not be perfectly explained by relying solely on the principals of Linnaean taxonomy. Therefore, specific areas of the genus that suggest that taxonomic revision is warranted need to be evaluated with large samples of individuals at narrower geographical scales of observation as was done in the first two chapters to better capture nuances.

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CHAPTER 1: INVESTIGATING A HYBRID MIXED POPULATION LEADS TO RECOGNIZING A NEW SPECIES OF *ARCTOSTAPHYLOS* (ERICACEAE)

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ABSTRACT

While investigating the potential for *Arctostaphylos* species to hybridize in the mixed populations of Point Sal and Burton Mesa in Santa Barbara County, California, we discovered that *Arctostaphylos* from the Nipomo Mesa (San Luis Obispo County), formerly considered a northern population of *A. rudis*, are genetically and morphologically distinct. We name this new taxon *A. nipumu* after the ytt (Northern Chumash language) word for the Nipomo Mesa region. For morphological and molecular analyses, we sampled 54 plants, focusing on *A. purissima*, *A. rudis*, and *A. crustacea* from multiple species and comparative single species populations. Parametric and nonparametric clustering analyses (STRUCTURE and PCA) of ddRADseq data show that *Arctostaphylos* from the Nipomo Mesa segregate from all other samples in the dataset. In mixed populations *A. purissima* and *A. crustacea* samples cluster with samples

from other unmixed populations of the same species but *A. rudis* samples form two distinct clusters. One is composed of the mixed populations in Santa Barbara County, and the other consists of the Nipomo Mesa population. Additionally, the Santa Barbara County *A. rudis* samples are admixed in STRUCTURE analysis unlike the samples from the Nipomo Mesa. A principal component analysis of eight morphological characters shows that *A. rudis* individuals from Santa Barbara County tend to be phenotypically variable, occurring in a wide morphological cluster that overlaps with the tight clusters formed by *A. purissima*, *A. crustacea*, and *Arctostaphylos* from the Nipomo Mesa. Based on this evidence we describe the Nipomo Mesa population as a new species of *Arctostaphylos*. Given its limited and fragmented distribution we believe that *A. nipumu* is of critical conservation concern.

INTRODUCTION

Arctostaphylos Adans. species in California have a potential to hybridize and there are multiple tetraploid taxa that are thought to have arisen from a hybrid origin (Wells 2000; Parker and Vasey 2016; Parker et al. 2020). It is therefore not surprising to find individuals with intermediate traits in a stand of multiple *Arctostaphylos* species. By the same token, it is surprising that *Arctostaphylos* species can remain distinct despite frequently occurring in mixed stands. Parker et al. (2020) studied pairs of sympatric *Arctostaphylos* species throughout California and made two key observations. First, they noticed that a significant portion of sympatric pairs were composed of a diploid and a

tetraploid *Arctostaphylos* species. This suggested that one mechanism of reproductive isolation was reduced meiotic compatibility for interploidal crosses. Out of the currently recognized 108 *Arctostaphylos* taxa, 68 (63%) are diploid taxa while 40 (37%) are tetraploid. The second key observation by Parker et al. was that the majority of sympatric diploid pairs were composed of *Arctostaphylos* species from different major ITS lineages. Molecular phylogenies of the Internal Transcribed Spacer (ITS)-locus revealed two major lineages of *Arctostaphylos*, the large ITS-clade containing ca. 78% of the diploid *Arctostaphylos* species, and the small ITS-clade containing the remaining ca. 22% diploid species (Markos et al. 1998; Boykin et al. 2005; Wahlert et al. 2009). Parker et al. also observed that the subset of sympatric pairs of *Arctostaphylos* species from the same ITS-clade were far more likely to include putative hybrid, morphologically intermediate individuals compared to sympatric pairs of *Arctostaphylos* species from different ITS-clades. This suggested that at least some reproductive isolation had formed between the two clades (hereafter the small and large clades). Thus, they hypothesized that the two primary reproductive isolation mechanisms in *Arctostaphylos* are isolation by genetic distance (ITS-clade incompatibility), and meiotic incompatibility between species of different ploidies. Although beyond the scope of this study, it should be noted that taxon-specific ploidy determination in *Arctostaphylos* has been based on chromosome counts of very few, generally single, individuals (see e.g. Wells 1968) and with the exception of Schierenbeck et al. (1992),

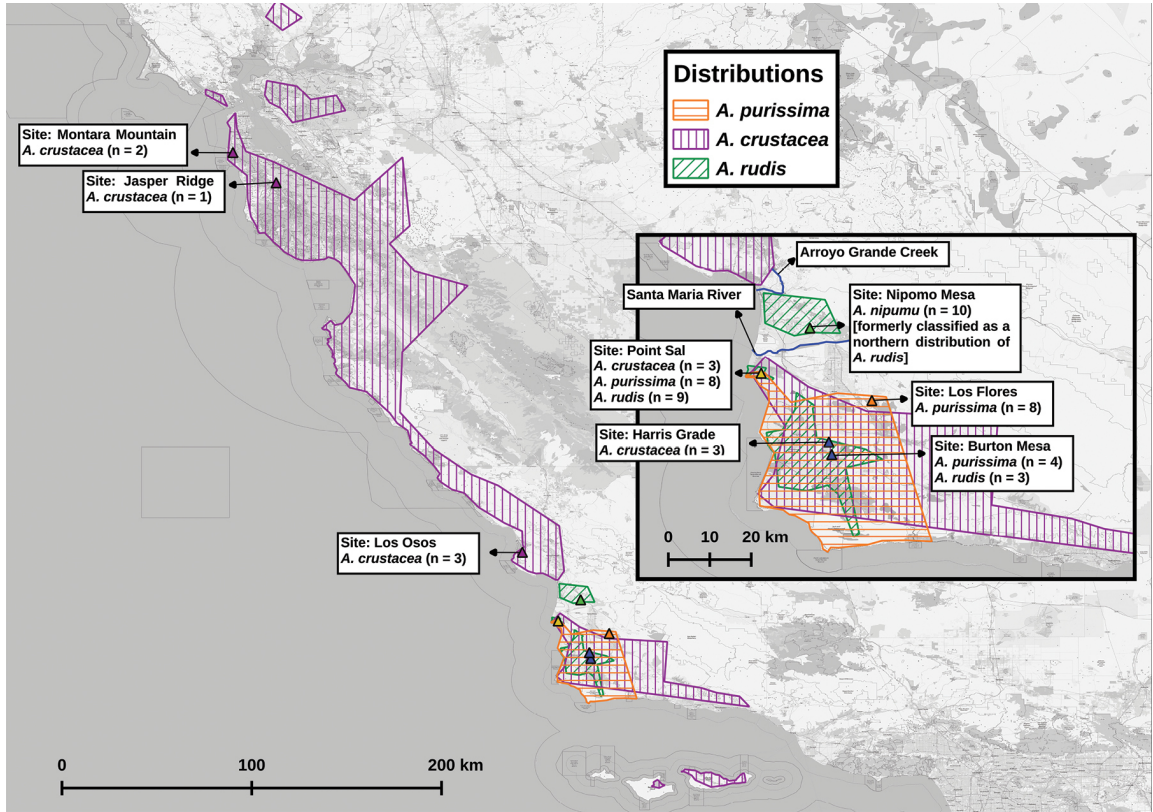


Figure 1.1: Distribution and sampling map generated in QGIS version 3.224. The background map of the central California coastline is from OpenStreetMap using the EPSG:3857 - WGS 84 / Pseudo-Mercator projection. The distributions of *Arctostaphylos purissima* (orange polygon), *A. crustacea* (purple polygon), and *A. rudis* (green polygon) were inferred using collection data from the Consortium of California Herbaria (CCH2 Portal 2024). The locations where we obtained samples for this study are marked with solid triangles, and site names, taxa sampled, and number of samples obtained for each site are annotated on the figure. The inset shows a closer view of the Nipomo Mesa and northern Santa Barbara County, and the key waterways of the Arroyo Grande Creek, and the Santa Maria River are highlighted in blue. Note that the northern green polygon was formerly considered a northern extension of *A. rudis* but herein is described as the new species *A. nipumu*.

there are no broad scale within taxon/population cytological studies in the genus. Therefore, the typical effects of hybridization on ploidy are unknown in *Arctostaphylos*.

The Central Coast of California is ideal for investigating interspecific gene flow in *Arctostaphylos*. This region hosts the largest number of taxa in *Arctostaphylos* (Cody 1986; Keeley 1992; Vasey and Parker 2014, Burge et al. 2016), especially local endemics, likely reflecting considerable edaphic diversity and a strong coast-to-inland temperature and precipitation gradient (Vasey et al. 2014). Further, stands of *Arctostaphylos* along the coast frequently contain multiple species. We investigated one such stand from central California, Point Sal in Santa Barbara County (Figure 1.1), containing two narrow endemic species, *A. rudis* and *A. purissima*, as well as a widespread species, *A. crustacea*. The endemic species in our study are generally restricted to sandy soils under a marine fog-moderated climate (Richerson and Lum 1980; Vasey et al. 2012, 2014) and are of conservation concern (CNPS 1.B.1 or 1.B.2).

The two narrow endemics are diploids and are from different ITS-clades, while *A. crustacea* is a tetraploid from the large clade (Wahlert et al. 2009), so we expected only limited gene flow. Despite these differences in likely ploidy and ITS-clade, a number of individuals at the site exhibited intermediate morphologies, indicating potential hybridization. Nevertheless, we hypothesized that we would find only limited gene flow among all these species in the mixed population. We tested our expectations using

genomic data to look for signatures of hybridization and assess if these varied between populations. During this investigation, we inadvertently learned that individuals currently classified as *A. rudis* varied morphologically and genetically among sites. We found that the type locality for *A. rudis*, Point Sal (Jepson 1938), as well as another population farther south, are part of an extensive hybrid zone. The *Arctostaphylos* population from the Nipomo Mesa, north of the Santa Maria River, segregated genetically and morphologically from these hybrid individuals to the south of the river. Here we present the evidence for hybridization, and recognize the distinct Nipomo Mesa population as a new species.

METHODS

SAMPLING

This study included 54 samples, summarized in Figure 1.1. All samples were from mature, adult shrubs. Our goal was to test the prediction of Parker et al. (2020) that species of differing ploidy and/or ITS-clades in a mixed population of *Arctostaphylos* would not exhibit interspecific gene flow. Therefore, Point Sal (Figure 1.1, yellow triangle), located at the northern tip of Santa Barbara County California, was our primary study site because *A. crustacea* (tetraploid, large clade), *A. purissima* (diploid, large clade), and *A. rudis* (diploid, small clade) occur there in sympatry. At Point Sal, we collected samples from three individuals of *A. crustacea*, eight individuals of *A.*

purissima, and nine individuals of *A. rudis*. The Burton Mesa and Harris Grade sites (Figure 1.1, blue triangles), located near Lompoc in Santa Barbara County, together serve as a pseudoreplicate of Point Sal because *A. purissima* and *A. rudis* occur there in sympatry, and a population of *A. crustacea* is close by. We collected samples from three individuals of *A. rudis*, and four individuals of *A. purissima*, at Burton Mesa and three individuals of *A. crustacea* at Harris Grade (ca. 5 Km from Burton Mesa). We selected the remaining sampling localities based on the distributions of *A. crustacea*, *A. purissima*, and *A. rudis* (Figure 1.1 polygons), which we estimated using herbarium collection records from the California Consortium for Herbaria (CCH2 Portal 2024). We used these distribution maps to devise a sampling scheme which accounted for both the ranges of our focal taxa and the regions where these ranges did not overlap. To account for potential genetic changes across its wide range, we split the remaining sampling of *A. crustacea* between the northern and southern edges of its core range. We collected samples from three individuals at the Los Osos population in San Luis Obispo County, and three individuals from San Mateo County, one of these from Montara Mountain, and two from the nearby Jasper Ridge. For *A. rudis*, we collected 10 samples from the Nipomo Mesa, a disjunct population in San Luis Obispo County north of Point Sal. Lastly, we sampled from eight individuals of *A. purissima* from Los [sic] Flores Ranch Park in northeastern Santa Barbara County. For all samples, we froze fresh material of flower buds and/or immature leaves; we stored this tissue at -20 C until DNA extraction.

For each sample we also collected a herbarium voucher specimen, dried and preserved for documentation and morphological analysis. We deposited voucher specimens at the UCR Herbarium, and when sufficient excess material was present, we deposited a duplicate at the Harry D. Thiers Herbarium at San Francisco State University. The appended Supplemental Materials 1 contains further sample information including GPS coordinates and collection numbers.

MOLECULAR SAMPLE PREPARATION AND SEQUENCING

From each sample we extracted DNA using the Qiagen DNeasy Plant mini kit (QIAGEN, Venlo, The Netherlands) following manufacturer's instructions and confirmed the presence of high molecular weight DNA on a 1% agarose gel. We subsequently used these DNA extracts to prepare ddRADseq libraries. Although we collected the majority of our samples in December, 2023, we collected some of our samples in the years 2018–2020 as part of an ongoing genus-wide, phylogenomic study (Supplemental Materials 1). For the four samples of *A. purissima* and three samples of *A. rudis* from Burton Mesa only (sample codes: I7, I8, J1, J2, J3, J5, J6; see Supplemental Materials 1), we used sequences we obtained in 2020 from the UCR Institute for Integrative Genome Biology Genomics Core. We prepared these libraries following the exact protocol published in Huang et al. (2020). We prepared the remaining 47 samples in 2024 using a modified version of the Huang et al. protocol. The main modifications we implemented were: 1) We increased the time that DNA samples were digested with restriction enzymes at 37 C

from three hours to six hours. In a set of bench trials, Morrison et al. (unpublished), observed that this increased digestion time seemed to yield a more complete digestion and had no observable down sides. 2) We carried out the size selection by gel excision earlier in the protocol to increase workflow efficiency. Here, we implemented a mini pooling strategy where we combined samples into mini pools of 8–10 samples, size-selected these pools via gel excision, and then combined them into a single pool after PCR amplification. 3) We increased the volume of DNA template solution from 1.25 μ l to 2.65 μ l per PCR reaction. We kept the volume of all other PCR reagents the same as Huang et al. by not adding additional water to the PCR master mix i.e. the increased DNA template volume corresponded to the amount of water added in the original protocol. Bench trials by Morrison et al. (unpublished) showed that this modification favored the amplification of DNA library material over a competing dimer reaction. 4) We eliminated the Evrogen (Moscow, Russia) Timmer Kit reaction. This Timmer Kit reaction was highly inefficient in terms of DNA recovery and therefore required a second PCR reaction which increased stochasticity of pooled sample amplification. The relatively smooth curve we detected in our Agilent Bioanalyzer (2100 expert High Sensitivity) quality control showed that it was unnecessary. These modifications improve the consistency of successful library preparation and the resulting sequence data remains compatible with batches prepared using the original Huang et al. (2020) methods. Abbo (unpublished) combined a larger set of data prepared with both methods and did not observe any batch effects. We then submitted the finished libraries to GeneWiz (Azenta,

New Jersey, USA) for sequencing on a partial lane of an Illumina NovaSeq sequencer with 150 bp paired-end configuration.

MOLECULAR DATA ANALYSIS

We quality controlled and aligned the raw sequence reads to the genome of *Arctostaphylos glauca* (Huang et al. 2022) using IPYRAD version 0.9.95 (Eaton and Overcast 2020). We kept parameters at default for paired ddRADsequencing reads with the exception of the “min_samples_locus” parameter which we set to 15, corresponding to ca. 28% of the total number of ingroup samples. Wagner et al. (2018) tested the sensitivity of inferred phylogenetic topology to changes in the “min_samples_locus” parameter and found that the extremely low default value of four or an extremely high value corresponding to 100% of samples in the dataset yielded unreliable results, and intermediate values only affected support values and not recovered topology. Consistent with Wagner et al.’s results, preliminary assemblies of other *Arctostaphylos* datasets showed that values greater than 25% maximize the amount of data without harming the reliability (Abbo, unpublished). We used the default maximum number of alleles in the consensus sequence as two despite having tetraploids in our dataset. Huang et al. (2020), using our same sequencing method, found equivalent results when treating tetraploid *Arctostaphylos* samples as diploid in the context of maximum number of alleles and recommended this approach for future studies in the genus. Burge et al. (2018) also used

two as their maximum number of alleles for a dataset of tetraploid *Arctostaphylos* samples using single digest RAD-sequencing.

A key difference between these previous studies and the current one is that Huang et al. and Burge et al. only examined one tetraploid *Arctostaphylos* species and we examined diploid *Arctostaphylos* species in combination with one tetraploid species. To validate using two as the maximum number of alleles in the consensus sequence (MA), we tested the effect of setting the MA to four on principal component analysis (PCA). This was not a perfect solution as IPYRAD still assumes diploidy for the purpose of heterozygous base calling when the MA is set to four, but our approach was consistent with other studies that used IPYRAD to process multiple-ploidy datasets (Wagner et al. 2020; Suissa et al. 2022). PCA results were consistent between the two and four MA datasets (Supplemental Materials 2); however, the issue described below of samples with higher missing data influencing PC1 was more severe in the four MA dataset (Supplemental Materials 2 B). The results were consistent between both datasets when we used K-means missing data imputation (Supplemental Materials 2 A, D) and when we did not use imputation but excluded the four samples with the highest amounts of missing data (Supplemental Materials 2 C, F). When we did not exclude samples nor impute missing data, both datasets showed similar results for PC0, but PC1 showed some informative clustering despite samples with higher missing data plotting as outliers in the two MA dataset, whereas PC1 appeared to only give information about sample-specific missing data in the

four MA dataset (Supplemental Materials 2 B, E). We found that the similar PCA results between the two MA and four MA datasets justified using the two MA dataset for STRUCTURE analysis, which produced results that agreed with PCA. All analyses depicted in the main text, herein, were generated using the two MA dataset.

We analyzed the aligned sequences using clustering analyses. We carried out nonparametric clustering as a PCA using the .pca function from the IPYRAD analysis tools (IPA). First, we ran .pca with single nucleotide polymorphisms (SNPs) present in at least 80% of individuals (minimum coverage = 0.8) and with no missing data imputation over 50 replicate runs, each with a different random draw of uncorrelated SNPs. The data separated into four clusters but with four samples with high missing data plotting as outliers. Therefore, we reran the analysis using the same minimum coverage and number of replicates but with K-means missing data imputation set to $K = 4$. Imputation refers to the process of inferring missing values in the data using patterns in the remainder of the data. We chose K-means imputation as it inferred the missing values from four clusters naturally present in the data rather than relying on our assumptions about taxonomy or geographic distributions.

We then performed parametric clustering using the STRUCTURE (Pritchard et al. 2000) distribution in the STRUCTURE_THREADER package version 1.3.10 (Pina-Martins et

al. 2017). We used STRUCTURE_THREADER to run multiple replicates at various K values simultaneously. To meet the assumptions in STRUCTURE of no linkage between sites, we used the .ustr file generated by IPYRAD from randomly selected unlinked SNPs as the input data file. We used standard parameters in STRUCTURE with admixture set to true, and supplied no population priors to avoid biasing the inferred groups with taxonomic and/or spatial assumptions. We ran STRUCTURE from K = 1 up to K = 10 with three replicates per run (30 runs total), each for 50,000 MCMC generations with the first 10,000 generations omitted as burn-in. We selected these MCMC chain and burn-in lengths based on Burge et al (2018); in the past we have also experimented with longer MCMC chains in *Arctostaphylos* (Abbo, unpublished) and did not notice changes to the results. We evaluated the best number of K ancestral groups by examining the changes in mean log-likelihood and changes in ΔK across each value of K *i.e.* the Evanno method (Evanno et al. 2005) in R version 4.4.1 using the values calculated by STRUCTURE HARVESTER (Earl and vonHoldt 2012). K = 2 was clearly best supported and the results for higher values of K were virtually identical to those of K = 2 with additional ancestral groups represented only by negligibly low percentages (Suppl. Material 3).

MORPHOLOGICAL DATA COLLECTION AND ANALYSIS

We analyzed morphological variability across species and populations in the data by measuring eight morphological characters, available for all samples; for each individual,

we recorded traits in the field or from their voucher specimens after collection. The characters we used were: 1) Estimated height of individual (continuous): the height of the individual from the ground to the tallest part of the canopy. This was estimated and recorded in the field, in meters. 2) Estimated width of individual (continuous): the length of the apparent widest portion of the individual's canopy. We estimated and recorded this in the field, in meters. 3) Presence or absence of a burl (categorical): a burl is a woody organ, containing meristematic tissue that develops at the base of the plant and provides a mechanism for regeneration after damage, especially fire. We recorded the presence or absence of this character in the field and numerically coded it as 0 (absent), and 1 (present). 4) Leaves isofacial or bifacial (categorical): leaves with similar color, hairiness, and presence of stomata on both leaf surfaces are considered isofacial, and leaves with different colors or hairiness on leaf surfaces and/or lacking stomata on one surface are considered bifacial. We assessed this character by examining leaves from voucher specimens under a dissecting microscope and numerically coded it as 0 (bifacial), and 1 (isofacial). 5) Twig hair length (categorical): the general trend of length of trichomes on young twigs in three binned categories, short (0–2 mm), intermediate (2–6 mm) and long (>6 mm). We assessed this character by examining young twigs from voucher specimens under a dissecting microscope and numerically coded it as 1 (short), 1.5 (intermediate), and 2 (long). 6) Length of the longest leaf blade (continuous): the length of the blade of the longest leaf on a voucher specimen, which we measured from the end of the petiole to the end of the blade to the nearest tenth of a centimeter. If the leaf was basally lobed or

auriculate, we measured the midrib length instead. 7) Length of the petiole (continuous): the length of the petiole of the longest leaf on a voucher specimen, which we measured from the stem to the start of the blade to the nearest millimeter. 8) Leaf base shape (categorical): the general trend of the shape of the base of the leaves on a voucher specimen. We coded this character as 0 (lobed), 1 (truncate), 2 (rounded), 3 (tapered). We coded categorical variables numerically in order to examine all variables using a PCA, which we generated in R version 4.4.1 using the `prcomp` function with both `scale` and `center` set to `true`.

FIELD SURVEYS FOR NEW SPECIES DESCRIPTION

Because the data support the *Arctostaphylos* population from the Nipomo Mesa as a new species, we carried out additional surveys throughout the Nipomo Mesa to assess its range. For each population that we found we collected a paratype voucher specimen, and we documented morphological characters with field notes and photographs (see Supplemental Materials 1). We then supplemented these field surveys with satellite data using ArcGIS Pro version 3.3.2 (Esri Inc 2024). We estimated the range and land occupied by *Arctostaphylos* from the Nipomo Mesa (Figure 1.5), by converting coordinate data for individual occurrences of plants to point data shapefiles, and we mapped these using the NAD 1983 (2011) StatePlane California V FIPS 0405 (US Feet) coordinate system. We calculated population surface area in hectares by loosely defining

population boundaries surrounding single individual occurrences and clusters of individuals within a contiguous area of associated habitat. We then calculated the surface area of the resulting polygons using ArcGIS Pro. Lastly, because the field surveys determined that the individuals from Nipomo Mesa lacked a basal burl, we performed an additional survey at Burton Mesa to confirm that the presence of a burl was not a mistake in the original description of *A. rudis*.

RESULTS

GENETIC ANALYSIS

Principal component analysis (PCA) reveals four groups of samples. PC0 explains the majority of variation in the data (15% without missing data imputation; > 30% using K-means, K = 4, missing data imputation) and can on its own be used to separate the samples into four clusters, but the two taxa from the large clade, *A. crustacea* and *A. purissima*, are very close together. We observe further separation along PC1, the second axis of variation; however, without imputing missing data, the four samples with the highest amounts of missing data (samples: I8, CZ6 DA3, and DC5; see Supplemental Materials 1) group on their own along PC1 (see Supplemental Materials 2 E). We observe no PC1 outliers using K-means imputation at K = 4 (Figure 1.2). The samples from the Nipomo Mesa and the samples of *A. purissima* form the tight clusters at the opposite bottom corners of the figure. The *A. crustacea* samples form a slightly wider

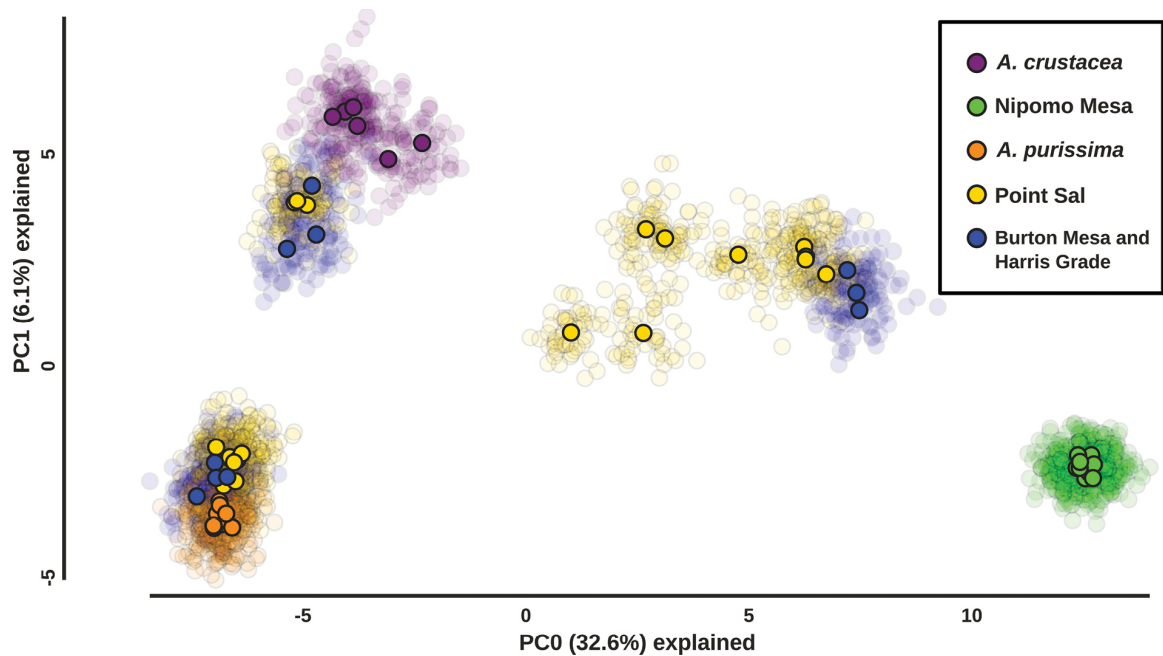


Figure 1.2: Principal component analysis (PCA) of ddRADseq data with SNPs present in at least 80% of individuals (minimum coverage = 0.8). The figure was generated using the IPYRAD analysis tools (IPYRAD version 0.9.95) and edited in Inkscape version 1.1.2. PC0 is given in the x axis and explains 32.6% of variation in the data, while PC1 in the y axis explains 6.1% of variation in the data. Solid circles are the results of the PCA for a random draw of uncorrelated SNPs for each sample, and the transparent circles are the results of 49 additional PCAs for distinct, independent random draws of uncorrelated SNPs. Missing data was imputed using K-means imputation at K = 4. Samples from the Nipomo Mesa (green), Point Sal (yellow), and Burton Mesa and Harris grade (blue) are color-coded by Sampling location, and samples of *Arctostaphylos purissima* (orange), and *A. crustacea* (purple) from control/nonfocal populations are color-coded by taxonomic identity.

cluster with low values of PC0 and high values of PC1. The fourth and widest cluster consists of 12 samples from Point Sal and Burton Mesa that we morphologically determined as *A. rudis*; this cluster is intermediate to the other three clusters in principal component space. The remaining samples from Point Sal, Burton Mesa and Harris Grade group with either *A. purissima* or *A. crustacea* consistent with the morphological identification of those respective samples. No other samples cluster with the samples from the Nipomo Mesa. The transparent points in Figure 1.2 are the results of 50 repeated principal component analyses using a different randomly selected set of unlinked single

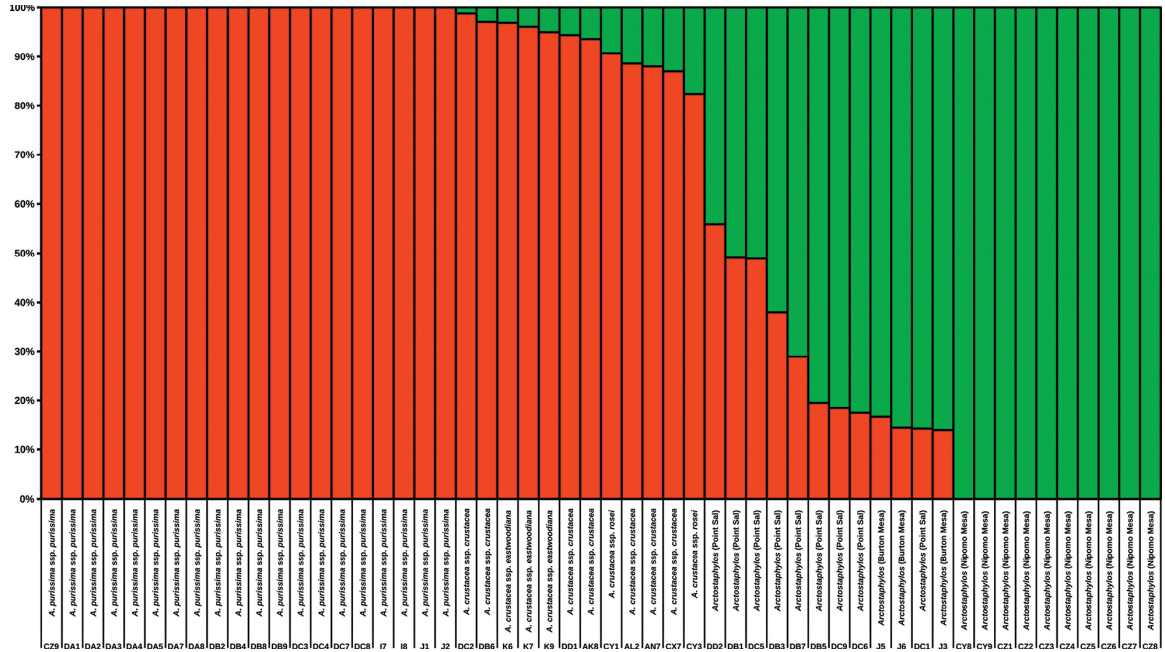


Figure 1.3: Structure plot/stacked bar graph of the $K = 2$ using uncorrelated SNPs from ddRADseq data. Structure was run using STRUCTURE_THREADER version 1.3.10 for 50,000 MCMC generations with the first 10,000 discarded as burn-in. The figure was generated using LibreOffice Calc version 7.3.7.2 and edited in Inkscape version 1.1.2. Each individual sample is represented by a unique 1-2 letter and single number code given at the bottom of the x axis. Above the code, we indicated the individual's source population, or its taxonomic identity for control/nonfocal populations of *Arctostaphylos purissima*, and *A. crustacea* (see Supplemental Materials 1 for more information about samples). The y axis indicates the genotype percentage of the two ancestral groups (colored as green and orange) inferred for each sample.

nucleotide polymorphisms (SNPs). We observe minimal variation among these replicate analyses suggesting that the results are robust.

The parametric clustering analysis, STRUCTURE, Figure 1.3 shows results for all samples at $K = 2$. Results for higher values of K are not supported, and the additional ancestral groups occur at such low percentages that their plots look practically identical to Figure 1.3 (Supplemental Materials 3). The two K clusters are correlated with major ITS-clade membership. All *A. purissima* (large clade) samples are 100% associated with the same ancestral group, depicted in orange. The *A. crustacea* samples, the other large

clade taxon, are mostly composed of the orange ancestral group but always have at least a small amount of admixture from the second ancestral group, depicted in green. The samples from the Nipomo Mesa are entirely associated with the green ancestral group. Although the samples currently classified as *A. rudis* (small clade) are primarily associated with the green ancestral group, the same 12 samples (from Point Sal and Burton Mesa) that are intermediate in the principal component analysis are recovered with substantial admixture from the orange ancestral group.

MORPHOLOGICAL ANALYSIS

The samples cluster morphologically in similar groups to those revealed by genetic analyses. Because all samples were not at the same phenological stage at the time of collection, we focused on a set of continuous and categorical vegetative traits that were available for all samples. Principal component analysis of these data (Figure 1.4) reveals a broadly similar pattern to the genomic PCA (Figure 1.2). The main distinction from the genetic data is the lack of a discrete cluster of hybrid samples. The data separate into three main morphological groups represented by the ellipses in Figure 1.4. *A. crustacea* and *A. purissima* samples separate into two clusters regardless of population of origin, and the third cluster consists of the samples from the Nipomo Mesa. The hybrid samples from Point Sal and Burton Mesa, depicted as yellow and blue circles respectively, have a

significant amount of morphological variability. These samples group with either samples from the Nipomo Mesa, *A. crustacea*, or they are intermediate between the three clusters.

We confirmed morphological distinctions between *Arctostaphylos* from the Nipomo Mesa and other populations of *A. rudis* during additional field surveys. At the Nipomo Mesa we found 10 multi-individual occurrences of *A. nipumu* fragmented by urban and agricultural development (Figure 1.5). We estimate that these occurrences amount to 300–700 individuals occupying 28.63 hectares of land. Plants from the Nipomo Mesa were readily distinguishable from other *A. rudis* by their lack of basal burls and different bark phenotype. All plants we surveyed at Burton Mesa had basal burls and also have varying degrees of smooth red bark in their younger branches. In contrast, plants from the Nipomo Mesa tended to be erect or layering but lack basal burls and generally have gray shredding bark even in younger branches.

The only exceptions we saw were in the undeveloped areas along the north side of Black Lake Canyon Drive in the vicinity of the Blacklake Golf Resort, which had a mixture of tall individuals lacking burls and with gray, shredding young branches, and short, mounding individuals, with smooth, red young branches and often with large, exposed basal burls. We subsequently verified that that area is a mitigation site required by the County of San Luis Obispo in conjunction with a nearby golf course and housing development (San Luis Obispo County 1982). There was no burn site at the Nipomo

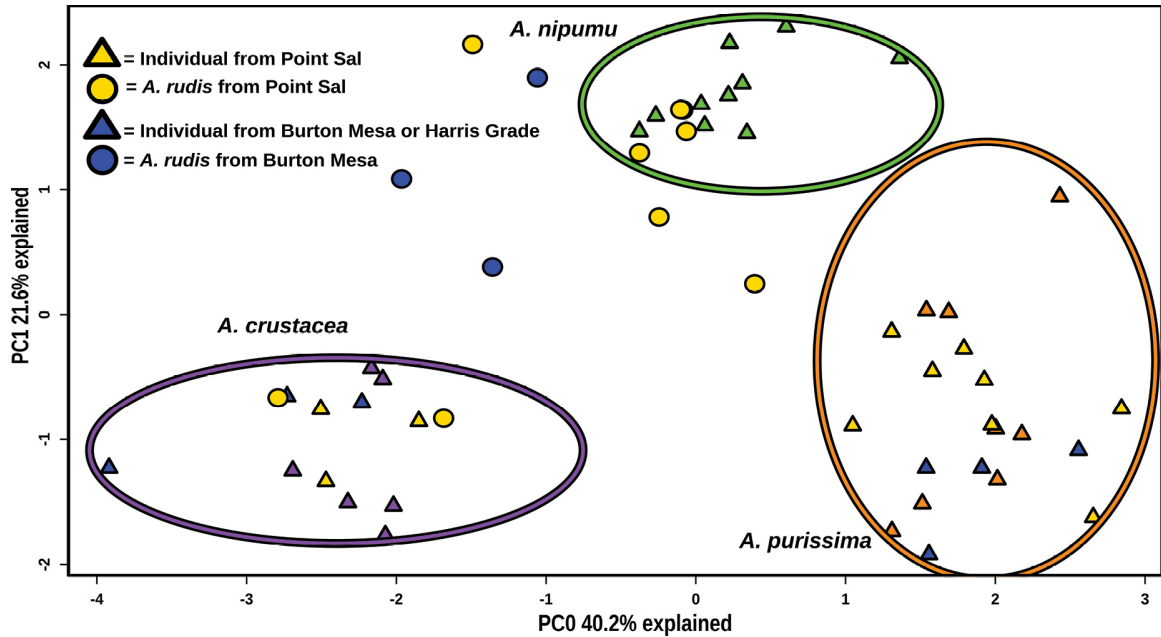


Figure 1.4: Principal component analysis (PCA) of morphological data. The figure was generated in RStudio version 2024.04.2, Build 764 and was edited in Inkscape version 1.1.2. PC0 is given in the x axis and explains 40.2% of variation in the data, while PC1 in the y axis explains 21.6% of variation in the data. Ellipses were added manually in Inkscape to highlight groups of points that align morphologically with *Arctostaphylos nipumu* (green), *A. crustacea* (purple), *A. purissima* (orange) and were based on the spread of green, purple and orange triangles (representing samples taxonomically identified as *A. nipumu*, *A. crustacea*, and *A. purissima* respectively). Samples from the focal populations of Point Sal (yellow), and Harris Grade and Burton Mesa (blue) are color-coded by their source populations. Yellow and blue triangles are samples of *A. purissima* or *A. crustacea* and have no evidence of hybridization in the molecular data. Yellow and blue circles are samples of *A. rudis* and are hybrids according to molecular data.

Mesa so we could not confirm that *A. nipumu* is unable to sprout after fire. The morphology at the *A. rudis* type locality, Point Sal, is more complex and less consistent than the morphologies observed at the Nipomo Mesa and Burton Mesa, consistent with the higher levels of introgression we detect at Point Sal compared to Burton Mesa (Figure 1.3).

DISCUSSION

We find unidirectional introgression from the large clade to the small clade at the type locality of *A. rudis* and at Burton Mesa. This is consistent with the hypothesis that some reproductive isolation exists between the three focal species but this isolation is incomplete. All samples of *A. rudis* from these populations form an intermediate cluster in principal component analysis of SNP data (Figure 1.2) and have highly to moderately admixed genomes according to STRUCTURE analysis (Figure 1.3). Furthermore, these samples are morphologically variable (Figure 1.4) as would be expected for early generation hybrids with unequal levels of introgression. Although some signatures of admixture were also detected in *A. crustacea*, these were detected in all sampled populations of this species suggesting this is an older fingerprint of hybridization, more or less stabilized in the genome, and not associated with our focal sites.

Therefore, we conclude that strong signals of hybridization are present in *A. rudis* from Point Sal and Burton Mesa, and this hybridization appears to be associated with multispecies populations because it is absent in the genome of the population from the Nipomo Mesa. Both the morphological and genetic data indicate that the *A. rudis* individuals from Point Sal and Burton Mesa are introgressed with genes from another taxon, potentially *A. crustacea*, or some locally extinct lineage. Meanwhile, the Nipomo Mesa population of *Arctostaphylos* is morphologically and genetically distinct from the other *A. rudis* populations. Because its type locality is Point Sal, the name *A. rudis* refers

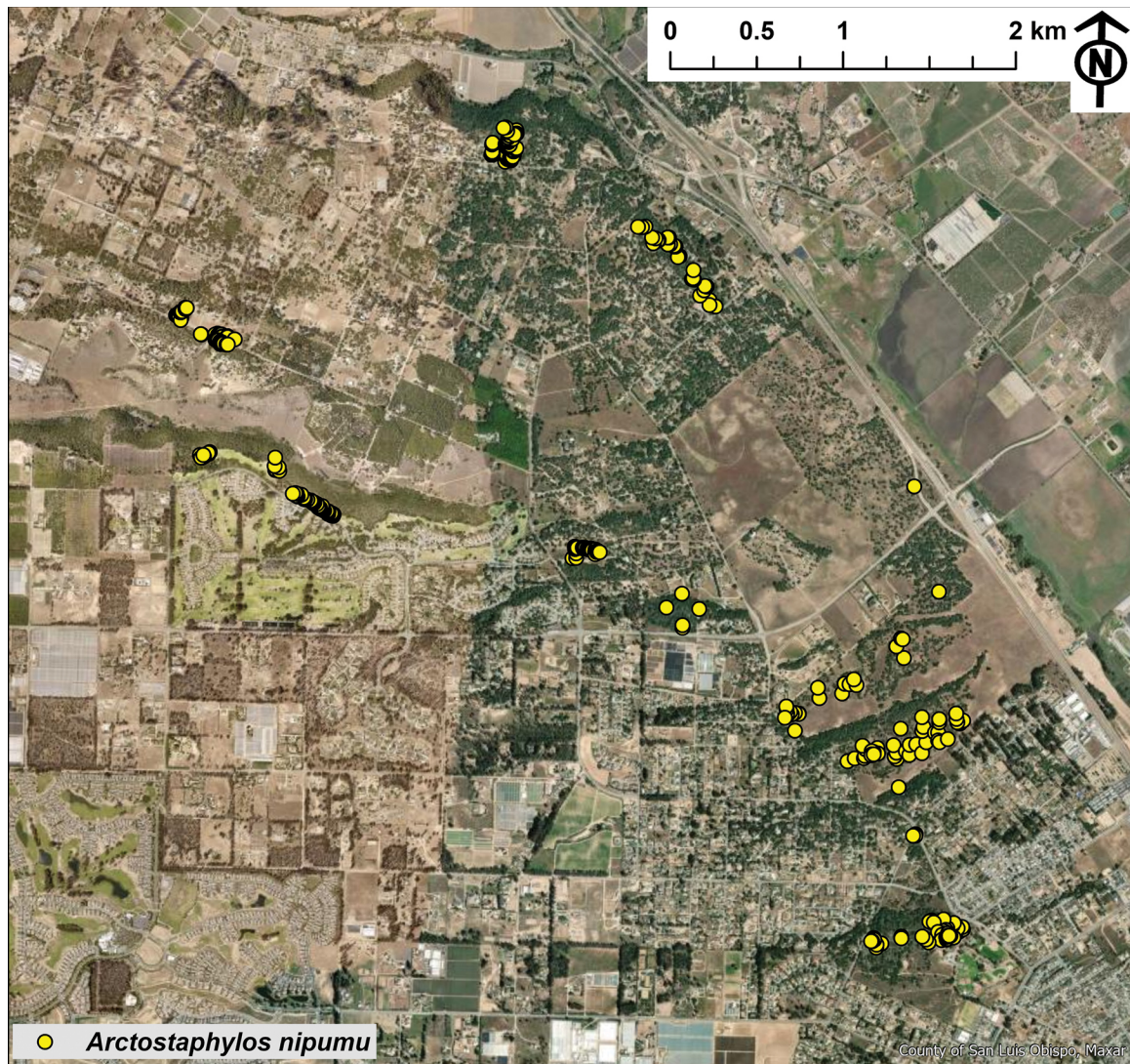


Figure 1.5: Known occurrences and distribution of *Arctostaphylos nipumu*. Nipomo Mesa, San Luis Obispo County, California. Map figure was produced using ArcGIS Pro 3.3.2 (Esri Inc 2024). Coordinate data for individual occurrences of *A. nipumu* were converted to point data shapefiles and mapped using the NAD 1983 (2011) StatePlane California V FIPS 0405 (US Feet) coordinate system.

to hybrid individuals within a hybrid zone. The *Arctostaphylos* species from the Nipomo Mesa is likely one of the parental species of these hybrid individuals but is distinct from, and lacks, the hybrid signal associated with *A. rudis*. We therefore assign this new species the name:

***Arctostaphylos nipumu* T.Abbo, M.A.Stickrod, A.Krohn, V.T.Parker, M.C.Vasey, W.Waycott & A.Litt., sp. nov.** urn:lsid:ipni.org:names:77355280-1 Figures 6, 7

Type. United States of America • California: San Luis Obispo County, Nipomo Regional Park, 200 m southwest (260°) from parking area exit onto Pomeroy Rd., Oceano 7.5' Quad: 35°01'57.5"N, 120°30'01.8"W, ± 55 m, 114–124 m alt., 28 Dec 2023, *MA Stickrod 135* (**holotype:** UCR!; isotype: SFSU!). **Paratypes** (all from San Luis Obispo County, for coordinates and collection dates see Suppl. Material 1): • Nipomo Regional Park, MA Stickrod 126 (UCR; SFSU) • same locality, MA Stickrod 128 (UCR; SFSU) • same locality, MA Stickrod 130 (UCR; SFSU) • same locality, MA Stickrod 131 (UCR; SFSU) • same locality, MA Stickrod 134 (UCR; SFSU) • same locality, MA Stickrod 128 (UCR;

SFSU) • same locality, T Abbo 147 (UCR; CAS; MO; NY; OBI) • same locality, T Abbo 148 (UCR; DAV; UCSB) • Blacklake, Golf Ball Rd., T Abbo 149 (UCR; IRVC; SBBG; SD) • Blacklake, Black Lake Canyon Dr., T Abbo 150 (UCR) • Los Berros Ridge, Dale Ave., T Abbo 153 (UCR; OBI; RSA) • southeast of Los Berros; Summit Station Rd., T Abbo 154 (UCR; UCSB).

Description. Shrub up to 2, 3 (5) m ht. and < 10 m width; often layering, rooting when branches contact soil; burl 0 but with branchlets sprouting infrequently on stems; bark (red tinged) gray and shredding, from large stems up to new growth; twigs and petioles with moderately to very dense, short, nonglandular hairs; leaves isofacial (with stomata on both surfaces), green, shiny, generally lanceolate to ovate (elliptic to rounded with mucronate tip); blade 1.4–3.2 cm length, 0.9–1.9 cm width; petiole 0.4–0.8 cm; inflorescence a raceme or few branched panicle (generally < 5-branched), nascent and mature inflorescences of similar length and thickness, 0.5 to 1.1 cm, and ca. 1 mm; bracts ± scale-like, often grading to ± leaf-like proximally, generally green, photosynthetic in summer; flowers 5-merous, urn-shaped, white to pinkish-white; fruit a multi-seeded drupe, generally reddish-orange, depressed axially; mesocarp mealy; endocarp generally rough, fused or separating into a variable number of nutlets.

Etymology. Based on discussions with the yak tityu tityu yak tilhini (Northern Chumash Tribe), we selected the name *Arctostaphylos nipumu* because nipumu is the ytt (Northern

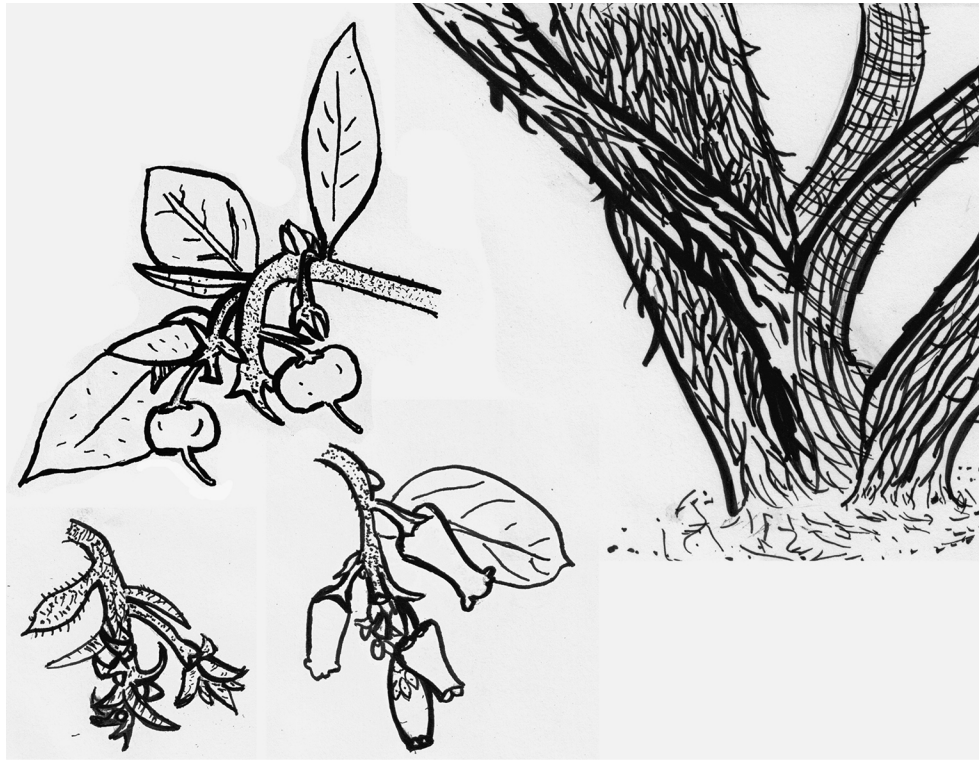


Figure 1.6: Line drawing of *Arctostaphylos nipumu*. Top right (mature main trunk): bark shredding; base lacking burl. Top left (fruiting branch): twigs and petioles with moderately to very dense, short, nonglandular hairs; leaves generally lanceolate to ovate; fruit depressed. Bottom Left (nascent inflorescences): nascent inflorescences short/compressed; bracts overlapping, $\pm$ scale-like. Bottom center (flowering branch): inflorescences short, $\pm$ same length as nascent inflorescences; flowers urn-shaped.

Chumash language) word for the Nipomo Mesa region. The word *nipumu* is literally translated to English as “of the big house”, so we treat the epithet *nipumu* as a noun in apposition; ergo, *Arctostaphylos nipumu* is translated as “*Arctostaphylos* of the big house”. We recommend that *A. nipumu* be referred to by the common name *nipumu manzanita* or *Nipomo Mesa manzanita*; the latter regional name, *Nipomo Mesa*, is more well known in current usage, but the word *Nipomo* is an inferior Spanish transliteration of the word *nipumu*.

Distribution. *Arctostaphylos nipumu* is one of the most narrowly distributed *Arctostaphylos* species, occurring exclusively on Oceano series soil (Soil Survey Staff, USDA) at 100–200 m elevation in the sandy maritime chaparral and adjacent *Quercus agrifolia* woodlands of the Nipomo Mesa. It is the only member of the genus found in this area, and its isolated population appears to be associated with two local waterways, the Santa Maria River to the south, and Arroyo Grande Creek to the north (Figure 1.1, inset). Its range is situated within a disjunction in the distribution of *A. crustacea* and just north of the northern edge of the distribution of *A. purissima*. Little is known about the biogeography of *Arctostaphylos* species, so it is unclear what has led to this distribution pattern, which is a topic we are currently investigating.

Notes. *A. nipumu* is most reliably distinguished from the two populations of *A. rudis* using its bark characteristics. *A. nipumu* has gray to reddish-gray bark that shreds in short

strips generally all the way to its young branches; the young branches may become progressively redder but the outer bark remains a dull gray or brown that contrasts with the inner bark. The older stem bark of *A. rudis* may appear similar to that of *A. nipumu* but often is redder and shreds in wider strips (Figure 1.7), and younger branches tend to be smooth dark red or if shredding, then the outer bark does not contrast with the inner

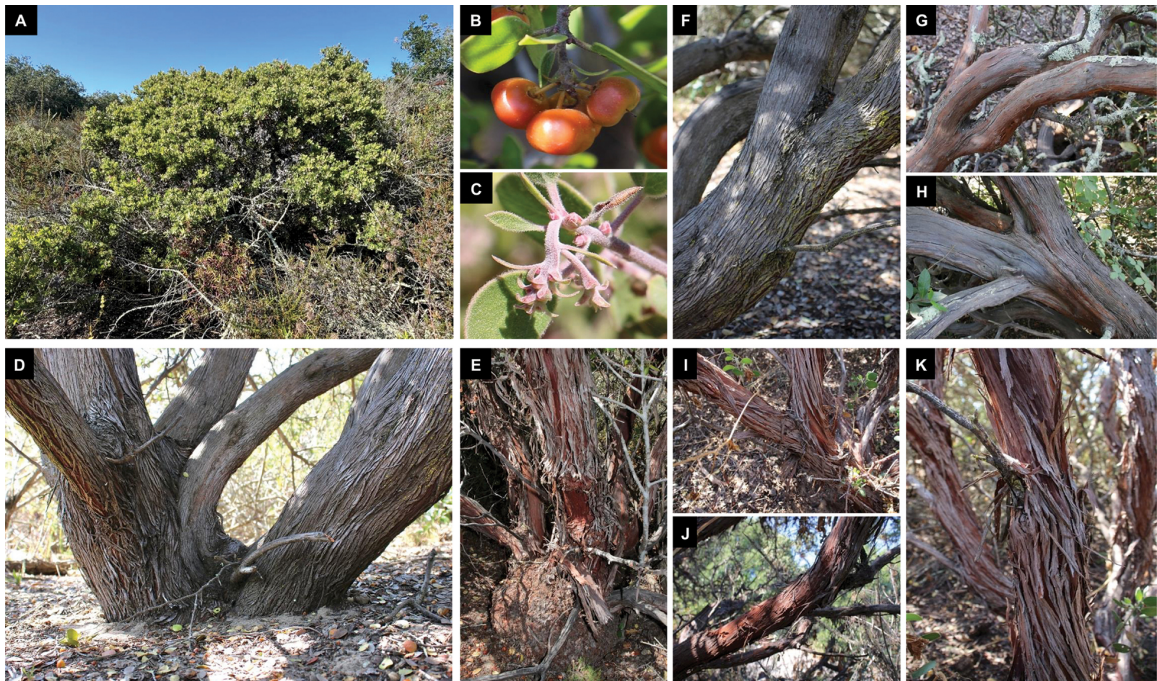


Figure 1.7: Diagnostic photographs of *Arctostaphylos nipumu* (Nipomo Mesa, San Luis Obispo County, CA) and *A. rudis* (Burton Mesa, Santa Barbara County, CA) **A** *A. nipumu* in situ, maritime chaparral habitat **B** depressed fruit of *A. nipumu* **C** nascent inflorescence, with leaf-like bracts proximally grading to scale-like bracts distally **D** *A. nipumu*, lacking a burl at the base of the stem **E** *A. rudis* with a pronounced burl at the base of the stem **F–H** gray to reddish gray, thinly shredding bark of *A. nipumu* **I–K** pronouncedly red to reddish-gray, coarsely and variably shredding bark of *A. rudis*.

bark. Consistent with its high levels of introgression (Figure 1.3), the Point Sal population of *A. rudis* is highly variable. Some individuals may appear very similar to *A. nipumu* but, in addition to having the described bark difference, will be shorter and $\pm$ mounded compared to the taller, $\pm$ erect *A. nipumu*. Other individuals may be strikingly

distinct from *A. nipumu* by having burls, long hairs, and/or truncate to lobed leaves resembling *A. crustacea*, whereas *A. nipumu* lacks a burl, always has short hairs, and typically has lanceolate to ovate leaves (never truncate or lobed). In contrast, *A. rudis* individuals from the Lompoc area (represented in our molecular data by the Burton Mesa population) tend to be more similar to each other as well as to *A. nipumu*. They are, nonetheless, easily distinguishable from *A. nipumu* because they have the pronounced bark difference and consistently have burls. The lack of a burl in *Arctostaphylos* from the Nipomo Mesa was independently observed by Keil and Hoover (2022) who noted in the Vascular Plants of San Luis Obispo County the inconsistency with Jepson's description of *A. rudis* and the plants on the Nipomo Mesa, writing: "repeated unsuccessful search has been made for the basal burl which is said in the original description to be present. Apparently the presence or absence of that structure is not a distinctive feature of this species." (p. 448).

While the Point Sal population appears to be composed of recent first and second generation hybrid individuals, the Burton Mesa (Harris Grade south) population of *A. rudis* has lower percentages of admixture (Figure 1.3). Currently, we are further investigating the Point Sal and Burton Mesa populations. The questions we are considering include: what is the extent of hybridization among individuals, are these populations genetically stable and uniform, and is monophyly present in a large sample of

individuals. For now, we recommend that these populations continue to be referred to under the name *A. rudis* with the understanding that the name refers to hybrid individuals.

Conservation considerations. *Arctostaphylos nipumu* is extremely limited and restricted to the Nipomo Mesa, and, consequently of great conservation concern, especially as the subpopulations are mostly surrounded by development. Based on our estimates from satellite images, the vast majority of wild lands on the Nipomo Mesa have been converted into dwellings and agricultural fields. Figure 1.5 shows the estimated land occupied by *A. nipumu* based on field surveys and satellite images. Thus, the range of *A. nipumu* consists of only 28.63 hectares distributed across 10 fragmented multi-individual occurrences, with a total population estimated to be 300–700 individuals. Similar land use change led to the near extinction of *A. franciscana*, which is now represented by a lone individual in the wild and clones of just a few genetic individuals (Gluesenkamp et al. 2010; Weiss et al. 2020). The genus *Arctostaphylos* reliably dates back to western Nevada in the Middle Miocene in a climate more similar to the California coast than present (Edwards 2004; Millar and Woolfenden 2016). Conserving the integrity of the genome of *A. nipumu* could facilitate future research testing the hypothesis that this species represents a case of paleoendemism that may provide insight into broader patterns of diversification in the genus.

We would like to emphasize that the characters used to separate *A. nipumu* from *A. rudis* are readily apparent. We were able to immediately distinguish the ca. 53 relictual *A. nipumu* individuals from the ca. 168 *A. rudis* individuals which we believe were planted as a mitigation measure to develop the Blacklake Golf Resort, as well as the restoration planting along Osage Street on the Nipomo Mesa. The ecological impact report in the Black Lake Specific Plan recommended that *Arctostaphylos rudis* be planted in parts of the Blacklake Golf Resort with low vegetation cover, not used as turf (San Luis Obispo County 1982: IV-33). Based on their burls and smooth red young bark, we propose that the majority of plants at these sites were planted cultivars originally sourced from the Burton Mesa or Point Sal; however, the origin of the nursery stock used for restoration at this site remains uncertain. Even when sound ecological considerations are implemented, mitigation and revegetation efforts such as these have often historically neglected to consider potential genetic impacts on native plants and population structure through processes such as genetic swamping, demographic swamping and outbreeding depression, among others (Hufford and Mazer 2003, Prentis et al. 2007, Byrne et al. 2011). The integrity of the genome of *A. nipumu* appears to depend on its isolation from other *Arctostaphylos* species, and these introduced plants have the potential to hybridize with *A. nipumu*. Because of their potential to hybridize, allopatry is hypothesized as an important factor maintaining the separation of *Arctostaphylos* species within the same clade (Parker et al. 2020). This stresses the importance of utilizing plants with local genotypes for propagation in restoration and mitigation projects (Maschinski and

Albrecht 2017). Below is a key of *Arctostaphylos* species from San Luis Obispo and Santa Barbara Counties to aid in the identification of *A. nipumu*.

Key

- 1a Burl present, plants resprouting after fire2
- 2a Leaves isofacial, similar in color and hairiness and with stomata on both surfaces3
- 3a At least some twig hairs glandular.....*A. glandulosa* ssp. *glandulosa*
- 3b Twig hairs nonglandular.....4
- 4a At least some twig hairs long.....*A. glandulosa* ssp. *mollis*
- 4b Twig hairs short.....5
- 5a Older stem bark gray, shredding.....*A. rudis*
- 5b Older stem bark red, smooth6
- 6a Endocarp fused into rough, depressed, often imperfectly symmetrical structure, nutlets requiring force to separate.....*A. glandulosa* ssp. *gabrielensis*
- 6b Endocarp of free or easily separable nutlets....*A. glandulosa* ssp. *cushingiana*
- 2b Leaves bifacial, surfaces differing in color or hairiness, with stomata only abaxially7
- 7a Older stem bark grey, shredding.....8

8a At least some twig hairs long.....	<i>A. tomentosa ssp. daciticola</i>
8b Twigs hairs short, tomentose only	<i>A. tomentosa ssp. tomentosa</i>
7b Older stem bark red, smooth	9
9a At least some twig hairs glandular.....	<i>A. crustacea ssp. subcordata</i>
9b All twig hairs nonglandular	10
10a At least some twig hairs long.....	<i>A. crustacea ssp. crustacea</i>
10b All twig hairs short	11
11a Leaves densely hairy abaxially	<i>A. crustacea ssp. insulicola</i>
11b Leaves glabrous to very sparsely minute pubescent abaxially	12
12a Pedicels and ovary hairy	<i>A. crustacea ssp. eastwoodiana</i>
12b Pedicels and ovary glabrous to very sparsely minute pubescent.....	
.....	<i>A. crustacea ssp. rosei</i>
1b Burl absent, plants not resprouting after fire or resprouting ability unknown (<i>A. nipumu</i> , <i>A. rudis</i> [Point Sal])	13
13a Leaves bifacial, surfaces differing in color or hairiness, with stomata only abaxially	14
14a Young leaves tomentose abaxially.....	<i>A. morroensis</i>
14b Young leaves glabrous to very sparsely minute pubescent abaxially.....	
.....	<i>A. insularis</i>

13b Leaves isofacial, similar in color and hairiness and with stomata on both surfaces	15
15a Endocarp always fused into smooth, sphere	16
16a Leaves deeply lobed, auriculate to subauriculate	17
17a At least some twig hairs glandular.....	<i>A. refugioensis</i>
17b Twig hairs nonglandular.....	<i>A. pechoensis</i>
16b Leaves unlobed to shallowly lobed, not auriculate or subauriculate	18
18a Leaves glaucous.....	<i>A. glauca</i>
18b Leaves bright green.....	<i>A. parryana ssp. parryana</i>
15b Endocarp of free to separable nutlets in at least some fruits, and when $\pm$ fused generally not a smooth sphere.....	19
19a Older stem bark gray, shredding.....	20
20a Leaves deeply lobed, auriculate to subauriculate	<i>A. osoensis</i>
20b Leaves unlobed to shallowly lobed, not auriculate or subauriculate	21
21a Younger stem bark smooth, red or if shredding, generally dark brown and outer bark not contrasting in color with inner bark	<i>A. rudis</i>
21b Younger stem bark generally shredding, often gray, sometimes lighter red, then outer bark strongly contrasting in color with inner bark.....	<i>A. nipumu</i>
19b Older stem bark red, smooth	22
22a Bracts scale-like	23

23a Plant prostrate to mounded.....	<i>A. hookeri</i> ssp. <i>hearstiorum</i>
23b Plant erect.....	<i>A. pungens</i>
22b Bracts leaf-like.....	24
24a At least some twig hairs glandular.....	25
25a Leaves deeply lobed, auriculate to subauriculate....	<i>A. purissima</i> ssp. <i>globosa</i>
25b Leaves unlobed to shallowly lobed, not auriculate or subauriculate	
.....	<i>A. confertiflora</i>
24b Twig hairs nonglandular.....	26
26a Plant prostrate to mounded.....	<i>A. cruzensis</i>
26b Plant erect.....	27
27a Leaves gray, canescent.....	28
28a Leaves deeply lobed, auriculate to subauriculate	<i>A. luciana</i>
28b Leaves unlobed to shallowly lobed, not auriculate or subauriculate	
.....	<i>A. obispoensis</i>
27b Leaves green.....	29
29a Leaves unlobed to shallowly lobed, not auriculate or subauriculate	
.....	<i>A. pilosula</i>
29b Leaves deeply lobed, auriculate to subauriculate	30
30a Pedicels with moderately dense nonglandular hairs	<i>A. viridissima</i>
30b Pedicels glabrous or with sparse nonglandular hairs.....	31

- 31a Leaves ≥ 2 cm; fruit ≥ 8 cm wide; nutlets generally requiring force to separate *A. pechoensis*
- 31b Leaves ≤ 2.5 cm; fruit ≤ 8 cm wide; nutlets easily separable to free.....
 *A. purissima* ssp. *purissima*

CONCLUSION

Hybridization plays a key role in the evolutionary dynamics of *Arctostaphylos* species. Differences in ploidy and genetic distance impose incomplete reproductive isolation between species that occur in sympatry. *Arctostaphylos purissima* and *A. crustacea* remain genetically and morphologically distinct in sympatry, while *A. nipumu* is replaced by the hybrid *A. rudis* when it occurs with *A. purissima* and/or *A. crustacea*. *A. nipumu* is able to persist as a distinct species apparently because of its geographic isolation from other *Arctostaphylos* on the Nipomo Mesa. Its habitat is fragmented and should be considered as a high priority for conservation efforts. Future work should investigate its demographic history to better understand the patterns that led to its isolation and the circumstances that led to its hypothesized extinction by hybridization south of the Santa Maria River.

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AUTHOR CONTRIBUTIONS

Conceptualization: AK, VTP, WW, MCV. Data curation: TA. Formal analysis: TA, AK, MAS. Investigation: WW, TA, MAS, AL, MCV. Methodology: AL, MCV, VTP, AK. Resources: AL, WW, MCV, AK. Supervision: AL. Writing - original draft: TA. Writing - review and editing: MCV, AL, WW, AK, MAS, VTP.

DATA AVAILABILITY

All of the data that support the findings of this study are available in the main text or Supplementary Information. Raw DNA sequences are publicly available in the NCBI Sequence Read Archive (SRA) under the project number PRJNA1208541.

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CHAPTER 2: CHAPARRAL SHRUBS EVEN MORE ENIGMATIC THAN
PREVIOUSLY THOUGHT: DEL MAR MANZANITA AND THE *ARCTOSTAPHYLOS*
'PROBLEM CHILDREN' OF SOUTHERN CALIFORNIA

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ABSTRACT

Molecular support for taxonomic boundaries is essential for conservation, especially when morphological evidence is inconclusive and hybridization is suspected as a confounding factor. Hyperdiverse plant genera commonly have subtle traits that distinguish constituent taxa, and not all authorities may agree on the number of taxa to recognize. *Arctostaphylos* (Ericaceae) is a charismatic genus that has also shown notorious difficulty in species identification and delimitation. *Arctostaphylos glandulosa* ssp. *crassifolia*, Del Mar manzanita (DMM), is one such case. It inhabits a threatened ecosystem along the coast of San Diego County, California, and is currently protected under the US Endangered Species Act, but previous research provided conflicting evidence regarding whether it should be considered a distinct taxon. We sampled 181 *Arctostaphylos* shrubs, focusing on Southern California narrow endemics A.

rainbowensis, *A. otayensis*, and DMM, as well as neighboring plants with affinities to *A. glandulosa*. We used ddRAD sequencing and analyzed the data using maximum-likelihood phylogenetic analysis, clustering, and principal component analysis. Results were ambiguous in that we found a distinct genetic signal for DMM, but it may be linked by hybridization with closely related taxa from the Peninsular Ranges. The signal for DMM is only present at its type locality on the coast of San Diego County.

Arctostaphylos glandulosa as a whole is recovered as paraphyletic throughout the sampled range and requires substantial further study in regions outside of the Peninsular Ranges and adjacent coast. We find evidence of genetic distinctiveness for DMM and clearly define its range, thereby identifying the areas land managers should prioritize for conservation.

INTRODUCTION

The ability to identify and distinguish species and other natural groups is fundamental to ecology, evolution, conservation, land management, and particularly classification and taxonomy. In some groups, especially those that have undergone extensive diversification, identification can be problematic. Related taxa may be similar in appearance, potentially only reliably told apart by the different environments they inhabit or reproductive characters that are only available for short windows of time. Molecular data and phylogenetic methods have significantly enhanced our ability to define species boundaries, and these methods are increasingly being applied to questions of species

circumscription that have a direct impact on conservation and management practices (e.g., Murray et al. 2012; Luo et al., 2018; Young et al., 2021; Douglas and Bouckaert, 2022). These methods rely on underlying similarities and differences in DNA sequences, which are inherently more objective than morphologically based methods. With the combined power of these identification methods, understanding the structure of genetic diversity is becoming more attainable, although there are still existing issues.

Arctostaphylos Adans., commonly known as manzanita, exhibits all of the complexities in morphological identification and delimitation mentioned above, making it an ideal system for using molecular approaches to evaluate species and subspecies delimitations. The genus is in the Arbutoideae subfamily of Ericaceae, and consists of approximately 61 species and 111 taxa (Parker et al., 1993+; Kauffmann et al., 2021; Parker et al., 2023; Abbo et al., 2025). Its center of biodiversity is the California Floristic Province (CFP). Most *Arctostaphylos* species are endemic to this region, and only a small number of species can be found outside of the CFP (Parker et al., 1993+; Kauffmann et al., 2021). Manzanitas have long been a subject of intrigue as an example of plant diversification (see eg: Raven and Axelrod, 1978 p. 78-81). The genus is known for its conspicuously red bark and sinuously twisting branches, with clusters of pendant, urn-shaped, white-pink flowers followed by small, red-brown fruits that give the plant its common name, which translates from Spanish to “little apple.” Although there is variation in features such as growth habit, leaf shape, and hair traits, manzanitas in general are similar

looking, and identification can be challenging. In fact, a common strategy for identifying manzanitas is to rely on location (i.e., where the plant was observed) to limit the number of candidate species and subspecies (Kauffmann et al., 2021). Hybridization is also documented and thought to be widespread, resulting in intermediate and variable phenotypes that further complicate identification and bring into question the boundaries among taxa (Schierenbeck et al., 1992; Parker et al., 2020; Serkanic et al., 2021; Abbo et al., 2025). Attempts to apply molecular data and phylogenetic methods to understanding manzanita diversity have focused on the nuclear internal transcribed spacer (ITS) locus, but have been unable to resolve evolutionary relationships among manzanitas other than to identify two early-diverging clades (e.g., Markos et al., 1998; Boykin et al., 2005; Wahlert et al., 2009). Furthermore, these studies generally included only one individual per taxon; therefore, they were unable to address questions of species and/or subspecies delimitation.

Arctostaphylos contains numerous taxa that are of conservation concern, with four listed as federally endangered (Fish and Wildlife Service, 2021; California Natural Diversity Database [CNDDB]), six state-listed as endangered (CNDDB), and 50 listed by the California Native Plant Society, Rare Plant Program as rare, threatened, or endangered (CNPS). Thus, the ability to identify these endangered and threatened taxa is critical for conservation resource allocation and for evolutionary biologists seeking to understand diversification within the group. An example of these difficulties in identification is

Arctostaphylos glandulosa spp. *crassifolia* (Jepson) P.V. Wells (Figure 2.1 A, B), a subspecies of *A. glandulosa* (Eastwood manzanita) commonly known as Del Mar manzanita (DMM), one of the federally listed endangered taxa. DMM was first described by Jepson (1922) as *Arctostaphylos glandulosa* var. *crassifolia* with the diagnosis: “leaves elliptic, thick, 8 to 12 lines long” and the type locality as “Del Mar” (San Diego County, California). This very general diagnosis and the lack of range details have led to a history of debate regarding how to identify and where to locate DMM. Adams (1940) opined that the key diagnostic feature was leaf color and that DMM was distributed in sandy areas in coastal San Diego County, possibly extending into Baja California. Knight (1981) proposed that DMM referred to various hybrid individuals rather than a distinct taxon, but Wells (1987) later defended his recognition of *A. glandulosa* spp. *crassifolia* based on its geographic isolation from other subspecies of *A. glandulosa*.

Between the 1970s and 1990s, multiple attempts were made to list DMM as a federally endangered taxon due to the increasing loss of its habitat, the maritime chaparral ecosystem, caused by urbanization and agricultural land conversion. However, the plant did not receive protection under the US Endangered Species Act (ESA) until 1996 (Fish and Wildlife Service, 1996). Ultimately, the primary argument for recognizing DMM as a distinct taxon in the successful 1996 ESA listing petition was its long history of recognition in the California flora; however, it was still unclear precisely what constituted DMM. It was listed with identifying characteristics of a relatively short height and

differences in hairiness between the upper and lower leaf surfaces (Fish and Wildlife Service, 1996). However, the identifying features adopted in current floras, including the Flora of North America North of Mexico (Parker et al., 1993+) and the Jepson eFlora (Parker et al., 2023), are based on Keeley et al. (2007), who pointed to dark green leaves (inconsistently with reddish margins), nonglandular hairs, and strongly depressed to flattened fruits as diagnostic morphological characters. The USFWS treatment also included several interior populations that fell outside the range specified by Keeley et al. (2007).

Previous molecular data have also led to conflicting or inconclusive interpretations regarding the status of DMM. Burge et al. (2018) applied phylogenomic methods in an effort to define what is and is not DMM. Using RADseq, they identified a signal of genetic differentiation that separated *A. glandulosa* individuals from in and around the DMM type locality from those collected elsewhere, such as interior populations and plants from Baja California, Mexico. This suggested that DMM might be a distinct taxon that does not extend more than around c.14 km beyond where Jepson first discovered it. In contrast, another ddRADseq study by Huang et al. (2020) did not find genetic distinctions between any of the subspecies of *A. glandulosa*, with the exception of *A. glandulosa* ssp. *gabrielensis*.

While carrying out preliminary phylogenomic genus-wide analyses, we observed a third result, which agreed that most *A. glandulosa* subspecies were not monophyletic, but also found that DMM formed a distinct clade that, unexpectedly, was most closely related to the Peninsular Range endemics *A. rainbowensis* (Figure 2.1 C) and *A. otayensis* (Figure 2.1 D, G), as well as a population of *A. glandulosa* ssp. *cushingiana* (Figure 2.1 F) from Otay Mountain. This preliminary finding, based on only three individuals of DMM, suggested that a more thorough sampling of the manzanitas in this region was needed to understand the nature and relationships of this critically endangered group of plants. The other closely related species include *Arctostaphylos rainbowensis*, a diploid (Keeley and Massihi, 1994), that is narrowly endemic to northwestern San Diego and southwestern Riverside Counties, California. *Arctostaphylos otayensis*, also diploid (Wells, 1968), is similar to *A. glandulosa* but lacks a basal burl, a woody structure that contains dormant buds, allowing some manzanita species to regenerate after fire. *Arctostaphylos otayensis* is endemic to the Otay and San Miguel Mountains in San Diego County, California, and is suspected to extend to adjacent slopes in Baja California, Mexico (Kauffmann et al., 2021). Throughout its range, it co-occurs with glandular- and nonglandular-haired forms of *A. glandulosa*, currently treated as *A. g.* ssp. *glandulosa* and *A. g.* ssp. *cushingiana*, respectively (Parker et al., 2023).

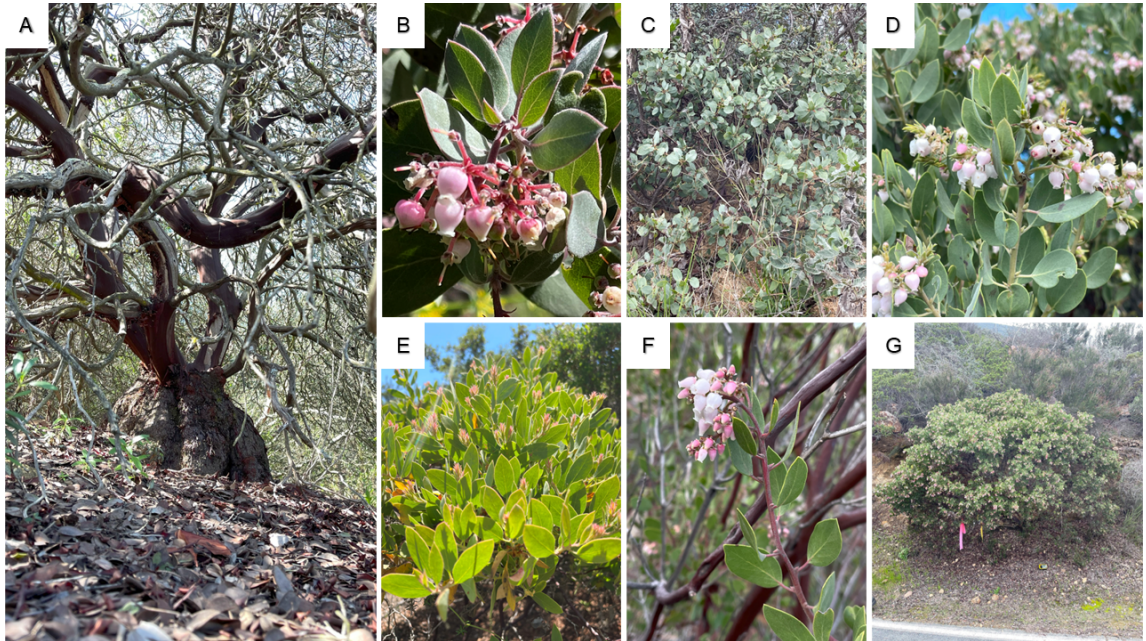


Figure 2.1: In situ photographs of manzanita species captured by Avrie Martello on an iPhone 12 Pro Max. **A.** *Arctostaphylos glandulosa* ssp. *crassifolia* (DMM) in southern maritime chaparral, Encinitas, California (33.03483 N, 117.24678 W). **B.** *Arctostaphylos glandulosa* ssp. *crassifolia* flowering inflorescence, San Diego, California (32.920657 N, 117.252721 W). **C.** *Arctostaphylos rainbowensis* in chaparral, Rainbow, California (33.412760 N, 117.119964 W). **D.** *Arctostaphylos otayensis* flowering inflorescences, Jamul, California (32.622778 N, 116.843189 W). **E.** *Arctostaphylos* aff. *glandulosa* nascent (young) inflorescences, Marine Corps Air Station Miramar, federal property within San Diego County, California (32.890176 N, 117.062594 W). **F.** *Arctostaphylos glandulosa* ssp. *cushingiana* flowering inflorescence, Jamul, California (32.622778 N, 116.843189 W). **G.** *Arctostaphylos otayensis* in *Adenostoma fasciculatum* dominant chaparral, San Miguel Mountain, San Diego National Wildlife Refuge, federal property within San Diego County, California (approximately 32.71367 N, 116.93158 W). The images were cropped and arranged into a seven panel figure in Microsoft PowerPoint Version 2505.

Our goal herein was to test our preliminary finding of support for DMM using a larger sample of individuals from the possible DMM localities and surrounding regions, including the Peninsular Ranges and its western foothills in San Diego and southwestern Riverside Counties, and the directly adjacent coast maritime chaparral. Due to its conservation concern, our primary research question is whether DMM is genetically distinct and, if so, what is the extent of its geographic range? Because hybridization and introgression appear to have been fundamental in the development of manzanita diversity, we also ask whether we find evidence of hybridization among manzanitas near

the Peninsular Ranges and beyond, and whether this helps explain the historical challenges of correctly identifying and/or classifying DMM and other neighboring manzanitas.

MATERIALS AND METHODS

COLLECTIONS AND FIELD WORK

SAMPLING

We sampled a total of 181 mature, adult shrubs in various vegetative and reproductive stages. Given our goal to test our preliminary results of close relationships between taxa from the Peninsular Ranges and South Coast of California, the majority of our sampling focused on our three focal taxa, *Arctostaphylos rainbowensis* (n=13), *A. otayensis* (n=13), *A. glandulosa* ssp. *crassifolia* (n=29), and other subspecies or individuals with affinities to *A. glandulosa* (n=69). Sampling of these taxa captured a broad geographic representation of the system starting from southwestern Riverside County to the coast of San Diego County, from Encinitas to Torrey Pines State Park, and from there inland via Poway to Otay Mountain (Figure 2.2). To understand the relationships of these taxa in the context of the overall genus, we also included samples of the widespread taxa from southwestern North America (*A. patula* [n=7], *A. pungens* [n=10], and *A. pringlei* [n=7]), a widespread California Floristic Province endemic (*A. glauca* [n=10]), and a narrow endemic to the central coast of California (*A. purissima* [n=20]). Our logic behind

selecting comparative taxa is twofold. Firstly, *A. patula*, *A. pungens*, *A. pringlei*, and *A. glauca* all have occurrences in Riverside and/or San Diego Counties, near the distributions of our focal taxa, and therefore are potentially closely related to and/or hybridize with our focal taxa. Secondly, we included *A. purissima*, as the sequence data available for this species suggests that it does not have a hybrid history (Abbo et al., 2025) and it could therefore function as a comparative control in case hybridization is detected in our three focal taxa. A map of our collected ingroup samples was generated using QGIS version 3.22.4 (Figure 2.2; QGIS Development Team, 2025). Our sampling primarily aimed to capture the ranges of our focal taxa within the Peninsular Ranges and South Coast of California; outside this region, our sampling was limited but represented a diversity of regions including the Sierra Nevada, the central coast of California, the San Francisco Bay Area, and sites in the North American southwest (including Arizona, New Mexico, and Utah). The majority of our samples were collected for this project specifically in 2024, but many samples were collected earlier (2018-2023) as part of a larger and ongoing genus-wide research project. We also sampled one individual of each of three outgroup taxa from the subfamily Arbutoideae: *Arbutus unedo* (cultivated on the UCR campus), *A. menziesii* (Santa Cruz County, California), and *Arctous alpina* (Alaska). Supplemental Materials 4 shows sample-specific metadata including GPS coordinates, collection dates, and, when applicable, information about herbarium voucher specimens.

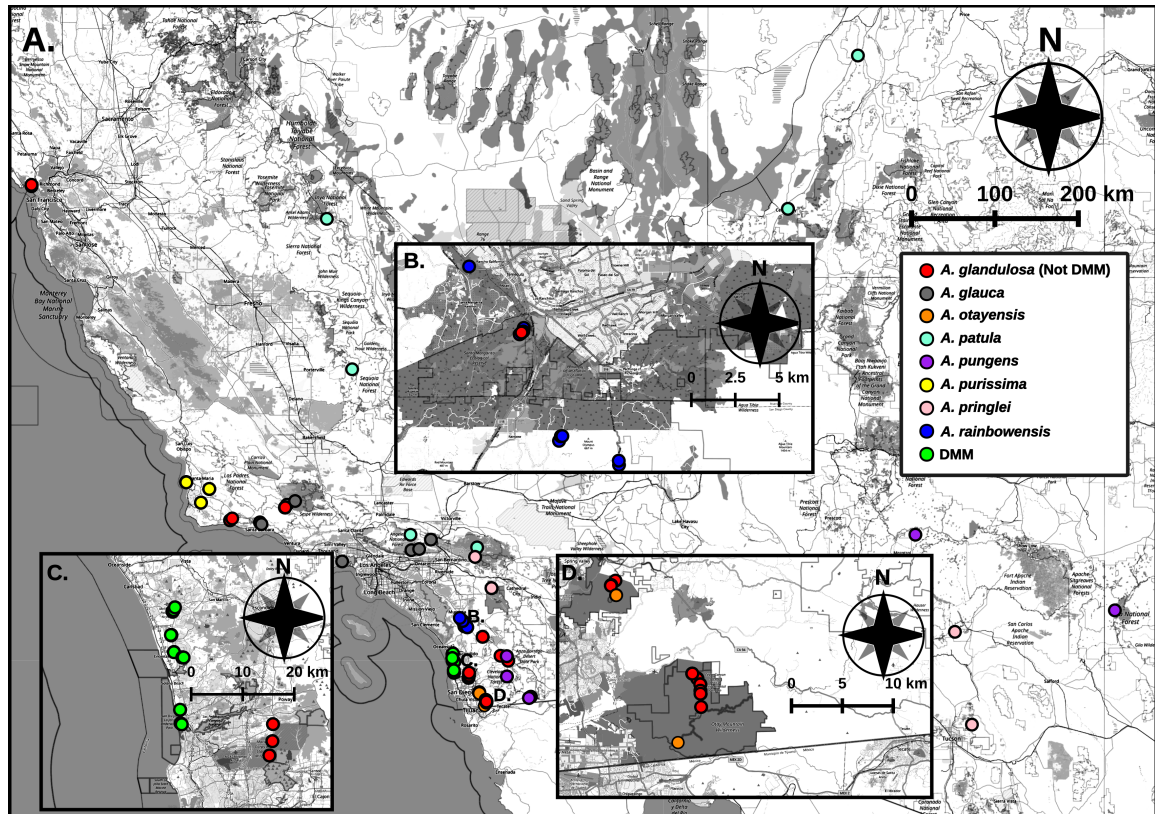


Figure 2.2: Map of sampling locations for ingroup samples. **A.** The background map of the southwestern United States was sourced from OPENSTREETMAP (OpenStreetMap contributors, 2025) using the EPSG:3857 - WGS 84 / Pseudo-Mercator projection. The acronym DMM stands for Del Mar Manzanita (*A. glandulosa* ssp. *crassifolia*). At the scales depicted here, not every single sampled point is visible when samples are <2 km apart; see Supplemental Materials 4 for a complete list and metadata of the 181 samples included in this study. Insets represent focal sampling areas in southern California (CA). **B.** Northwestern Peninsular Range, adjacent to Temecula, CA, north to Rainbow, CA, south. **C.** Coast of San Diego County, CA. **D.** Otay and San Miguel Mountains, CA. Generated in QGIS version 3.22.4 and edited in INKSCAPE version 1.1.2 (Inkscape Project, 2025).

Plant tissues were collected as described in Abbo et al. (2025), with fresh leaves or flower buds stored at -20 °C until DNA extraction. We collected a representative voucher specimen for each plant. We deposited vouchers at the UCR Herbarium, and specimen-associated data and images have been uploaded to the Consortium of California Herbaria data portal (CCH2, data portal).

ECOLOGICAL AND ABIOTIC CONTEXT

Our focal taxa are found within the Peninsular Ranges and its western foothills in San Diego and southwestern Riverside Counties, California, or are from the directly adjacent coast in maritime chaparral. The Peninsular Range is a north-south mountain range that extends from Riverside County in Southern California through the entirety of the Baja California peninsula with a maximum elevation of ca. 3300 m. It is characterized by infrequent wind-driven fires and shallow erosion-prone soils, which limits water availability for chaparral plants such as *Arctostaphylos* species to deep water stored in weathered bedrock (Graham and O'Geen, 2016 p. 64-65). In Southern California, the Peninsular Ranges also create a 'rain shadow' effect which separates coastal and mountain California Floristic Province ecosystems from the Mojave and Sonoran deserts (Minnich et al., 2000; Peryam et al., 2011). On the coastal side of the range, a climatic gradient occurs from the coast to the higher mountain elevations (Parker, 2020). The immediate coast is dominated by maritime chaparral and coastal scrub ecosystems, characterized by low rainfall and a summer marine cloud layer that ameliorates

temperature and drought stress (Vasey et al., 2012). In contrast, the higher elevations of the Peninsular Ranges experience more rainfall but greater water stress due to higher summer temperatures.

FIELD NOTES AND MORPHOLOGICAL DESCRIPTIONS

For each sample, we made detailed field observations of site characteristics, habitat, associated species, and plant-specific morphology. Additionally, we recorded the GPS coordinates and approximate elevation of each sampled plant using a Garmin model “etrex 10”. The morphological observations included the approximate height and width of the individual shrub, presence or absence of a basal burl, bark color and texture, twig hair length, density and glandularity, leaf color, phenology, and characteristics of available reproductive characters when present such as bract color and morphology, color of corolla, fruit shape and color, whether the nutlets (endocarp in *Arctostaphylos*) were fused or free, as well as miscellaneous unique features. A summary of this data is included on the label of each voucher specimen and can be accessed via CCH2.org through the links included in Supplemental Materials 4. Individuals are referred to throughout the manuscript by a unique one to three character code where at least one character is a number; more information about these samples, including links to images of herbarium specimens, is available in Supplemental Materials 4.

DDRAD SEQUENCING

From each sample, we extracted DNA using the Qiagen DNeasy Plant Mini Kit (QIAGEN, Venlo, The Netherlands) and quality-controlled the extracts using the same method as Abbo et al. (2025). We used these high-molecular-weight DNA extracts to prepare ddRADseq libraries. Given our ongoing long-term genus-wide research project, this study includes sequences from multiple sequencing batches, prepared with various iterations of our library preparation protocol. We prepared 34 samples using our original library preparation protocol published in Huang et al. (2020), and these samples were sequenced at the UCR Institute for Integrative Genome Biology Genomics Core in 2019 on an Illumina NextSeq 500 V2 (San Diego, California, USA), sequencer. This original protocol employed individual sample-PCR amplification, which was a labor-intensive process. We prepared the three outgroup samples in 2022 using a pilot modified protocol. This protocol was the same as in Abbo et al. (2025), except that instead of the minipooling strategy described therein, all libraries were pooled, size-selected, and amplified as a single large pool distributed over six replicate amplifications reactions following DNA barcode ligation. These samples were sequenced at Novogene (Beijing, China) at their facility at Davis, California, United States, on an Illumina (San Diego, California, USA) HiSeq platform. In 2023, we sequenced 18 samples at Novogene on the same sequencer, prepared with the same modified protocol. The drawback of the method was that we experienced high sample dropout. Therefore, the protocol was further optimized using the minipooling strategy, as implemented in Abbo et al. (2025), in which

samples were amplified in pools of 8-10 individuals, which were then combined after PCR. The 20 *A. purissima* samples were the same samples from that paper (NCBI project accession number: PRJNA1208541). Finally, the 106 most recent samples were prepared using the same protocol with a minor modification: the number of individuals per minipool was increased to 14. These samples were sequenced in 2025 at the UCR Institute for Integrative Genome Biology Genomics Core on an Illumina NextSeq 2000 sequencer.

MOLECULAR DATA ANALYSIS

READ PROCESSING

We processed raw reads using the IPYRAD version 0.9.95 pipeline (Eaton and Overcast 2020). As in Abbo et al. (2025), we aligned reads to the *A. glauca* reference genome (Huang et al., 2022) and used the default IPYRAD settings, except for the “min_samples_locus” (MSL) and “max_alleles_consens” (MA) parameters. The MSL was set to 30% of individuals in each dataset (Abbo et al., 2025). We analyzed three datasets: one which included all samples (“all samples” dataset, n=181, MSL=54), one which excluded samples of *A. glandulosa* (“no *A. glandulosa*” dataset, n=82, MSL=24), and one which only included samples in what we called the Peninsular Range-clade (“PR-clade” dataset [refers to a clade of samples from the Peninsular Range recovered in phylogenetic analysis], n= 116, MSL=35). *Arctostaphylos glandulosa* is considered

tetraploid, whereas our other samples are considered diploid (Parker et al., 2023).

However, we consider ploidy assignment in *Arctostaphylos* to be uncertain due to the lack of sample replication in available chromosome counts, an issue we elaborate on in the discussion. We analyzed the data with and without *A. glandulosa* samples to assess the extent to which inferences were sensitive to the inclusion of these samples. We also ran the “all samples” and “PR-clade” datasets, setting the maximum number of alleles in the consensus sequence (MA) to 4 (4MA dataset) as well as 2 (2MA dataset) to compare the results obtained assuming all samples are diploid vs allowing tri- and tetra-allelic loci in the consensus sequence.

CLUSTERING ANALYSIS

We used ADMIXTURE version 1.3.0 (Alexander et al., 2009) as a maximum-likelihood clustering analysis to identify genotypic groupings and potential signs of hybridization in the “all samples” and “no *A. glandulosa*” datasets. We initially filtered VCF files generated by IPYRAD in VCFTOOLS version 0.1.17 (Danecek et al., 2011) for biallelic single-nucleotide polymorphisms (SNPs) with a minor allele frequency of 1% or higher, and then thinned them to 1150 bp apart (approximately one randomly selected SNP per RAD locus) to account for linkage disequilibrium. We then converted the filtered VCFs to BED files using PLINK version 1.90b6.25 (PLINK 1.9; Chang et al., 2015). We then ran ADMIXTURE on the BED files for K values of one through ten using fivefold cross-validation (CV). The optimal K value was selected as the value with the lowest CV score

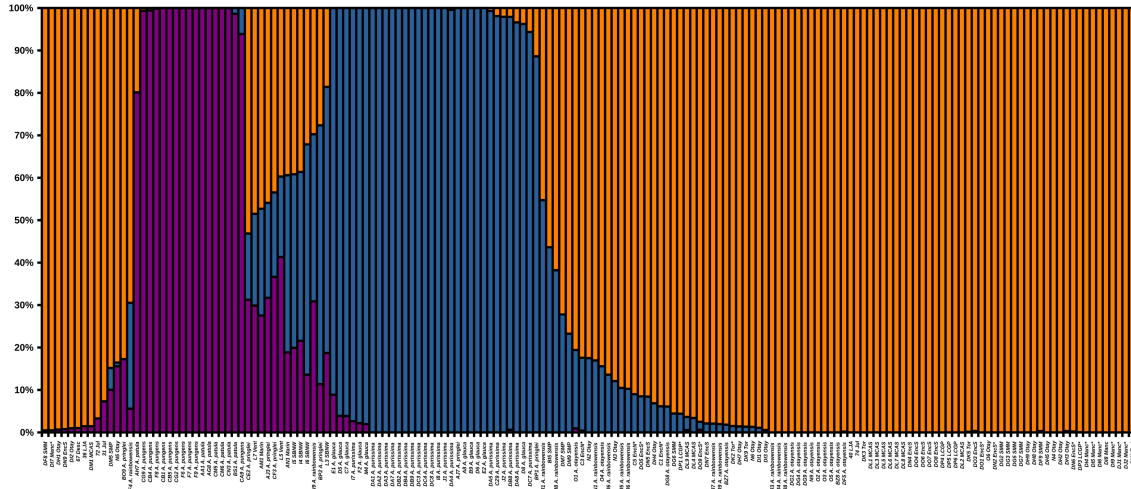


Figure 2.3: ADMIXTURE plot/stacked bar graph at $K = 3$ using SNPs from ddRADseq data thinned to 1150 bp apart. The y-axis indicates the genotype percentage of the three ancestral groups (depicted in blue, orange, and purple) inferred for each sample. Individual samples are labeled on the x-axis by a unique one to three character sample ID code (containing at least one number) followed by the sample's taxonomic identity (with the exception of *Arctostaphylos glandulosa* samples). For *A. glandulosa* samples, an abbreviated population label is provided after the sample ID code. Samples matching the current morphological description of *A. glandulosa* ssp. *crassifolia* (Del Mar manzanita) are marked with an asterisk (*). More information about each sample is available in Supplemental Materials 4. The figure was generated using LIBREOFFICE CALC version 7.3.7.2 and edited in INKSCAPE version 1.1.2 (Inkscape Project, 2025).

(Alexander and Lange, 2011). We also examined the pattern of deceleration in log-likelihood improvement, which agreed with the CV score method; i.e., increasing K to a value higher than the K value with the lowest CV score caused only relatively minor improvements in the log-likelihood score. The optimal values of K were three and four for the “all samples” and “no *A. glandulosa*” datasets, respectively (Supplemental Materials 5, 6). We visualized the results for the best supported K values as stacked bar graphs generated using LIBREOFFICE CALC version 7.3.7.2 (The Document Foundation). Additionally, for the “all samples” dataset, we averaged the ADMIXTURE results across populations of samples at least 6.5 km apart and plotted this result as

floating pie charts at the average GPS coordinates of the constituent samples using R version 4.5.0 (The R Foundation for Statistical Computing).

PHYLOGENETIC ANALYSIS

We conducted phylogenetic analysis using IQ-TREE2 version 2.2.0.5 (Minh et al., 2020), which utilized concatenated multiple-sequence alignment phy files generated by IPYRAD for the 4 and 2 MA “all samples” datasets and the “no *A. glandulosa*” dataset. First, we selected the best substitution model for each dataset using MODEL FINDER (Kalyaanamoorthy et al., 2017). MODEL FINDER selected the TVM+F+I+R10 model for both the 2MA and 4MA “all samples” datasets and the TVM+F+I+R7 model for the “no *A. glandulosa*” dataset. We used these models to generate maximum-likelihood phylogenetic trees for each dataset using default settings and 1000 ultrafast bootstrap iterations (Hoang et al., 2018). Both “all samples” datasets had the same topology and only differed in support values at specific nodes (Supplemental Materials 8, 9). We rooted phylogenetic trees using our three outgroup samples (*Arbutus unedo*, *A. menziesii*, and *Arctous alpina*) with APE version 5.8-1 (Paradis and Schliep, 2019). We visualized the trees with GGTREE version 3.16.0 (Yu et al., 2016).

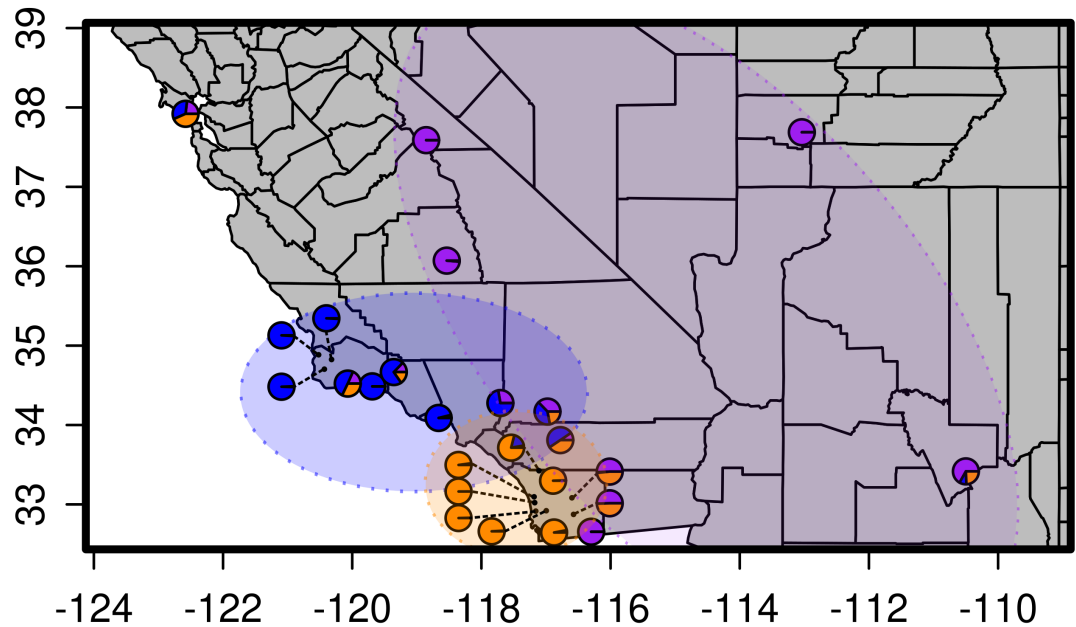


Figure 2.4: Map of ADMIXTURE results summarized by population. The results of ADMIXTURE at $K=3$ in Figure 3 are averaged for each sample within a population, such that each population would be at least 6.5 km apart. The average ADMIXTURE results for each population is plotted at the location of that population. The colors of the ADMIXTURE groups (blue, orange, and purple) correspond to the same colors of the groups in Figure 3. The GPS position of each population is the average decimal degree latitude and longitude of that population's constituent samples (individual plant's GPS coordinates were recorded at the time of sampling; these coordinates are given in Supplemental Materials 4). Overlapping pie charts have been moved for clearer differentiation; a dotted line leads to the center of the pie chart's original position (represented by a black dot). The semitransparent ellipses were added manually to represent the inferred sphere of dominance and area of overlap of each of the three ADMIXTURE groups. The base plot was generated in R version 4.5.0 using the packages MAPS version 3.4.2 (Becker et al., 2025) and PLOTrix version 3.8-4 (Lemon, 2006). The background map is sourced from the MAPS US County database, and each subdivision outlined in black is a US County. This figure was edited in INKSCAPE version 1.1.2 (Inkscape Project, 2025).

PRINCIPAL COMPONENT ANALYSIS OF THE PR-CLADE

Phylogenetic analysis revealed close relationships between samples from the Peninsular Range and the coast of San Diego County, which we refer to as the PR-clade. The structure of relationships in the PR-clade suggested a strong geographic signal, but did not entirely align with current taxonomic delimitations. To better understand genetic clustering within this group, we carried out a principal component analysis (PCA) on a separate dataset, which only included these samples (the “PR-clade” dataset) using the IPYRAD ANALYSIS TOOLS (IPA) .pca function. This method is sensitive to missing data (Abbo et al., 2025), so we first ran .pca within an IMAP dictionary that excluded the 16 samples with the most missing data (*A. otayensis* n=7, sample codes: DG8, BZ7, BZ8, N9, O1, O3, and O4; *A. glandulosa* n=9 [Descanso population n=1, sample code: 67; Marine Corps Air Station (MCAS) Miramar population n=1, sample code: DL8; Encinitas South population n=4, sample codes: DO1, DO2, DO4, and DO5; La Costa Glen Preserve population n=1, sample code: DP8; Otay Mountain population n=2, sample codes: N2, and N5]; Supplemental Materials 4). The run was repeated across 50 random draws of uncorrelated SNPs, with a minimum coverage of 90 (i.e., a SNP must be present in at least 90% of samples to be included in the draw). We then repeated the run with the same parameters for all samples in the “PR-clade” using K-means missing data imputation for K=2, 3, and 4. The value of K represents the assumed number of primary clusters in the data used to infer the identity of missing SNPs based on the SNP

identities at those positions in other samples within the same cluster group. We generated figures for each run using IPA, representing the initial draw of SNPs as solid points and replicate draws as transparent points; we combined each these into a multi-pannel figure using INKSCAPE version 1.1.2 (Inkscape Project).

RESULTS

CLUSTERING ANALYSIS FINDS THREE GROUPS DEFINED BY GEOGRAPHIC RANGE

Using the “all samples” dataset, ADMIXTURE analysis clusters samples into three major geographically coherent groups (Figure 2.3). For the most part, species showed little evidence of admixture. One cluster (purple) primarily consists of *A. pungens* and *A. patula* samples. The remaining two clusters, blue and orange, respectively, include westward samples within and north of the Transverse Range (TR) (*A. glauca* and *A. purissima*) or south of the TR (*A. otayensis*, *A. rainbowensis*, and *A. glandulosa* from Riverside and San Diego Counties). In contrast, *A. glandulosa* from north of the TR have substantial admixture with genotypes that are combinations of the three clusters (e.g., samples: AM1, AN1, I4, I5, I6, L5, and L7). A similar pattern of admixture is observed in samples of *A. pringlei*. The genotypes of other admixed samples (e.g., 36, DM9, O1, etc) are dominated by either the blue or orange cluster, consistent with these samples’ geographic origins. The geographic signal in the data is visualized in Figure 2.4. The

purple cluster has an eastern-inland sphere of dominance, the blue cluster predominates in south-central coastal California within and north of the Transverse Range (TR), and the orange group dominates the southern coast of California, south of the TR. Mixed-ancestry pie charts primarily occur where these spheres of dominance meet. The four exceptions to this pattern (the pie charts with all three clusters in Santa Barbara and Marin Counties, California, and southern Arizona) include the heavily admixed samples of *A. pringlei* or *A. glandulosa*, suggesting that these samples are exceptions to the geographic pattern.

We recover an overall similar pattern in the “no *A. glandulosa*” dataset, with two key differences (Supplemental Materials 7). Firstly, the best supported K is K=4, and this additional ADMIXTURE group separates *A. glauca* and *A. purissima* samples into their own individual clusters. Secondly, we do not observe consistently admixed groups of samples; instead, *A. pringlei* samples cluster with *A. rainbowensis* and *A. otayensis* samples.

PHYLOGENETIC ANALYSIS DOES NOT CONSISTENTLY SUPPORT CURRENT TAXONOMIC HYPOTHESES

Finer-scale taxonomic relationships are revealed in maximum-likelihood phylogenetic analysis shown in Figures 2.5 (the “no *A. glandulosa*” tree) and 6 (the “all samples” tree).

For ease of interpretation, the long outgroup branches are removed using the prune function in APE, well-supported (≥ 95 ultrafast bootstrap support [UFBS]) single-taxon clades are collapsed as color-coded triangles, and the remaining well-supported nodes (≥ 95 UFBS) are marked with a dark green circle. The unedited trees are included in Supplemental Materials 8-10. The only noticeable impacts of varying the MA (maximum alleles) were changes to support values in specific nodes of phylogenetic trees (Supplemental Materials 9, 10). This result is consistent with previous studies in *Arctostaphylos* that suggest that assumptions of ploidy in ddRADseq read processing do not noticeably affect results (Huang et al., 2020; Abbo et al., 2025). All taxa in the “no *A. glandulosa*” tree are monophyletic, and the tree is divided into two major clades, one containing *A. pungens* and *A. patula*, and the other containing the remaining taxa.

The inclusion of *A. glandulosa* samples in the “all samples” tree results in two main topological differences relative to the “no *A. glandulosa*” tree. Firstly, *A. glauca* and *A. purissima* form a clade (they are part of a grade in the “no *A. glandulosa*” tree). Secondly, the position of *A. pringlei* is changed, thus there is a closer relationship between *A. rainbowensis* and *A. otayensis* in the “all samples” tree. *Arctostaphylos glandulosa* (red tip labels) is recovered as paraphyletic, appearing in multiple locations throughout the tree. We refer to the clades that include the majority of “*A. otayensis*” and “*A. rainbowensis*” samples as the “*A. otayensis* clade” and “*A. rainbowensis* clade” respectively even though each one includes samples of *A. glandulosa*. In the phylogeny,

Arctostaphylos glandulosa from San Diego County and southwestern Riverside County are nested within the clades of *A. otayensis* and *A. rainbowensis*, respectively, meaning these two taxa are also resolved as paraphyletic. The *A. glandulosa* samples from coastal San Diego County (From Encinitas to Torrey Pines; Figure 2.2 and Figure 2.7) group together in a clade (highlighted in green) with two samples of *A. otayensis*, which resolve separately from all other samples of *A. otayensis* (highlighted in orange). The green-highlighted clade is sibling to the remaining *A. glandulosa* and *A. otayensis* samples from San Diego County, California, and resolves with moderate to high uncertainty (UFBS=94 in the 2MA tree [Supplemental Materials 9], and UFBS=59 in the 4MA tree [Supplemental Materials 10]). This clade includes all samples classified as DMM according to current taxonomic treatments. Although the current morphological diagnosis of DMM excludes glandular-haired individuals, these DMM samples (marked with an * in their tip labels; Supplemental Materials 4) do not resolve as phylogenetically distinct from morphologically similar, glandular-haired individuals with whom they co-occur. Further, the glandular and nonglandular-haired individuals in this clade tend to cluster geographically rather than morphologically, meaning that glandular and nonglandular individuals from the same population tend to be more closely related to each other than either is to the nearest neighboring population.

PCA ANALYSIS REVEALS SOME GEOGRAPHIC CLUSTERING WITHIN THE PR-CLADE

Principal component analysis of genomic SNPs from samples in the clade containing *A. rainbowensis*, *A. otayensis*, and *A. glandulosa* from San Diego County and southwestern Riverside County (hereafter the Peninsular Range clade or PR-clade) confirms some geographic clustering of samples, but with substantial overlap. The four panels in Figure 2.8 show the results of the PCA analysis (a color vision conscious version of this figure is available in Supplemental Materials 13). Panel A is filtered for missing data while panels B-D use different numbers of clusters to impute missing data. In all four cases, samples show slight separation, but the separation that is present relates to geography. *A. rainbowensis* samples tend to group with *A. glandulosa* samples from southwestern Riverside County/northwestern San Diego County; *A. otayensis* samples tend to group with *A. glandulosa* samples from southern inland San Diego County, and the remaining samples from coastal San Diego County also tend to group together. Although the pattern is present in all four analyses, all points tend to cluster more tightly together with increasing values of K in panels B-D. The results of these repeated draws in the reduced dataset without missing data imputation (Figure 2.8 A) indicate that the majority of SNPs yield the same result; however, many SNPs show a different pattern. The discrepancy among the results of randomly selected SNP draws is significantly influenced by missing data, as the results vary with the value of K when K-means missing data imputation is applied. There is clear agreement among SNP draws when K is set to two (Figure 2.8 B),

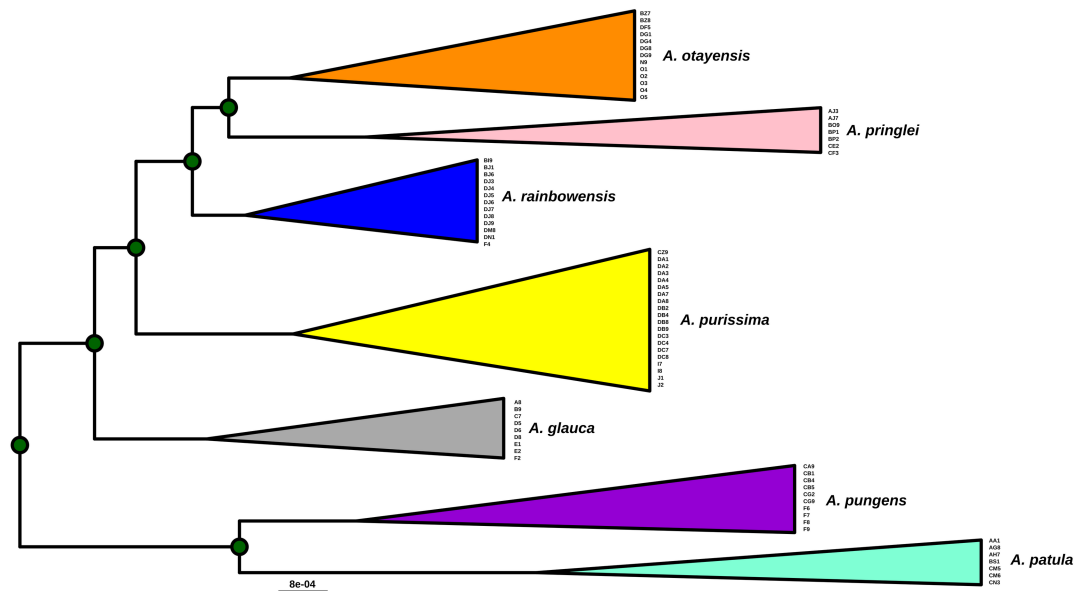


Figure 2.5: Maximum-likelihood phylogeny of *Arctostaphylos* samples excluding *A. glandulosa* (the “no *A. glandulosa*” dataset) generated from a concatenated multiple sequence alignment of ddRADseq data. The base phylogenetic tree was generated in IQ-TREE2 version 2.2.0.5 using the TVM+F+I+R7 model with 1000 ultrafast bootstrap iterations. The resulting tree-file was rooted at the three outgroups *Arbutus unedo*, *A. menziesii*, and *Arctous alpina* in R version 4.5.0 using the APE package version 5.8-1, and the outgroup branch was then removed from the rooted tree using the APE prune function (the unpruned version of the tree is included as Supplemental Materials 8). Samples of the same taxon formed single clades with ≥ 95 ultrafast bootstrap support (UFBS) and were collapsed and color-coded using GGTREE package version 3.16.0. The base of each collapsed triangle includes the list of samples included in the clade, followed by a label for the taxon they represent (gray corresponds to *Arctostaphylos glauca*, orange to *A. otayensis*, teal to *A. patula*, pink to *A. pringlei*, purple to *A. pungens*, yellow to *A. purissima*, and blue to *A. rainbowensis*). Individual samples are labeled by a unique one to three character sample ID code (containing at least one number), and more information about each sample is available in Supplemental Materials 4. All nodes had ≥ 95 UFBS and are marked by a dark green circle. The scale bar at the bottom of the figure is proportional to sequence evolution. The figure was generated using the GGTREE and edited in INKSCAPE version 1.1.2 (Inkscape Project, 2025).

marginal agreement when K is set to three (Figure 2.8 C), and there is substantial disagreement among draws when K is set to four (Figure 2.8 D). The points from coastal San Diego County include samples that match the current morphological description of DMM (green points) and co-occurring plants with glandular hairs (red points); these red and green points are not distinct from each other in PCA space. Likewise, A.

rainbowensis samples (blue points) are not distinct from co-occurring *A. glandulosa* (yellow points), and *A. otayensis* samples (orange points) overlap substantially with nearby and co-occurring samples of *A. glandulosa* (pink points).

MORPHOLOGICAL ANALYSIS REVEALS SOME DISTINGUISHABILITY AMONG FOCAL TAXA BUT ALSO FREQUENT, SOMETIMES EXTENSIVE, PHENOTYPIC OVERLAP

Qualitative traits for each collection were described in field notes. These observations reveal some morphological trends within the PR-clade. *Arctostaphylos rainbowensis* plants are readily distinguishable by their glaucous leaves, basal burls, and fused nutlets. However, plants frequently differ from the expected twig morphology (“minutely glandular, appearing glabrous”, Parker et al., 2023) and have a variety of hair types. We observed a morphological continuum from plants that fully matched the current description of *A. rainbowensis* (e.g., samples: BI9, BJ1, DJ6, DJ7, DN1 [see Supplemental Materials 4]) to occasional plants matching the description of *A. glandulosa* ssp. *glandulosa*. Intermediates had increasingly greener leaves, and longer and denser glandular hairs (e.g., samples: F4, BI5, DM5, DM7, DM8), and we hypothesized that these plants represented introgression into *A. rainbowensis* from *A. glandulosa*. We continued to observe individuals within populations that exhibited

varying and overlapping phenotypes on San Miguel and Otay Mountains, in coastal San Diego County, and at MCAS Miramar.

On the Otay and San Miguel Mountains, we observed three main phenotypes of plants. *Arctostaphylos otayensis* was readily distinguishable by its lack of a basal burl, and when available for examination, small globose fruits generally with fused nutlets. We also observed two classes of burl-forming plants: one completely nonglandular-haired form, which we determined as *A. glandulosa* ssp. *cushingiana*, and a glandular-haired form. The glandular form is traditionally treated as *A. glandulosa* ssp. *glandulosa* based on its green leaves and glandular hairs, but we noted substantial similarities between these plants and neighboring *A. otayensis*, mainly the presence of intermixed glandular and nonglandular hairs along twigs and similar rigid, more or less triangular or ovate bracts. Because these plants appear intermediate, with fruit and burls similar to *A. glandulosa* ssp. *cushingiana* and hairs and bracts more similar to *A. otayensis*, we hypothesized in the field that these plants were hybrids.

In coastal San Diego County, we observed two phenotypes of plants. These plants all had burls or layering growth (branches rooting when touching the soil) and appeared overall similar to each other, except that some were completely nonglandular and others had variable amounts of glandular hairs mixed with the nonglandular hairs. In the field, we

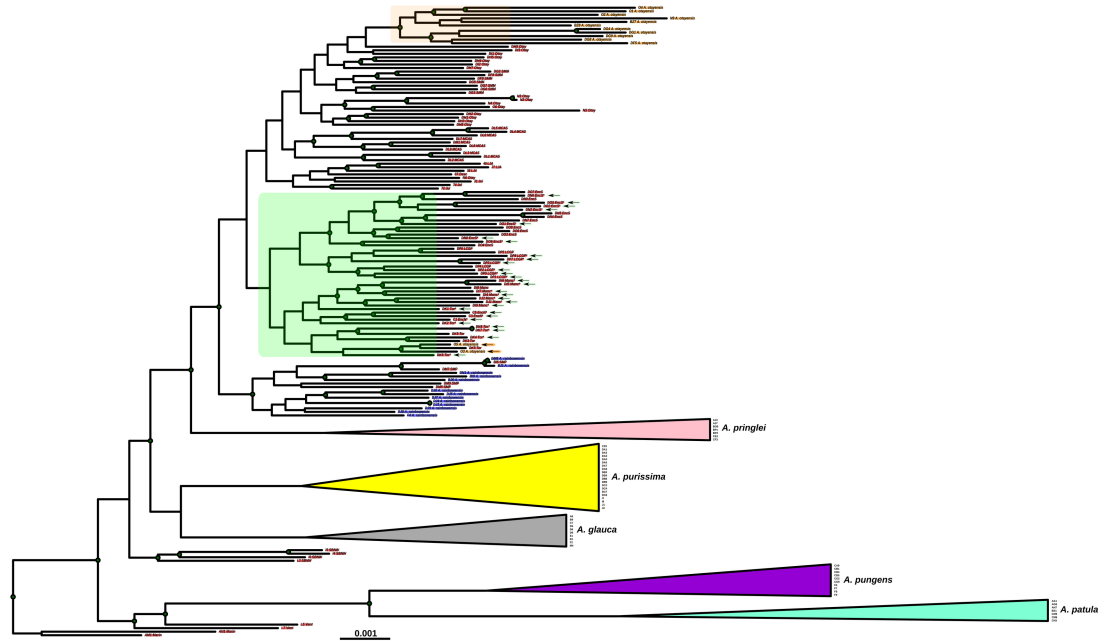


Figure 2.6: Maximum-likelihood phylogeny of *Arctostaphylos* samples generated from a concatenated multiple sequence alignment of ddRADseq data. The base phylogenetic tree was generated in IQ-TREE2 version 2.2.0.5 using the TVM+F+I+R10 model with 1000 ultrafast bootstrap iterations. The tree was rooted and pruned as in Figure 5 (see Figure 5 figure legend; the unpruned version of the tree is included as Supplemental Materials 10). The underlying sequence alignment was generated with the IPYRAD parameter max_alleles_consens set to four, but the same topology is recovered when this parameter is set to two (Supplemental Materials 9). When all samples of the same taxon formed a single clade with ≥ 95 ultrafast bootstrap support (UFBS), these clades were collapsed as a color-coded triangle using GGTREE version 3.16.0. Nodes with ≥ 95 UFBS are marked with a dark green circle. Individual samples are labeled by a unique one to three character sample ID code (containing at least one number) and more information about each sample is available in Supplemental Materials 4. The base of each collapsed triangle includes the list of samples included in the clade, followed by a label for the taxon they represent (grey corresponds to *Arctostaphylos glauca*, teal to *A. patula*, pink to *A. pringlei*, purple to *A. pungens*, yellow to *A. purissima*). Tip labels in uncollapsed clades include sample ID codes followed by the sample's taxonomic identity in the case of *A. otayensis* and *A. rainbowensis*, or an abbreviated population label in the case of *A. glandulosa* (Supplemental Materials 4). These labels are highlighted with a color-coding where orange corresponds to *A. otayensis*, blue to *A. rainbowensis*, and red to *A. glandulosa*. The well-supported clade that includes all but two samples of *A. otayensis* is highlighted in transparent orange. The remaining two *A. otayensis* samples, emphasized with orange-highlighted, black arrows, resolve in a clade, highlighted in transparent light green, composed of *A. glandulosa* samples from the coastal San Diego County, California. This green-highlighted clade includes samples that match the current morphological description of *A. glandulosa* ssp. *crassifolia* (Del Mar Manzanita), and these samples are denoted by black arrows highlighted in green. The scale bar at the bottom of the figure is proportional to sequence evolution. The figure was generated using the GGTREE and edited in INKSCAPE version 1.1.2 (Inkscape Project, 2025).

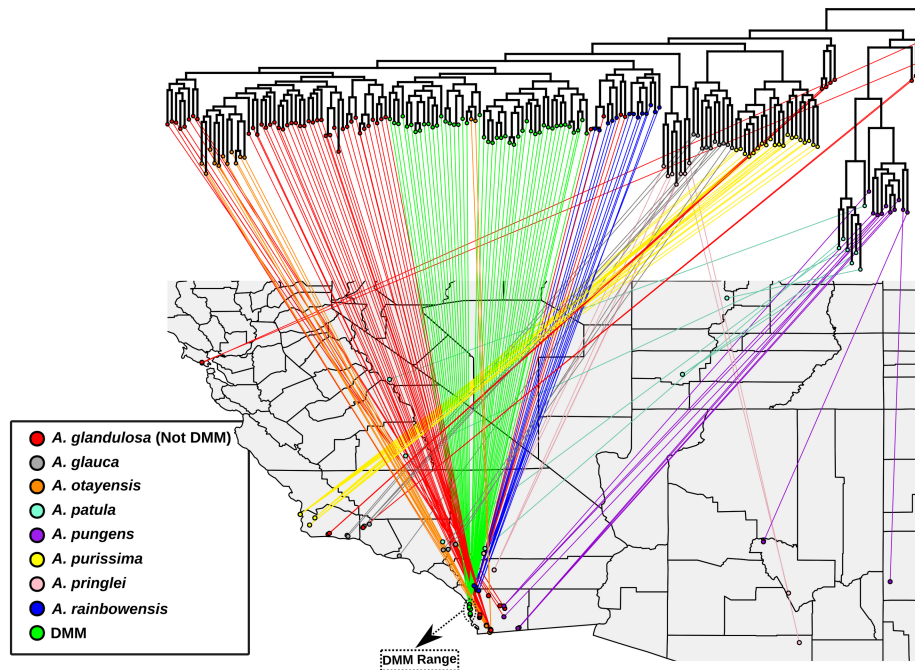


Figure 2.7: Phylogenetic tree with tips mapped to collection sites. The maximum-likelihood phylogeny in Figure 6 was combined with the GPS coordinates of sampled plants (Supplemental Materials 4), and a map of southwestern United States Counties from the MAPS R package version 3.4.2 (Becker et al., 2025) using the PHYLO.TO.MAP function from PHYTOOLS R package version 2.4-9 (Revell, 2012). The background map is sourced from the MAPS US County database, and each subdivision outlined in black is a US County. Each tip in the phylogeny is connected to each sample's collection location on the map via a straight line. Samples are color-coded by taxonomic identity, where gray corresponds to *Arctostaphylos glauca*, orange to *A. otayensis*, teal to *A. patula*, pink to *A. pringlei*, purple to *A. pungens*, yellow to *A. purissima*, blue to *A. rainbowensis*, and both red and green to *A. glandulosa*. The green samples correspond to *A. glandulosa* samples from the coast of San Diego County, California, labeled in the figure as “DMM” (Del Mar Manzanita = *A. glandulosa* ssp. *crassifolia*), and the red samples represent all other samples of *A. glandulosa* in the dataset, which are labeled in the figure as “*A. glandulosa* (not DMM)”. Note that the green samples include plants with glandular hairs and therefore do not match the current morphological diagnosis for *A. glandulosa* ssp. *crassifolia*. The green samples are circled and labeled as “DMM range” to highlight the narrow geographic extent of their distribution. Map generated in R version 4.5.0 and edited in INKSCAPE version 1.1.2 (Inkscape Project, 2025).

determined the nonglandular plants as DMM and the glandular ones as putative hybrids. Plants near Encinitas tend to manifest the glandular and nonglandular phenotypes at roughly equal frequency, whereas plants further south, represented in our data by Torrey Pines State Park and the CNLM Manchester Preserve, tend to have mostly nonglandular hairs. In contrast, plants further inland in the vicinity of Poway (represented in our data by the populations from MCAS Miramar), which have traditionally been speculated to be DMM, are almost exclusively glandular; we observed only one nonglandular plant (DL4), which otherwise looked identical to its glandular neighbors.

In light of the molecular results indicating the paraphyly of *A. glandulosa* and the close relationships of samples in the PR-clade, we compared the morphologies of burl-forming plants in coastal San Diego County with those of the MCAS Miramar population and plants from San Miguel and Otay Mountain. As these plants are quite similar to each other, we wanted to see if a closer examination would reveal consistent morphological distinguishing characters; however, we found that distinguishing features are subtle and inconsistent. Supplemental Materials 11 shows a selection of leaves from various sampled individuals from these geographic regions (these leaves are linked to their source voucher specimen by the collector's initials and number). There is substantial overlap in leaf color and shape; however, the more inland leaves arguably tend to be smaller and more frequently have truncate bases, whereas coastal leaves tend to be larger and more oblong. A similar montage of reproductive structures is depicted in Supplemental

Materials 12. Plants from Otay and San Miguel Mountains are distinguishable in that plants with nonglandular hairs tend to have appressed, more or less dry, almost scale-like bracts (e.g., Supplemental Materials 12 page 3, panels A and P), and plants with glandular hairs tend to have rigid *A. otayensis*-like leafy bracts as described above. Plants closer to the coast tend to have shorter, more or less spreading, and more widely ovate bracts. When glandular hairs are present, plants from the coast are not readily distinguishable from those at MCAS Miramar (compare, e.g., Supplemental Materials 12, page 2, panels L and J). In the context of populations, the MCAS Miramar plants are readily distinguishable from coastal populations only by geography or the frequency of the nonglandular phenotype outlined above.

DISCUSSION

OVERVIEW

Successful conservation of taxa requires understanding species boundaries of focal taxa. Molecular approaches are a means to test morphological treatments, especially when morphological data has multiple valid interpretations and/or when there are compounding factors, such as when characters vary spatially and it is unclear whether this is due to hybridization or the inherent instability of those characters. Molecular data can also reveal differences among individuals or populations that are not visible in morphology, such as in the case of cryptic species or genetic differentiation along a spatial cline (e.g.,

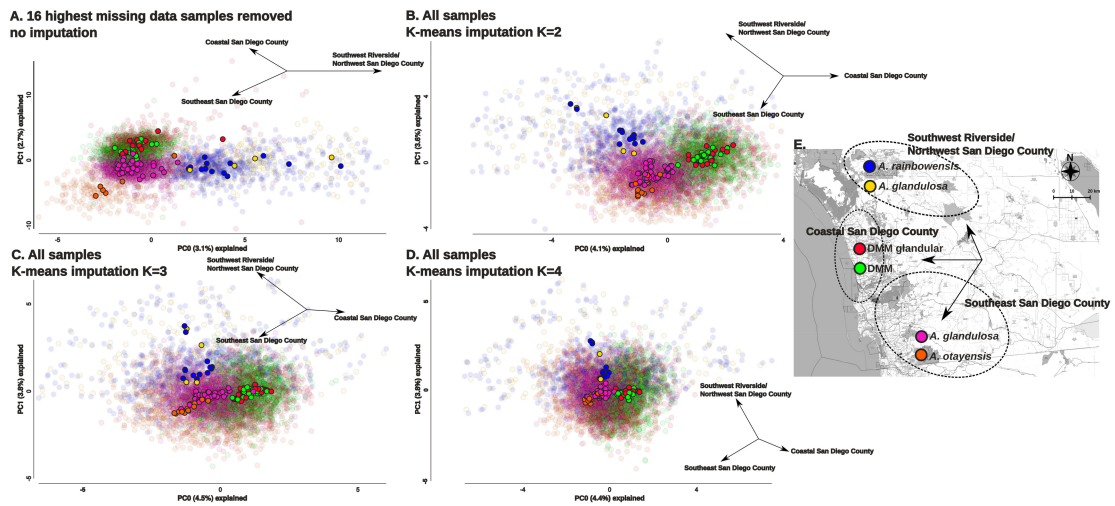


Figure 2.8: Principal component analyses (PCAs) of uncorrelated SNPs using ddRADseq data for samples in the Peninsular Range (PR)-clade. The PR-clade is a group of closely genetically and geographically related samples (Figure 6, 7). It includes the two narrow endemic taxa *Arctostaphylos rainbowensis* (color-coded in blue) and *A. otoyensis* (color-coded in orange), as well as the samples of *A. glandulosa* from south of the Transverse Range. The *A. glandulosa* samples were recovered as paraphyletic in phylogenetic analysis (Figure 6) and are color-coded according to geographic locality or morphology. Yellow represents *A. glandulosa* from southwest Riverside County to northwest San Diego County, fuchsia represents *A. glandulosa* from southeast San Diego County, and both green and red (labeled in the figure as “DMM”, and “DMM glandular” respectively) represent *A. glandulosa* ssp. *crassifolia* (Del Mar Manzanita = DMM), and the red samples are co-occurring plants that fail to meet the current description of *A. g. ssp. crassifolia* because they have glandular hairs. Each PCA was generated using IPYRAD ANALYSIS TOOLS (IPA) with 50 replicate draws of uncorrelated SNPs at a minimum coverage of 90 (all included SNPs are present in at least 90% of samples). Solid points represent the results of a single random draw, while the transparent points represent the results of the additional 49 draws. The Black arrows were added manually to emphasize the geographic structure in the data. **A-D.** For each panel PC0 (first principal component) is given in the x-axis, PC1 (second principal component) is given in the y-axis, and the amount of variation explained by each PC is labeled on its corresponding axis in the figure. **A.** PCA excluding 16 samples with the highest amounts of missing data and no missing data imputation. **B.** PCA of all PR-clade samples using K-means missing data imputation at K=2. **C.** PCA of all PR-clade samples using K-means missing data imputation at K=3. **D.** PCA of all PR-clade samples using K-means missing data imputation at K=4. **E.** Simplified distribution map; the background map was generated in QGIS as in Figure 2; the ellipses represent the approximate geographic range of the samples labeled inside of them; the arrows that point to each of the three ellipses signify the same geographic areas as labeled in panels **A-D**. The raw PCA figures generated using IPA were exported as SVG files, edited, and combined into a 5-panel figure in INKSCAPE version 1.1.2 (Inkscape Project, 2025).

Johnson and Cairns-Heath, 2010; da Costa et al., 2011; Preckler-Quisquater et al., 2023).

However, results that are entirely inconsistent with longstanding morphological understanding may lead to complex or unintuitive reorganizations. For example, the molecular-based reorganization of Scrophulariaceae into several smaller families without

clear morphological distinctions makes it difficult to recognize by sight what family an unfamiliar plant in the Lamiales belongs to (Simpson, 2010, p. 410, 412). Our study shows both the utility of molecular systematics for conservation but also its limitations.

In the case of the federally endangered DMM, whose status has long been debated due to conflicting opinions and conflicting data (including molecular data), our study finds a much stronger signal of distinctiveness than previous molecular analyses, suggesting DMM is a real entity meriting conservation. Nonetheless, the precise boundaries between DMM and other manzanitas in the Peninsular Range are fuzzy, which provides some context for the longstanding ambiguity and debate surrounding DMM. *Arctostaphylos glandulosa*, which currently includes DMM as a subspecies, is recovered as paraphyletic. Small populations of *A. glandulosa* appear throughout the phylogeny with very distant relationships to one another. The differences are most pronounced when comparing lineages from either side of the Transverse Range which appears to be a major source of differentiation for the genus and as a biogeographic barrier for organisms in general (e.g., Calsbeek et al., 2003; Spinks and Shaffer, 2005; Ortego et al., 2015). Because *A. glandulosa* appears throughout the entire phylogeny, its inclusion has an impact on the

topology of the entire phylogeny, as can be seen in the differing trees recovered when samples with affinity to *A. glandulosa* are excluded from the dataset compared to when they are included. The idea that every population of *A. glandulosa* could be an independent lineage is counterintuitive from a morphological standpoint, as floras have long recognized similarities among *A. glandulosa* subspecies: a basal burl (one exception [*A. g. ssp. atumescens*]), stomata on both leaf surfaces, and typically leaf-like inflorescence bracts. On the other hand, the variability within and among *A. glandulosa* subspecies across the taxon's wide range is considered substantial, and this seems to be a morphological clue that individuals currently classified as *A. glandulosa* are not as cohesive as previously thought. This result also highlights the limitations of our study, both in the limited range of our sampling of *A. glandulosa* outside of southern California and in the limited proportion of the genome sampled using ddRADseq, a reduced representation sequencing method. To fully understand *A. glandulosa* will require a large-scale study across its entire range using more advanced sequencing to capture more fine-scale structure. Nonetheless, similar problems were not revealed in other members of the genus we sampled, suggesting that ddRADseq is a viable option for understanding species relationships within the genus.

THERE IS DISTINCT SIGNAL FOR DMM THAT IS RESTRICTED TO TYPE LOCALITY AND ADJACENT AREAS

A distinct genetic signal for manzanitas is established around the type locality of DMM, but individuals that do not match the current morphological description of DMM are included within that signal. The evidence for the genetic distinctiveness of these plants is that they all cluster together in a single clade (green highlighted clade, Figure 2.6) and tend to separate from closely related samples in PCA (green and red points, Figure 2.8). However, these coastal samples include individuals that do not match the current description of DMM because they have varying amounts of gland-tipped hairs, and DMM is currently described as a nonglandular-haired taxon (Parker et al., 2023). In PCA, the red points, which represent glandular-haired individuals that co-occur with DMM, are not distinguishable from the green points, which perfectly match DMM's current description, and individuals that do match the description of DMM (marked with an asterisk) tend to be more closely related in the phylogeny to their glandular-haired immediate neighbors than they are to nonglandular individuals at other sites. Expanding the description of DMM to include glandular-haired individuals should make identification easier for land managers, as DMM occurs within a narrow range and is the only manzanita that occupies it.

The distinct genetic signal for DMM is only present along the coastline of San Diego County (Figures 2.7, 2.8). Our result is consistent with Burge et al. (2018), who found a

distinct signal for DMM near its type locality, provided that the diagnosis of DMM is expanded to include forms with glandular hairs. DMM individuals are distinguishable from interior resprouting manzanitas in San Diego County by bract type and leaf color, though not so readily distinguishable from mid-coastal/interior populations at MCAS Miramar (Supplemental Materials 11, 12). We did observe a trend of a lower frequency of glandular hairs among individuals on the immediate coast compared to those at MCAS Miramar, but this only helps to distinguish between populations rather than on an individual-by-individual basis. At MCAS Miramar, all but one individual we observed had glandular hairs, whereas coastal populations tended to have a mix of glandular and nonglandular-haired individuals, or the nonglandular phenotype was dominant. This trend makes sense given that the lack of glandular hairs is emphasized in the current description of DMM, even though we have shown that the character is not strictly diagnostic. The plants at MCAS Miramar have previously been hypothesized to be DMM based on morphological similarities; however, they were shown in Burge et al. (2018) to be excluded from DMM. Our study agrees with this conclusion but also recognizes that there is a very close genetic relationship between these two populations, for they resolve in sibling clades in the phylogeny (Figure 2.6) and the edges of both populations overlap in PCA (MCAS Miramar plants are among the lavender points, and DMM is represented by the green and red points, Figure 2.8).

The genetic boundaries between taxa in the PR-clade overlap at their edges, but DMM, *A. otayensis*, and *A. rainbowensis* are distinguishable from one another at their cores. The primary counter evidence for the distinctiveness of DMM in our data is that the clade containing DMM has only moderate to weak support (UFBS<95 Supplemental Materials 9, 10), the clade includes two apparently rogue samples of *A. otayensis* (O3 and O5), and samples from the PR-clade do not separate completely in PCA but rather overlap at their edges. The PR-clade is the most newly diverging clade in the phylogeny, suggesting that speciation has occurred relatively recently compared to other sampled manzanitas so reproductive barriers may be weaker. The taxa in this clade more or less occur in sympatry (Figures 2.2, 2.7), making at least some gene flow between them likely. When samples with affinity to *A. glandulosa* are excluded from the dataset, *A. otayensis* and *A. rainbowensis* samples are reciprocally monophyletic, and even appear more distantly related to each other; *Arctostaphylos glandulosa* samples seem to attract these two narrow endemic samples suggesting shared introgression with *A. glandulosa*.

Arctostaphylos rainbowensis is not distinct in the phylogeny from the *A. glandulosa* samples it cooccurs with. The majority of *A. otayensis* samples resolve in their own clade, which diverges from *A. glandulosa* samples from interior San Diego County but is not reciprocally monophyletic with the *A. glandulosa* samples that grow next to them on Otay and San Miguel Mountains. The DMM samples occur in their own clade, which is sibling to the *A. otayensis* and *A. glandulosa* clade. The two *A. otayensis* samples (O3

and O5) that resolve in the DMM clade do not appear morphologically different from other *A. otayensis* samples and, interestingly, do not noticeably cluster with DMM in PCA (Figure 2.8). These two *A. otayensis* samples are the exception to what is otherwise a clear pattern of geographic signal, where *A. rainbowensis* forms a clade with its neighboring *A. glandulosa* samples, the coastal samples (DMM) form a clade, and *A. otayensis* forms a clade with its neighboring southern interior *A. glandulosa* samples (Figure 2.7). The PCA (Figure 2.8) supports this pattern by dividing the PR-clade samples into three geographic clusters that overlap at their edges but are distinct from each other (the pattern is significantly diminished when K is set to 4 in K-means missing data imputation, but remains discernible). Overall, this suggests that there are three distinct taxa: *A. rainbowensis*, *A. otayensis*, and DMM, each narrowly endemic but potentially not reproductively isolated, and the remaining samples with affinity to *A. glandulosa* are enigmatic. Some of the *A. glandulosa* samples could potentially be recent hybrids or backcrosses, and/or could be completely separate taxa. The results are certainly biologically complex and intriguing, but we conclude that the case for treating DMM as genetically distinct is stronger than the alternative of treating PR-clade samples as only one or two highly morphologically variable entities.

Our results suggest that all *Arctostaphylos* along the San Diego County coast from Encinitas to Torrey Pines State Park be treated as DMM, regardless of hair morphology, and only plants within this narrow geographic range should be called DMM. Although

future studies are required to formally address the status and rank of DMM, our results significantly clarify precisely what constitutes DMM. The key information needed to conserve DMM is both a clear understanding of its range and clear metrics to distinguish it from other manzanitas. Our study clarifies the range and broadens the morphological understanding of DMM, but identification without the consideration of range still poses a challenge. In order to confirm hypotheses of hybridization among the PR-clade taxa and to better understand the evolutionary origins of DMM, future studies should capture greater genomic representation ideally via a high-resolution pan-genome approach (e.g., Fang et al., 2024).

ARCTOSTAPHYLOS GLANDULOSA IS PARAPHYLETIC AND IMPACTS THE INTERPRETATION OF THE ENTIRE *ARCTOSTAPHYLOS* PHYLOGENY

The enigma of *A. glandulosa* stretches beyond San Diego County, impacting the broader *Arctostaphylos* phylogeny. *Arctostaphylos glandulosa* is paraphyletic unlike other widespread species which suggests that the primary complexities in our study system are associated with *A. glandulosa*. While our results support the need for future taxonomic reorganization, a better understanding of the number of independent lineages of *A. glandulosa*, their ploidies, and evolutionary origins is required to inform an appropriate taxonomic revision. The impact of *A. glandulosa* is best illustrated by comparing the results to those when *A. glandulosa* is excluded from the dataset. Figure 2.5 depicts the phylogeny without *A. glandulosa* samples, where samples segregate neatly into well

supported clades based on taxonomic identity. When all samples are included (Figure 2.6), there are large changes to the PR-clade described above. The recovered relationship between *A. glauca* and *A. purissima* is altered, and the branch length separating *A. patula* and *A. pungens* from the remaining samples is more or less inflated (Supplemental Materials 8-10). A similar change is seen in ADMIXTURE, where without *A. glandulosa*, the optimal K value increases from 3 to 4, and the inference of admixture between clusters is much lower (compare Figure 2.3 and Supplemental Materials 7). Because *A. glandulosa* samples are distributed throughout the phylogeny, it is not surprising that their impact is felt throughout it.

The three major ADMIXTURE groups (Figure 2.3) consist of one dominated by *A. pungens* and *A. patula* (purple), one dominated by the PR-clade samples and *A. pringlei* (orange), and a third dominated by *A. purissima* and *A. glauca* (blue). Figure 2.4 shows the geographic distribution of these ADMIXTURE groups, with the purple group having an eastern sphere dominance, and the blue and orange groups having respective northerly and southerly spheres of dominance overlapping at the Transverse Range (TR). The purple group represents the small clade observed in ITS phylogenies, which consistently includes *A. patula* (Boykin et al., 2005; Wahlert et al., 2009). The placement of *A. pungens* between the large and small clades has been uncertain. It was placed in the large clade in Boykin et al. (2005), but in Wahlert et al. (2009), the *A. pungens* from California was placed in the small clade, and the sample from Arizona was placed in the large clade.

Our study included *A. pungens* samples from California, Arizona, and New Mexico, and all resolved as a monophyletic group that is sibling to *A. patula*. This result is also consistent with the chloroplast gene-tree in the recent study by Sweeten and Parker (2025).

The other two ADMIXTURE groups divide the large clade samples into northern and southern lineages. The samples of *A. glandulosa* from north of the TR and *A. pringlei* have ADMIXTURE-genotypes that are mixtures of the three ADMIXTURE groups, suggesting these samples are either the product of a complex history of hybridization among the three ADMIXTURE groups or, more simply, that the ADMIXTURE model was uncertain about how to classify them. *Arctostaphylos pringlei* is consistently recovered as monophyletic in phylogenetic analysis and does not appear admixed when *A. glandulosa* samples are excluded from ADMIXTURE analysis, so they do not consistently appear to have hybrid genotypes. In comparison, the *A. glandulosa* samples from north of the TR form a complex grouping in the “all samples” tree, reflecting their mixed classification in the ADMIXTURE analysis. Some of the admixed *A. glandulosa* samples form a grade at the base of the tree, and others form a clade that is sibling to the remaining large clade samples. Huang et al. (2020) found a genotypic gradient separating *A. glandulosa* samples into two groups: one from south of the TR and the other from the TR and north of it. Combined with their results, our findings suggest that the TR may represent a key biogeographic boundary for *Arctostaphylos* species within the large clade,

rather than a feature unique to *A. glandulosa*. However, this biogeographic hypothesis needs to be verified with a more comprehensive taxonomic representation. Furthermore, this relationship exists in the data only when *A. glandulosa* samples are included.

At a minimum, the divide between *A. glandulosa* samples across the TR suggests the *A. glandulosa* lineages from around and north of the TR are very different genetically from the *A. glandulosa* samples found in the PR-clade. The purple ADMIXTURE group ancestry found in the *A. glandulosa* samples from around the TR and north, and their early diverging placement in the phylogeny, suggests that they may have a hybrid ancestry between the two major ITS-clades, similar to what was recently shown for *A. rudis* (Abbo et al., 2025). The outlier pie chart in the northwest of Figure 2.4, with ancestry from all three groups, represents the *A. glandulosa* samples from Marin County (AM1 and AN1) and preliminarily suggests a widespread geographic signal of mixed ancestry among *A. glandulosa* samples.

Uncertainty in the ploidy of *A. glandulosa* samples is an essential complicating factor. Due to the difficulty of determining ploidy, we have cautiously assumed the ploidies of taxa as given in the Parker et al. (2023) Jepson eFlora treatment (derived primarily from Wells, 1968, and a few other studies for later described taxa, e.g., Keeley and Massihi, 1994; Keeley et al., 1997; Parker and Vasey, 2004; Vasey and Parker, 2008). The

weakness of this assumption is that the majority of these ploidy determinations – excluding Schierenbeck et al. (1992) for *A. patula*, *A. viscida*, and *A. mewukka*– were based on only one to three individuals per taxon, meaning that any within-taxon ploidy variation, a condition not uncommon in plants (e.g., Eriksson et al., 2017; Kovanda, 2002; Měsíček, 1970; Barbour, 1969), would not be readily detectable. Moreover, rejecting the monophyly of *A. glandulosa* makes it unclear which lineages of this paraphyletic complex are represented by the currently available chromosome counts. To our knowledge, there are no published chromosome counts that correspond geographically with the *A. glandulosa* samples that resolve in the PR-clade, so the ploidy of these samples should be considered undetermined. This uncertainty highlights the need for a broad-scale cytological study in *Arctostaphylos*. Furthermore, given that *A. glandulosa* is so widespread and samples do not cluster predictably, especially around and North of the TR, any future research on *A. glandulosa* needs to be broad in both geographic scope and sample size.

CONCLUSION

Current genomic methods can resolve species relationships in manzanitas to a much greater degree than was previously achieved. This is extremely promising for addressing future questions of species delimitation in the genus, but significant challenges remain. This study used a reduced-representation genomic sequencing approach, which yields exponentially more data than Sanger sequencing but does not capture the entirety of the

information available in the plants' genomes. A broad-scale understanding of the ploidies and more advanced sequencing should help illuminate the genetic similarities and differences among the various *A. glandulosa* lineages and inform a strategy for their delimitation. Other challenges are philosophical in nature, such as what defines a species or subspecies, and may not have a single, objective best answer at present. In Darwin's view, there was no set threshold for species delimitation as he noted that the distinction between species could be much more substantial in some lineages than others (Darwin, 1872 p: 56-58). As living organisms experience diverse biotic and abiotic environments that vary in space and time, it seems logical that different groups will not speciate uniformly and that the changes and circumstances required for speciation will be equally diverse. We have shown here that three closely related taxa (DMM, *A. rainbowensis*, and *A. otayensis*) are genetically distinguishable but overlap. The overlap between these three taxa is not substantial by itself, but rather the three taxa are bridged by grades of other potentially independent lineages of *A. glandulosa*. We hypothesize that this pattern represents independent taxa linked by limited introgression. Thus, they merit continued recognition and governmental protection.

In the case of DMM, conservation efforts should be limited to coastal San Diego County. The distinct signal of DMM disappears rapidly towards the interior of the county. Conversely, manzanitas throughout the Peninsular Range and coastal San Diego County overlap substantially, are very closely related genetically, and may even hybridize. This

can be interpreted as taxonomic boundaries not being entirely clear, further compounded by evidence of numerous *A. glandulosa* lineages without clear limits on their number, range, or boundaries. In short, we find a geographic signal within San Diego County that distinguishes *A. rainbowensis* in the north from DMM on the coast, to *A. otayensis* in the southern interior, along with numerous, potentially indefinite, other lineages of *A. glandulosa*. There is a core signal throughout these distributions, but the boundaries between these signals are blurred and uncertain. Future studies can help clarify this situation by examining manzanitas more closely from a cytological perspective and by gathering a more comprehensive representation of the *Arctostaphylos* genome using advanced, cutting-edge technologies and analytical methods. Such studies would serve as a model for addressing complicated species delimitations in plants, especially those with similar morphologies and incomplete reproductive isolation.

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AUTHOR CONTRIBUTIONS

T.A. and A.M.: Primary field work, bench work, analysis, writing manuscript.

G.R.M., A. B., A.C.S., V.T.P., M. C. V., J. E. K., and A. L.: Assisted with result interpretation and editing manuscript.

T.A., G.R.M., V.T.P., and A.L.: Conceptualizing, generating and interpreting initial preliminary data.

T.A., A.M., and A.L.: Funding acquisition, and permitting.

G.R.M., A.B., A.C.S., and V.T.P.: Assisted with field collection, scouting, or sampling.

G.R.M. and A.B.: Assisted with benchwork, and wetlab methods conceptualization and improvement.

V.T.P., M.C.V., and J.E.K.: Provided special expert evaluation of taxonomy and ecological context.

A.C.S.: Managed curation and preparation of voucher specimens.

A.L.: Principal instigator; managed resources and funds, and provided supervision, and mentorship.

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CHAPTER 3: THE END OF THE 25 YEAR *ARCTOSTAPHYLOS* (ERICACEAE)
POLYTOMY: A CASE STUDY IN WOODY PLANT EVOLUTION IN THE
CALIFORNIA FLORISTIC PROVINCE

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ABSTRACT

Resolving species relationships in hyperdiverse genera is a fundamental first step in understanding the driving forces that lead to asymmetrical distribution of biodiversity in lineages, space, and time. We present a phylogenetic study in the genus *Arctostaphylos* (Ericaceae), a hyperdiverse woody genus composed of a majority of narrow endemic species confined to the biodiversity hotspot, the California Floristic Province (CFP), and a small number of species that are widespread outside of the CFP. Although the majority of woody species in the CFP are hypothesized to be paleoendemics, *Arctostaphylos* is thought to be an exception that radiated relatively recently within the CFP. It has been proposed that a combination of unique life history traits and adaptive introgression allowed the genus to evolve at a rate that is atypical for similar slow growing woody plants. However, lack of molecular phylogenetic resolution has thus far precluded the

evaluation of its diversification and species relationships at the genus-wide level. This combined with its complex and historically debated taxonomy means that little is known about the evolution of *Arctostaphylos*. We use maximum likelihood phylogenetic methods to evaluate the monophyly of currently recognized species, and examine the phylogenetic distribution of taxonomically informative morphological traits using a ddRADseq dataset that includes 88% of currently recognized *Arctostaphylos* species (our full dataset includes 430 individual samples with a majority of taxa represented by three or more replicates). We further generate a Bayesian time-calibrated phylogeny for a subset of representative species from major lineages identified in our maximum likelihood phylogenies to estimate the divergence times of these lineages and test the hypothesis of neoendemism for CFP endemics. We found at least partial support for the overwhelming majority of currently recognized species with the most notable exceptions being widespread tetraploid taxa for which individual samples often resolve chaotically, appearing in multiple, sometimes very distant, regions of the phylogeny. Despite their support overall, a significant amount of taxa have small subsets of replicate samples that do not cluster with the majority of replicates of their respective taxa suggesting that hybridization is widespread and often is cryptic. We find widespread homoplasy among the majority of taxonomically informative characters suggesting that they repeatedly arose independently and possibly were acquired via horizontal gene transfer. Our estimates of divergence times have large margins of error but suggest that *Arctostaphylos* diversified during the mid to late Miocene in a rapid radiation. Our study provides

context for complicated evolutionary processes that occur in the CFP and provide a foundation for future conservation and evolutionary studies in the genus.

INTRODUCTION

Understanding evolutionary relationships in diverse lineages is an essential step in understanding the processes that drive speciation. Biodiversity varies taxonomically, temporally and spatially. Regarding taxonomic variation, some lineages such as the Coleoptera and cichlids among animals (see eg: Brawand et al., 2014; Salzburger, 2018; Zhang et al., 2018; Matschiner et al., 2020), or the Orchidaceae (Stewart and Griffiths, 1995) and Asteraceae (Funk et al., 2009) among Angiosperms, may be disproportionately species rich compared to species-poor lineages, such as Buphagidae (Oxpeckers) (Lovette and Rubenstein, 2007), Nautilidae (nautilus) (Vandepas et al., 2016), Amborellaceae (*Amborella* Genome Project, et al., 2013), and Fouquieriaceae (Soto-Trejo et al., 2022) to name a few. Temporal variation is seen in key innovations and/or favorable conditions that can facilitate rapid species radiations. Typically, these events hinge on opportunities to occupy available niches such as the colonization of a recently formed archipelago, the ability to utilize a resource such as oxygen, or the transition between terrestrial and aquatic environments (Heckman et al., 2001; Rota-Stabelli et al., 2013; Martin and Richards, 2019; Gillespie et al., 2020). Such opportunities are context dependent, for as environments change and new innovations arise, the distribution of lineage specific biodiversity shifts. For example, the trilobites that dominated Cambrian

paleofaunas and remained important throughout the Ordovician period and onwards were fully extinguished by the end of the Permian-early Triassic (Andrian et al., 1998; Sundberg et al., 2020; Hopkins et al., 2023), or the Equisetaceae family, which exhibited several radiations and extinctions from the Carboniferous through the Jurassic periods, is now only represented by a few extant species (Elgoriaga et al., 2018). The global inventory of extant biodiversity is distributed asymmetrically in space where biodiversity hotspots support disproportionately high species density (Myers, 1988, 1990; Marchese, 2015).

Understanding the factors that promote increased biodiversity in so-called hotspots is of central importance to understanding evolution and diversification. The Mediterranean climate region which extends from southern Oregon, through most of California to northern Baja California is known as the California Floristic Province (CFP) because it supports nearly 5,800 plant species, and over 30% are endemic (Burge et al., 2016). Numerous explanations for the high plant diversity of the CFP were proposed (see eg: Raven and Axelrod 1978; Ackerly, 2009; Thornhill, et al., 2017), and it is likely to due to a combination of numerous factors including Mediterranean climate, high topographic and substrate diversity, and an abundance of stable refugia (Baldwin, 2014). Our understanding of the relative importance of these factors is enhanced by the phylogenetic analysis of plant groups that inhabit the CFP, which in turn informs our universal understanding of the factors that support biodiversity. Regarding endemic diversity in the

CFP, Raven and Axelrod (1978, p. 54-78) originally proposed that woody species such as *Sequoiadendron giganteum* and *Abies magnifica* are so-called paleoendemics that were once widespread and now are restricted to stable refugia, whereas endemics that evolved recently within the CFP, neoendemics, are generally herbaceous species that diversified in the Central Valley of California, which was submerged for at least most of the Tertiary era (Stanley et al., 2002; Graham and O'Geen, 2016, p. 51-52). Phylogenetic and biogeographic research has largely supported these hypotheses (Baldwin, 2014; but see Thornhill, et al., 2017). Herein, we investigate the genus *Arctostaphylos*, the most diverse woody genus in North America that is long thought to be an exception to this life-history-endemism type association in the CFP but whose species relationships have remained largely mysterious.

Widely proposed woody neoendemics in the CFP are shrubs that mainly reside in chaparral. Woody plants have been shown to have lower rates of evolution compared to herbaceous species, a phenomenon attributed to their relatively longer generation times (Smith and Donoghue, 2008), so exceptions are of evolutionary interest. The chaparral is a young CFP ecosystem that arose approximately 15 Mya during the Miocene epoch (Axelrod, 1958a; Rundel, et al., 2016). Plants would either require preadaptations or novel adaptations to occupy this fire-prone, xeric environment. The biome is dominated by densely packed evergreen sclerophyllous shrubs (Parker, et al., 2016, p. 479; Rundel, 2018). The hot dry summers of the CFP lead to frequent fires and the success of woody

plants in the chaparral relies on physiological and life-history adaptations to high intensity fires (see eg: Keeley and Zedler, 1978; Keeley, 2006; Pausas and Kelley, 2014). The typical physiological adaptation is a hardened basal storage organ known as a burl which contains dormant buds that allow the plants to resprout after fire.

The three main genera suspected to have radiated in the CFP chaparral are *Quercus* (Fagaceae), *Ceanothus* (Rhamnaceae), and *Arctostaphylos* (Ericaceae). The shrub phenotype arose twice in *Quercus* from an ancestral arborescent habit, once in section *Quercus* (McVay et al., 2017) and again with a smaller radiation in section *Lobatae* (Hauser et al., 2017). Although there are several arborescent *Quercus* species in the CFP which dominate the oak woodland and oak savannah biomes, they are generally thought to be paleoendemics and are less species rich than their chaparral-shrub counterparts. Weighing in at ca. 400-600 species globally (Manos and Hipp, 2021, Jepson Flora Project, 2026), *Quercus* is a highly successful genus in the northern hemisphere but only has ca. 20 species in the CFP (Sork et al., 2016, Jepson Flora Project, 2026) where *Ceanothus* and *Arctostaphylos* have ca. 45 and ca. 67 species respectively (Parker et al., 1993+; Kauffmann, et al., 2021; Burge et al., 2011). The high diversity in the two latter genera is primarily represented by narrow endemics in the CFP chaparral. Their success may in part be due to a unique life-history adaptation to fire. The majority of *Arctostaphylos* and *Ceanothus* species lack burls and instead repopulate after fire via fire-stimulated seedbanks; this is known as an obligate seeder life-history. Wells (1969)

hypothesized that the obligate seeder life-history facilitated diversification in *Ceanothus* and *Arctostaphylos* by setting their generation times to the fire return interval thereby creating opportunities for natural selection to act after each fire, while adult generations of plants that resprout (sprouters) could persist through indefinite fires and their seedlings need to compete for space with the already established adult generation. Raven and Axelrod (1978, p.78-81) agreed with Wells' assessment but also noted putative frequent hybridization in the two genera and proposed that their diversification was also driven by adaptive introgression. North American *Vitis* were shown to exchange resistance genes to Pierce's disease, a bacterial pathogen with exponentially higher generation time than their woody hosts, suggesting that adaptive introgression is a viable mechanism to accelerate evolution relative to mutation rate in woody plants (Morales-Cruz et al., 2021). Numerous reports of and studies on hybridization in *Quercus*, *Ceanothus* and *Arctostaphylos* suggest that it is a common phenomenon (see eg.: Schierenbeck, et al., 1992; Burge et al., 2011, 2019; Hauser et al., 2017; Parker et al., 2020; Serkanic et al., 2021; Abbo et al., 2025).

In contrast to *Quercus* (Hauser et al., 2017; McVay et al., 2017), and *Ceanothus* (Burge, et al., 2011), there are no molecular phylogenetic studies in *Arctostaphylos* that combine both high taxon sampling and multiple within-taxon replication making it difficult to assess the monophyly of currently recognized species, which by extension limits our ability to test hypotheses about the diversification of the genus. Early phylogenetic

studies found that *Arctostaphylos* is divided into two major clades known as the small and large clades but were unable to resolve species relationships within the two clades (Markos et al., 1998; Boykin et al., 2005; Wahlert et al., 2009). Said experiments were principally designed to assess species relationships and typically did not include multiple replicates of taxa. More recent studies included higher replication of taxa, making them able to assess the monophyly of named taxa but were limited to a small subset of species (Huang et al., 2020; Serkanic et al., 2021; Abbo et al. 2025, in prep; Sweeten and Parker, 2025). Two studies that combined nuclear and chloroplast loci suggest that widespread chloroplast capture, and therefore hybridization, complicates phylogenetic resolution (Serkanic et al., 2021; Sweeten and Parker, 2025).

The genus *Arctostaphylos*, commonly known as manzanitas, has a circumpolar distribution but only a single species, *A. uva-ursi*, extends outside of North America. The genus is hyperdiverse due to numerous species that are endemic to the CFP, which unlike the prostrate *A. uva-ursi*, are typically shrubs or small trees (Supplemental Materials 21 B). They are best known for having smooth red bark with twisting branches but a few species have shredding gray bark (Supplemental Materials 21 C). The common name manzanita (little apple in Spanish) is a reference to their characteristic small red fruits (Figure 3.4 C); although, they are technically multi seeded drupes rather than pomes. The species richness of *Arctostaphylos* declines precipitously upon exiting the CFP. A few species such as *A. nevadensis* and *A. glauca* expand short distances outside of the CFP

but only five species have significant ranges outside the CFP.¹ Within the CFP, especially its Central Coast, the genus is primarily represented by a series of narrow endemics of conservation concern often with distributions of only a few square kilometers or less (Vasey and Parker, 2014 p. 192-193; Kauffmann, et al., 2021; Jepson Flora Project, 2026).

The reasons for this shift in diversification pattern are unknown due to limited evidence for evolutionary hypotheses. *Arctostaphylos* has a sparse fossil record and a controversial taxonomy that lacks broad scale molecular evaluation (Paleo Biology Database; Wells, 1988; Hileman et al., 2001; Parker et al., 2007; Wahlert et al., 2009; Keeley et al., 2017; Kowalski and Worobiec, 2018). *Arctostaphylos* is a member of the Arbutioideae subfamily of Ericaceae; it contains a handful of genera that all produce ursinate flowers (Supplemental Materials 21 D). The genera in the Arbutioideae other than *Arctostaphylos* are comparatively species poor (Fernald, 1914; Diggs, 1986; Hileman et al., 2001; Yatsenko et al., 2025). The genus *Arbutus* has a Medrean-Tethyan disjunction which is estimated to predate the emergence of *Arctostaphylos* by approximately 10 million years

¹*Arctostaphylos uva-ursi* which is found throughout alpine and subarctic habits throughout the Northern Hemisphere and has a small disjunct population in the Mountains of Guatemala (Wells, 1968; Parker et al., 1993+; Graham, 1998; Sweeten and Parker, 2025), *A. pungens* which is found throughout southwestern North America (Parker et al., 1993+; Kauffmann, et al., 2021; Martínez-Calderón et al., 2022; Jepson Flora Project, 2026), *A. patula* which is found throughout the Sierra Nevada, Southern Cascades, Klamath, Transverse, and Peninsular CFP mountain ranges, the US Inter-Mountain West, and a small portion of the US Pacific Northwest (Parker et al., 1993+; Ackerfield, 2015, p. 342; Hitchcock and Cronquist, 2018, p.403; Kauffmann, et al., 2021; Jepson Flora Project, 2026), *A. pringlei* which occurs throughout the Peninsular and northern Madrean sky island mountain ranges (Parker et al., 1993+; Vasey and Parker, 1999; Kauffmann, et al., 2021), and *A. columbiana* which follows the Pacific coast from northern California to Vancouver Island, British Columbia (Parker et al., 1993+; Hitchcock and Cronquist, 2018, p.403; Jepson Flora Project, 2026).

(Hileman et al., 2001). Although *Arbutus* occurs in an analogous habitat to CFP chaparral in the Mediterranean Basin, it did not hyper diversify in either the CFP nor the Mediterranean, suggesting that the hyper diversification of *Arctostaphylos* was due to unique characters and/or unique environmental circumstances. One hypothesis, mentioned above, that the presence of obligate seeders may have uniquely facilitated *Arctostaphylos* diversification via higher rates of evolution compared to resprouting *Arctostaphylos*, could not be tested in previous studies due to insufficient variability in the nuclear ITS locus (Wahlert et al., 2009).

The current study aims to evaluate the monophyly of taxa and resolve the evolutionary relationships among individuals in the genus *Arctostaphylos*, which will serve as a foundation for understanding hyperdiversification within the CFP and within woody plants in general. Additionally, the results have tangible implications for conservation as assessing the support for rare taxa which currently lack molecular evaluation will aid land managers in prioritizing the allocation of limited conservation resources. A growing number of studies have used RAD-seq or ddRAD-seq, a popular method of reduced representation genomic sequencing for molecular systematics and population genetics (see eg: Peterson et al., 2012; DaCosta and Soreson, 2016; Ma et al., 2025), to investigate *Arctostaphylos* and have achieved far more promising results than those achieved with Sanger-sequencing (Burge et al., 2018; Huang et al., 2020; Abbo et al., 2025; Abbo et al., in prep). These have been gradually increasing in taxonomic scope beginning with an

investigation of a small subset of subspecies of *A. glandulosa* (Burge, et al., 2018), expanding to an investigation of the majority of subspecies of *A. glandulosa* (Huang et al. 2020), and culminating in investigations of small groups of *Arctostaphylos* species in two subregions of the CFP (Abbo et al., 2025; Abbo et al., in prep). The latter two studies benefit greatly from the reference genome of *A. glauca* assembled as part of the California Conservation Genomics Project (Huang et al., 2022). As a natural progression from these prior studies, we use reference-aligned ddRAD sequences to investigate *Arctostaphylos* from a genus-wide context including ca. 88% of described species and ca. 71% of described infraspecific taxa. Although primarily a diploid genus ($2n=26$), *Arctostaphylos* includes several tetraploid taxa ($2n=52$), and Abbo et al. (in prep) found that *A. glandulosa*, currently treated as tetraploid, was paraphyletic and affected the inferred species relationships among, and in two cases led to the paraphyly of, a set of diploid taxa, which resolved neatly when *A. glandulosa* samples were excluded from the dataset. We hypothesized that this might be the case for other tetraploid taxa and therefore carried out both analyses that used all available samples and one which excluded potential tetraploids based on historically documented chromosome counts. Our primary research questions are: 1) To what extent are currently described *Arctostaphylos* species monophyletic supporting the hyperdiversity hypothesized in the current morphological-based treatment? Here we seek to test a null hypothesis of oversplitting in the genus, for challenges in species identification and numerous historic taxonomic reorganizations have led several botanists to speculate that the genus has unreliable taxa;

most famously, *Arctostaphylos* taxonomist Roof decided near the end of his career that the entire taxonomy of the genus should be lumped into only six species (Roof, 1976, 1979a, 1979b, 1980, 1982). 2) How are *Arctostaphylos* species related to one and other and can we establish the order of their diversification within the CFP? 3) To what extent do the primary morphological characteristics used to identify *Arctostaphylos* species have single origins making them good indicators of species relationships which recapitulate the molecular phylogeny? 4) To what extent does the data support the hypothesis of neoendemism for *Arctostaphylos* species endemic to the CFP?

METHODS

SAMPLING

Over the course of six years (from 2018 to 2025) we sourced a total of 426 samples of individuals in the genus *Arctostaphylos* as well as four other individuals used as outgroups (*Arbutus unedo* [n=1], *A. menziesii* [n=2], *Arctous alpina* [n=1]). In terms of taxa represented, the 426 *Arctostaphylos* samples are distributed among 59 species (54 with three or more replicates, three with two replicates, and two represented by a single sample) or 76 taxa including subspecies (63 with three or more replicates, nine with two replicates, and four represented by a single sample), and 27 samples represent currently unrecognized taxa or putative hybrid individuals. Sampled locations are summarized in Figure 3.1, and the detailed metadata for each sample including which datasets it was

used in and links to voucher specimen data is given in Supplemental Materials 15. Our aim was to sample a minimum of three replicate individuals for narrow endemic *Arctostaphylos* species, and a minimum of five samples well-distributed across their ranges for widespread *Arctostaphylos* species. This is true for the vast majority of taxa represented in the dataset but some exceptions apply where procuring material was not feasible or material did not yield viable DNA after multiple extraction attempts. Most samples were obtained in the field during collection events that included one or more of the authors. Two types of exceptions apply: one for donated material and one for cultivated material. The outgroup sample of *Arctous alpina* and three samples of *Arctostaphylos pungens* from Mexico were collected by and sent to our laboratory by other researchers. Due to conservation concerns or logistic difficulties arranging field collections, some samples were obtained from the living collections at the Tilden Regional Parks Botanic Garden, Tilden, United States (n=19), and the University of California (UC) Berkeley Botanical Garden, Berkeley, United States (n=17); *Arctostaphylos* individuals from these institutions were originally sourced as cuttings from wild individuals and their accessions are well documented. Additionally, the *Arbutus unedo* outgroup sample, native to the Mediterranean Basin, was obtained from an individual cultivated on the UC Riverside campus. Early trials showed that air-dried material of *Arctostaphylos*, such as that found in herbarium specimens, did not yield high molecular weight DNA which ruled out the option to supplement the dataset with museum specimens (unpublished).

In general, for each sample we collected young leaves, and/or when available unopened flower buds and young inflorescences, which we stored on ice for short term transport (seven days maximum) to our laboratory at UC Riverside where the material was kept at -20 °C until DNA extraction. Occasionally, it was not possible to transport a cooler into the field and we instead dried material in silica gel and it remained in the silica gel at room temperature until DNA extraction. In our long term experience extracting DNA from *Arctostaphylos*, we have obtained higher success rates and DNA concentrations using fresh-frozen material, our preferred storage method, compared to silica-dried, but both material types work with the same DNA extraction protocol. Additionally, for each sample, we collected morphologically diagnostic material to make a voucher specimen which we deposited at the UCR Herbarium. Images of and label-data from these voucher specimens was added to the publicly accessible data portal, CCH2.org and can be quickly accessed from the links provided in Supplemental Materials 15. If excess material was available after mounting the voucher specimen, one or more duplicates were made and distributed to various herbaria as part of the UCR Herbarium's exchange program.

DNA EXTRACTION AND SEQUENCING

For the majority of samples, we extracted DNA using the Qiagen DNeasy Plant mini kit (QIAGEN, Venlo, The Netherlands) according to manufacturer's instructions. For a

small minority of our samples in 2019-2020, we extracted DNA using a CTAB method; we abandoned this method as the Qiagen kits had a higher success rate with our material. The presence of high molecular weight DNA was confirmed for each sample via gel electrophoresis (using 1% agarose gels with ETBR as a UV-visible stain). We ascertained an estimate of the DNA concentration of each extract using an Eppendorf BioSpectrometer Basic Model #6135. Prior to 2022 we used a Qubit 3.0 Fluorometer, and for a short period in 2024 a Nanodrop One, while the device was available in a shared temporary laboratory space, to quantify DNA concentration, but we found no real world improvement in library preparation when DNA was quantified using these more modern methods. Successful extracts were prepared for ddRAD sequencing using an applicable version of our library preparation protocol.

This study is the fruit of multiple years of research and consequently samples were sequenced in various batches and were prepared with different versions of our library preparation protocol which has undergone iterative optimizations to balance labor intensity with observed sample drop out in sequencing. Additionally, some sequences have been used in previous manuscripts; Supplemental Materials 15 indicates the citation for the publication a sequence was first used in if different from the current one and cites the SRA database project accession number that includes the sequence. A detailed write up of our first library preparation protocol can be found in the supplemental materials of Huang et al. (2020). The samples labeled sequencing batch ‘UCR early batches’ in

Supplemental Materials 15 were prepared using this original protocol and were sequenced between the years 2019 and 2020 on an Illumina NextSeq 500 V2 (San Diego, California, USA) sequencer at the UCR Institute for Integrative Genome Biology Genomics Core. Samples with sequencing batch labels of “Novogene 1” or “Novogene 2” in Supplemental Materials 15 were sequenced in 2022 and 2023 respectively on an Illumina HiSeq platform-sequencer at Novogene (Beijing, China) in Davis, California, United States. We prepared these batches of sequenced libraries with a redesigned library preparation protocol that eliminated the need for several labor intensive steps. Instead of removing excess unligated adaptors using magnetics beads and amplifying each sample individually, the samples were pooled following barcoded adaptor ligation and both size selection and ligation product cleanup was achieved in the same step via a gel excision and subsequent gel extraction using the NEB Monarch Spin DNA Gel Extraction Kit (Ipswich, USA). Then the size-selected pool was amplified via PCR in six replicate reactions. The PCR product was purified using a one-to-one bead cleanup using Beckman Coulter Life Sciences AMPure XP Beads (Brea, USA). The Evrogen (Moscow, Russia) Timmer Kit reaction was deemed unnecessary as explained in Abbo et al. (2025), and the cleaned amplified pool was submitted directly for sequencing. The main drawback of this modified protocol was that amplification among samples was uneven leading to a large number of samples failing in sequencing due to low read recovery. This was mitigated by implementing the minipooling strategy explained in Abbo et al. (2025) where gel excisions and amplifications were carried out in pools of eight to ten samples, and the

minipools were combined into a single pool prior to sequencing. We included a portion of the samples prepared for Abbo et al. (2025) here; they are labeled as “Genewiz” in the batch column of Supplemental Materials 15. The final two batches labeled “UCR 2” and “UCR 3” respectively were both sequenced in 2025 at the UCR Institute for Integrative Genome Biology Genomics Core using an Illumina NextSeq 2000 sequencer. We prepared the last two batches of libraries with the most current version of the protocol, which only modifies the Abbo et al. (2025) version by increasing the minipool size to 14 samples per pool.

PROCESSING READS FOR PHYLOGENETIC ANALYSIS

We used the IPYRAD version 0.9.95 pipeline to generate multiple sequence alignments from raw reads. We set the assembly method parameter to “reference” to align the reads to the reference genome of *Arctostaphylos glauca* (Huang et al., 2022). Other parameters were kept at default for ddRAD sequences digested with MspI and PstI enzymes with the expectation of the “min_samples_locus” and “max_alleles_consens” parameters. The “min_samples_locus” parameter controls how many samples need to include a rad-locus for the locus to be included in the final alignment; following the methods from our previous research in *Arctostaphylos* (Abbo et al., 2025; Abbo et al., in prep) we selected a value for this parameter equal ca. 30% of ingroup samples (128 for the 2 MA and 4MA “all samples” datasets and 77 for the “diploids only” dataset). The “max_alleles_consens” parameter controls the maximum number of alleles allowed at any given site in the

consensus sequence. For the full dataset, we produced two separate sets of alignments, one with this parameter set to two (default) and the other with the parameter set to four to allow for triallelic or tetraallelic sites in the consensus sequence as these could potentially be prevalent in tetraploid samples; we referred to these datasets as the “all samples 2MA” and “all samples 4MA” datasets representatively. Previous studies in *Arctostaphylos* did not observe significant changes to the results when changing this parameter from two to four (Abbo et al., 2025; Abbo et al., in prep), and Haung et al. (2020) observed similar results using different sequencing pipelines. These studies, however, examined fewer taxa than herein, only two of which (*A. crustacea* and *A. glandulosa*), are considered tetraploid in the current taxonomic treatment (Jepson Flora Project, 2026), so we aimed to test these findings in the full dataset.

Additionally, Abbo et al. (in prep) found the widespread tetraploid *A. glandulosa* to be paraphyletic and its inclusion affected the recovered topology of their phylogenetic tree, so we examined a third dataset containing only diploid taxa (the “diploids only” dataset), which we processed with “max_alleles_consens” set to two. We selected samples for this dataset by reviewing all published ploidy counts in the genus to identify taxa with confirmed diploid chromosome counts. The results of this ploidy-count review are summarized in Supplemental Materials 17. All samples of taxa with unknown ploidy, with polyploid counts, or, with the exception of *A. pungens*, with conflicting ploidy counts were excluded from the “diploids only” dataset. *Arctostaphylos pungens* was

treated differently because there are multiple chromosome counts that suggest it is diploid and conflicting counts were only reported in Monterey and San Benito Counties, California which is suspected to be a hybrid zone for this taxon and individuals from there have historically been treated as different species, now subsumed into *A. pungens* (Roof, 1978; Wells, 1988; Parker et al., 2023). Additionally, the results herein show that *A. pungens* samples from these two neighboring Counties resolve separately from the remaining *A. pungens* samples (Figure 3.2; Supplemental Materials 18, 19). Therefore, we excluded from the “diploids only” dataset samples of *A. pungens* from Monterey and San Benito Counties but included all other *A. pungens* samples. Ideally, the ploidy of each individual sample would be tested via flow cytometry (Bourge et al., 2018; Wagner et al., 2020; Crowl, et al., 2022) but pilot trials were unsuccessful and suggested that the technique was nonviable for frozen or dried *Arctostaphylos* tissue (unpublished).

Lastly, the “diploids only” dataset was further subsampled to create a dataset for estimating the divergence times of major lineages, the “time calibration” dataset. Maximum likelihood phylogenetic analysis revealed nine clades composed of geographically consistent taxa and a single species, *A. klamathensis*, resolved on its own at the base of the small clade (Figure 3.3). We selected a set of taxa including the *Arctous alpina* outgroup, *Arctostaphylos klamathensis* and representatives of each of the other nine clades proportional to the number of taxa in each of those clades, typically two or three taxa but six from the “North Central Coast and Northern Coastal” clade because it

had the largest number of member taxa. Samples used in building this dataset are identified with “yes” in the “time calibration dataset member” column of Supplemental Materials 15. We excluded any sample identified as a putative cryptic hybrid in the “diploids only” dataset maximum likelihood phylogeny (Figure 3.3). Ingroup taxa were selected that had the most number of replicated samples, had reasonable phylogenetic distance from one another, and which together captured the majority of the geographic range of their respective clades with minimal redundancy; in the event of a tie we selected the taxon with the highest average number of rad-loci in the assembly according to the IPYPRAD summary statistics. In the case of *A. viscida* which in the “diploids only” dataset maximum likelihood phylogeny had one apparent hybrid sample and the remainder of its samples divided into two sibling clades, one including only *A. viscida* samples and the other including *A. nissenana* nested within the remaining *A. viscida* samples, we excluded the hybrid sample and only included *A. viscida* samples from the clade composed exclusively of *A. viscida* samples. We represented the *A. viscida* plus *A. nissenana* clade using only the samples of *A. nissenana*. We then created a multiple sequence alignment of consensus sequences for each taxon in the “time calibration” dataset by supplying a list of all scaffolds in the reference genome along with a PYTHON dictionary associating all replicate individuals to their respective taxon (referred to as an “imap” in the IPA documentation) to the “window_extractor” function from the IPYRAD analysis tools (IPA). We ran “window_extractor” with the additional arguments of “mincov=0.8” to restrict the alignment to locus shared by at least 80% of samples in the

dataset, and “consensus_reduce=True” which insured that consensus sequences would be built for each taxon from their replicate samples. This reduced the size of the dataset to 26 samples. We only used this dataset for an analysis of the divergence time of taxa, rather than individuals, and individuals used to create each consensus sequence were shown to be monophyletic in the maximum likelihood phylogeny. This data reduction both made the downstream analysis less computationally intensive but increased the quality of data for ingroup samples as missing data in any one or more individuals of a given taxon could be nonmissing in the consensus sequence sample of the taxon provided that the data was nonmissing in at least one of the individual replicates for the taxon.

PHYLOGENETIC ANALYSIS

We built maximum likelihood phylogenies for the “all samples 2MA” and “4MA”, and the “diploids only” datasets by supplying the concatenated alignment matrix in PHYLIP format (generated by IPYRAD) for each dataset to IQTREE version 2.2.0.5 (Minh et al., 2020) using the TVM+F+I+R10 substitution model and 1000 ULTRAFAST BOOTSTRAP iterations (Hoang et al., 2018). We ran MODEL FINDER (Kalyaanamoorthy et al., 2017) on the alignment matrix for the “diploids only” dataset to confirm that TVM+F+I+R10 was the most supported model out of the list of models available in IQTREE. We could not carry out this step for the “all samples” datasets due to the size of the alignments which made MODEL FINDER computations prohibitively slow; however, our preliminary testing shows that MODEL FINDER always selects from

the similar models TVM+F+I+R7, TVM+F+I+R8, TVM+F+I+R9, or TVM+F+I+R10 for our data with TVM+F+I+R10 being the most common, especially for longer alignments (unpublished) as was the case for the larger dataset in our most recent analysis (Abbo et al., in prep). We rooted the tree files generated in IQTREE at the clade of outgroups (*Arbutus unedo*, *A. menziesii*, and *Arctous alpina*) in R version 4.5.0 using APE version 5.8-1 (Paradis and Schliep, 2019). We visualized and annotated the rooted trees with GGTREE version 3.16.0 (Yu et al., 2016) and made final design edits in INKSCAPE version 1.2.2 (Inkscape Project).

We generated a time calibrated Bayesian phylogeny for the “time calibration” dataset using BEAST2 version 2.7.1 (Bouckaert, et al., 2019). We created the .xml file supplied to BEAST2 in BEAUTI2 version 2.7.8 (*ibid*) using the multiple sequence alignment in nexus format generated by IPA “window_extractor”. Long branches for samples of *Arbutus* in the maximum likelihood trees (Supplemental Materials 18-20) suggested a significant loss of restriction sites in these samples relative to the ingroup samples, so we only included the *Arctous*, the closest relative to *Arctostaphylos* (Hileman et al., 2001), outgroup sample for this analysis. We allowed BMODELTEST version 1.3.3 to infer the best substitution model for our dataset with uninformative priors (Bouckaert, and Drummond, 2017). *Arctostaphylos* has a sparse fossil record with only seven fossils documented in the Paleo Biology Database (Paleo Biology Database; Figures 3.6, 3.7), and although fossils in Montana and Wyoming suggest that genus could be up to 48.1

million years old, fossils in the Arbutoideae subfamily are considered unreliable due to the limited morphological characters they provide and the specimen of the extinct taxon *A. mohavensis* Axelrod (1939) is considered the most plausible oldest record for *Arctostaphylos* (Kowalski and Worobiec, 2018). Hileman et al. (2001) estimated the age of the *A. mohavensis* fossil to 15.8 Mya and argued that it could be placed anywhere along the branch connecting the most recent common ancestor (MRCA) of *Arctous* and *Arctostaphylos* to the MRCA of *Arctostaphylos*. Axelrod (1958b) also described the fossil taxon *A. verdiana* from material found in a 5.3-2.6 Mya formation. Using these two pieces of evidence we set a prior exponential distribution with a mean of 5.9 and an offset of 4.0 for the age of the MRCA of *Arctostaphylos*, setting the minimum age at 4.0 Mya but with a 95% probability density that included up to 21.7 Mya.

We used an Optimized Relaxed Clock Model for the molecular clock (Douglas et al., 2021). Yoichi et al. (2016) estimated the mutation rate of *Rhododendron weyrichii* (Ericaceae) to be 1.581×10^{-9} per site per year by dividing the geometric mean of estimated mutation rates of *Arabidopsis* (herbaceous) and *Populus* (woody) by the generation time of *R. weyrichii* estimated at 15 years. We used this same approach but noting that the generation time of obligate seeder *Arctostaphylos* species is tied to the fire return interval which is variable among sites (17-100 years across ecosystems occupied by *Arctostaphylos*, typically closer to 35 years [Stafford and Van De Water, 2014]) in the CFP, and the generation time of resprouting *Arctostaphylos* species is indefinite, we

reasoned that 50 years was a usable starting point estimate for the mean *Arctostaphylos* generation time but the estimated mutation rate should be allowed vary by plus or minus one order of magnitude. Thus, we used 4.7×10^{-4} mutations per site per 106 years ($\sim \{ [1.581 \times 10^{-9} \mu/y \times 15y] / 50y \} \times 106y$) as both the estimated initial value and mean value for an exponential distribution for the mean clock rate prior. We also used an exponential distribution for the standard deviation of clock rate prior, which controls the extent to which branches can deviate from the assumption of a strict molecular clock, with a mean of 0.5 leaving the initial estimated value at default; this distribution allowed for a range of weak to more significant deviations from clock-like behavior.

We used a Calibrated Yule model tree prior (Heled and Drummond, 2012) and set the birth rate prior to a log normal distribution with a mean in realspace of 0.11 and a standard deviation of 0.2; we estimated the birth rate prior based on the recommendations in the BEAST2 documentation (Centre for Computational Evolution) whereby we selected the mean of the distribution by dividing the natural log of half of the number of taxa in the dataset ($\ln[26/2]$) by an estimate of the root height (23 million years, allowing the divergence between *Arctous* and *Arctostaphylos* to occur 1 million years before our latest estimate for the MRCA of *Arctostaphylos*) and chose a standard deviation that allowed for a 95% probability density of one order of magnitude variation from the mean. We kept all other parameters/priors at default and carried out three replicate runs with different random seed numbers with MCMC chain lengths of 100 million, logging every

5000 samples. We assessed the completeness and convergence of the three runs using TRACER version 1.7.2 (Drummond et al., 2018). Then we generated a maximum credibility tree using TREEANNOTATOR version 2.7.7 for the first run discarding the first 10% of trees as burn-in (Rambaut and Drummond, 2024). We visualized and annotated the tree using GGTREE along with the “coord_geo” function from the DEEPTIME package version 2.3.1 (Gearty, 2025) and made final design edits in INKSCAPE.

ASSESSMENT OF SUPPORT FOR TAXA

We used a criterion of monophyly, which is consistent with a phylogenetic species concept (Donoghue, 1985), to evaluate the support for each taxon included in this dataset. Our threshold for considering a taxon to be fully supported was that all samples of a given taxon must form a single clade with $\geq 95\%$ UFB support that does not interfere with another taxon i.e. does not cause another taxon to be paraphyletic. Additionally, we required that a taxon must be supported by at least three samples and the samples must account for the taxon’s geographic range. Thus, some taxa could not be fully supported by default due to deficiency in sampling. We considered a taxon partially supported if it exhibited few minor violations of these criteria; for example, a taxon-clade could have close to but less than 95% UFB support, the taxon could be monophyletic but nested within another taxon, the taxon could include putative hybrids in its clade, or the taxon could be supported in most of but not all of its geographic range. We considered a taxon

unsupported if it exhibited multiple, severe violations of our criteria such as a taxon appearing in multiple places on the tree lacking a clear pattern.

PHYLOGENETIC DISTRIBUTION OF MORPHOLOGICAL CHARACTERS

We selected nine characters that are essential to the identification of *Arctostaphylos* species (Jepson Flora Project, 2026) to assess their distribution in the “all samples 2MA” phylogeny. We constructed a table assigning categorical values for each character to each sample in the dataset and plotted this information on the phylogeny using GGTREE and made final edits in INKSCAPE. The characters we selected are summarized as follows.

Inflorescence bracts: leaf-like— unambiguously foliaceous; scale-like— dry, scarious, typically browning at anthesis; mixed— in a predictable sequence from scale-like to leaf-like; intermediate—ambiguously foliaceous often fleshy/succulent; deciduous— ephemeral, senescing at fruiting or prior. Leaves: auriculate— with highly reduced or absent petioles and deeply lobed blades, clasping the stem or nearly so; entire— with readily apparent petioles and blade margins entire or shallowly lobed. Burl: present— has woody basal structure with dormant buds which allows the plant to resprout after fire; absent— lacks a basal burl, plant is an obligate seeder; sometimes present— only some individuals produce a burl, the taxon contains a mixture of obligate seeders and resprouters. Stomatal distribution: amphistomatous— stomata present on both leaf surfaces in $\pm$ equal proportions; abaxial— stomata only present on the abaxial leaf surface; weakly amphistomatous— stomata present on both leaf surfaces but noticeably

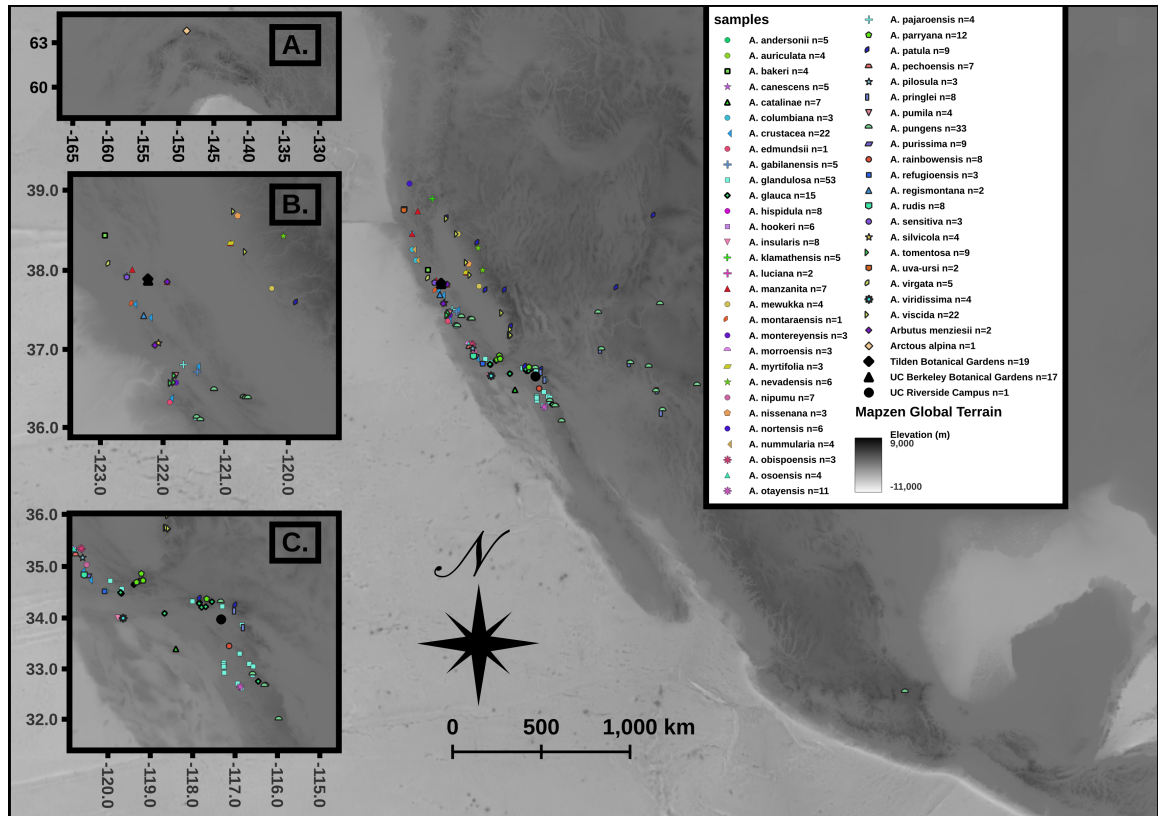


Figure 3.1: Simplified sampling map. Sample collection locations throughout California and the number of individuals per species collected (n) are presented on a topographic map. Elevation is presented in meters relative to sea-level (darker shades are higher). Taxa are only represented at the species rank and putative morphological hybrids and some morphologically nonconforming samples are omitted (see Supplemental Materials 15 for a complete sample list). Each species is represented by a unique color, as shown in the key to the right of the map (If readers can easily tell them apart, they would probably also enjoy the *Arctostaphylos* key [Jepson Flora Project, 2026]). Inset axes are in degrees latitude (y) and degrees longitude (x). **Inset A** shows the collection site for the outgroup sample of *Arctous alpina* in Alaska. **Inset B** shows the area around the Tilden and UC Berkeley botanical gardens, who provided study samples from their living collections. **Inset C** displays the UC Riverside campus (source of out cultivated *Arbutus unedo* outgroup sample) and collection sites in southern California. The map was generated in QGIS using MAPZEN as background layers for the main map and insets.

sparser on the adaxial side. Nutlets: fused— endocarp consisting of a single smooth sphere; free— endocarp consisting of multiple units separating on their own or after minimal manipulation; weakly fused (variable)— fusion of nutlets may vary between individuals or between fruits within individuals, when fused are typically imperfect spheres, rough and can often be separated by force. Bark: smooth, red— reddish on a spectrum of orange-brown to purple-brown with minimal tearing or peeling; grey, shredding — gray to reddish-grey with substantial tearing and/or peeling at least at the base of the stem. Habit: erect— plant typically $\pm$ tall, may be decumbent but not creeping along ground; prostrate— plant short, creeping along the ground. Inflorescence dormancy: present— produces inflorescences (referred to as nascent inflorescences) in the late season, typically fall, which arrest development until late winter or early spring; absent— inflorescences do not arrest development. Corolla: four-merous— with four lobes; five-merous— with five lobes.

RANGE MAPS

We downloaded collection records from the IDIGBIO portal for all taxa in each geographic clade in the “diploids only” dataset maximum likelihood phylogeny as well as the coordinates from each *Arctostaphylos* fossil from the Paleo Biology Database (IDIGBIO Portal; Paleo Biology Database). We imported these data points into QGIS version 3.34.4 over a base world map using the default projection (QGIS). We estimated the distribution of each clade by manually tracing polygons around the data points of the

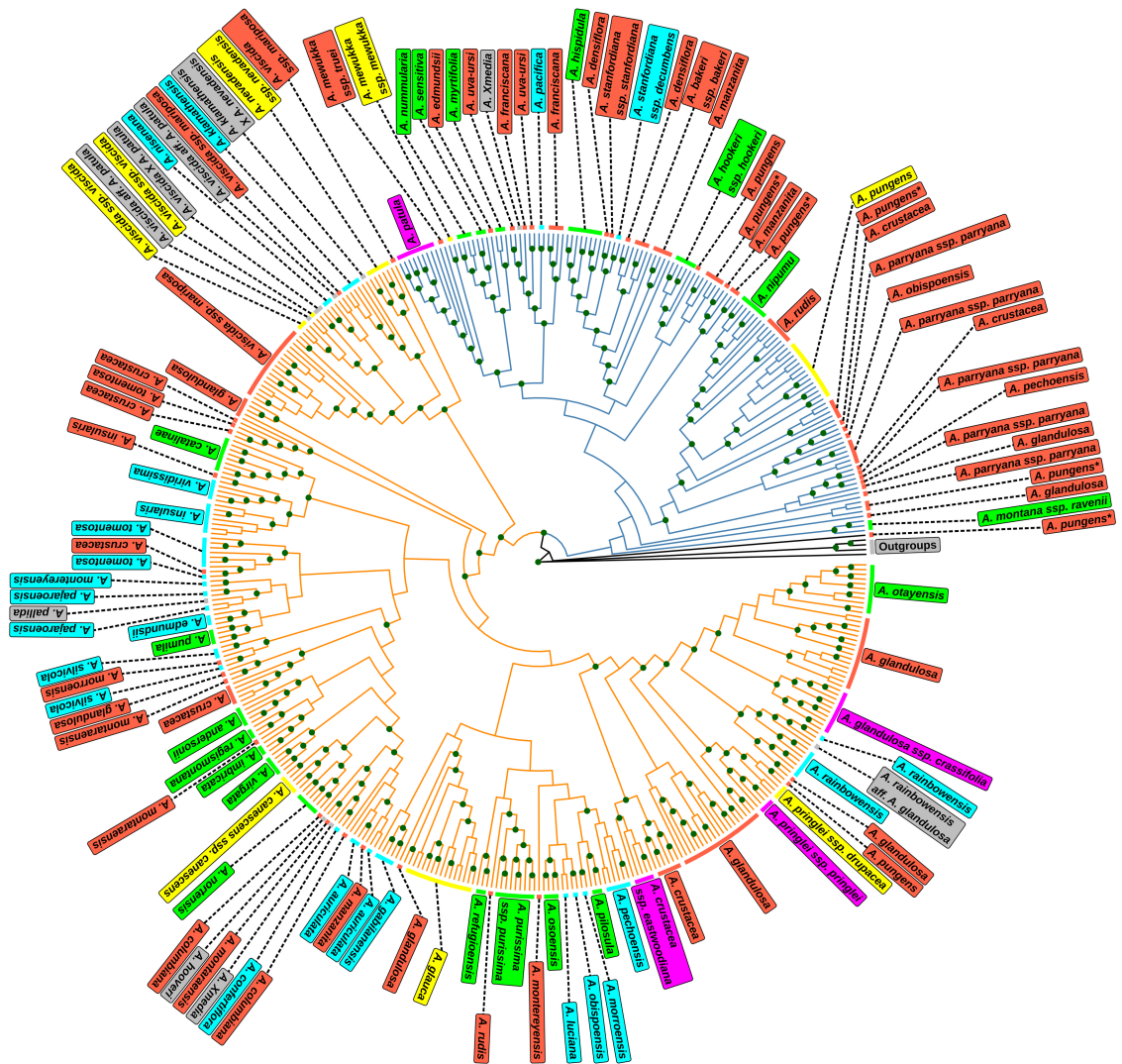


Figure 3.2: Genus-wide maximum likelihood phylogeny for the “all samples 2MA” dataset (includes diploid, tetraploid or lacking chromosome counts [see Supplemental Materials 17]). Branches leading to outgroup samples and a single, likely hybrid, sample of *Arctostaphylos pungens* from San Benito County, which resolves as sibling to the phylogeny, are in black. Branches of the small and large clades are blue and orange respectively. Nodes marked with a green circle have ≥ 95 UFBootstrap support. Color-coded tip labels indicate taxonomic support (based on monophyly of replicate samples). Narrow endemic taxa with strong support are in bright green, well-supported widespread taxa are in magenta. Narrow endemics and widespread taxa that are not fully supported are in cyan and yellow, respectively. Unsupported taxa are in Red, and outgroups, putative morphological hybrids, unreplicated taxa for which support can not be evaluated are in gray.

members of each respective clade excluding outlier points. For each map we added scale bars, north arrows, legends and other design elements in the QGIS print layout.

RESULTS

PHYLOGENETIC ANALYSIS SHOWS STRONGER SUPPORT FOR TAXA IN THE “DIPLOIDS ONLY” TREE

The maximum likelihood phylogenies for the “all samples 2MA”, “all samples 4MA”, and “diploids only” datasets agree that *Arctostaphylos* is divided into two major clades and generally agree that subclades within the two clades have strong geographic signal but disagree on the relative placement of geographic subclades (Figures 3.2, 3.3; Supplemental Materials 19). The branch leading to *Arbutus* outgroup samples is extremely long relative to *Arctous alpina* and the ingroup samples (Supplemental Materials 18-20) indicating a distant relationship to *Arctous* and the ingroup samples but we question whether the branch length may be inflated due to reference bias and/or loss of restriction sites (Lee et al., 2018; Akopyan, et al., 2025). Also *Arbutus menziesii* is curiously recovered as paraphyletic as the *A. unedo* sample resolves as sibling to one of the two *A. menziesii* samples. Support values differ strongly between the three trees. The “diploids only” tree (Figure 3.3) has a majority of strongly supported nodes ($\geq 95\%$ UFB), whereas the “all samples” trees have a higher proportion of nodes with low support, especially for internal nodes (Figure 3.2; Supplemental Materials 19). The “all

samples 4MA” has the highest proportion of nodes with low support, indicating limited resolution (Supplemental Materials 19). The 2MA and 4MA “all samples” trees mostly agree on topology with two notable differences. *Arctostaphylos refugioensis* samples are more closely related to *A. glauca* samples in the 4MA tree than in the 2MA tree, and *A. auriculata* samples associate with geographically neighbouring taxa in the 2MA tree but are sibling to a clade of *A. catalinae* (a Santa Catalina Island endemic) and *A. edmundsii* samples in the 4MA tree. Unsurprisingly given that the alignment was also generated with a maximum of two alleles per site allowed in the consensus sequence, the “diploids only” tree agrees with the “all samples 2MA” tree as to the placement of these two taxa.

There is a large disparity regarding the proportion of taxa that meet our criteria for full taxonomic support between the “diploids only” and “all samples” trees. We provide a full assessment of the support for all taxa included in the study in Supplemental Materials 17 but a simplified presentation is given with the color-coded tip labels in Figures 3.2 and 3.3 where fully supported taxa are in green or magenta, partially supported in yellow or cyan, and unsupported in red. The most striking examples of unsupported taxa are the widespread tetraploids (Supplemental Materials 17) *A. glandulosa*, *A. crustacea*, and *A. tomentosa* (to a lesser extent) which appear in multiple areas throughout the tree, some more or less geographically consistent with where they were collected and others seemingly random, and often the three taxa will appear in clades or form adjacent grades together. Not all tetraploids are unsupported; for example, the narrow endemic *A.*

montana ssp. *ravenii* is fully supported, the widespread *A. nevadensis* is mostly supported but is nested within *A. viscida*, and the samples of the moderately widespread *A. mewukka* all form a grade at the base of *A. patula* samples where the two representatives of *A. mewukka* ssp. *mewukka* resolve as monophyletic. Diploid taxa are generally at least weakly supported but there are notable exceptions such as *A. columbiana*, *A. densiflora*, and *A. montaraensis*. Several taxa that are weakly supported or unsupported in the “all samples” trees are fully supported in the “diploids only” tree because the samples that interfere with support are excluded from the latter dataset; some examples include *A. glauca* and *A. auriculata* which both include a sample of a tetraploid in the clades formed by their respective samples in the “all samples” trees, and *A. franciscana* which forms a complex with *A. pacifica*, *A. uva-ursi*, and *A. Xmedia* in the “all samples” tree but is the only taxon in the complex included in the “diploids only” tree and therefore resolves as monophyletic.

There are multiple indicators of hybridization in the maximum likelihood trees and these often prevent taxa from being fully supported due to our criteria of monophyly. Some cases of hybridization are overt such as each of the two samples of the hybrid species *A. Xmedia* which resolve near a different one of its hypothesized parents *A. columbiana* and *A. uva-ursi* respectively, or the specimen Abbo 087 which was identified as a putative hybrid between *A. klamathensis* and *A. nevadensis* due to morphological intermediacy observed in the field and resolves intermediate to the two hypothesized parental taxa in

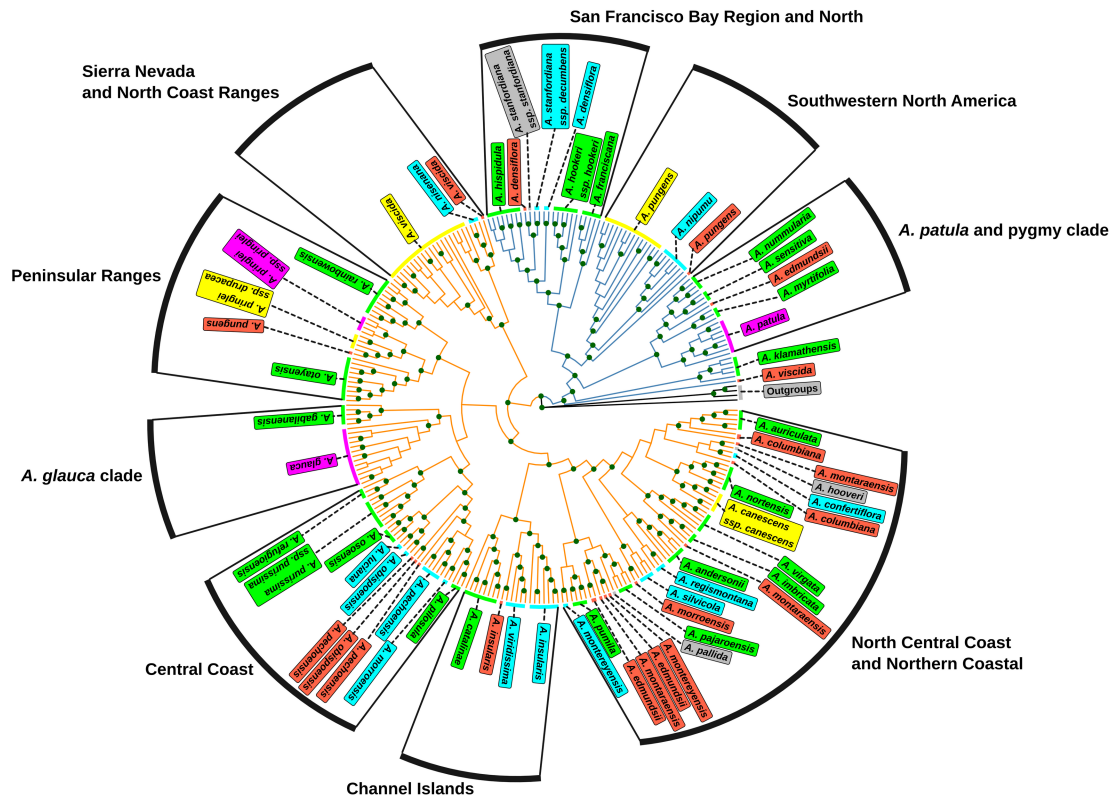


Figure 3.3: Maximum likelihood phylogeny of only ingroup samples from taxa with exclusively diploid chromosome counts in the literature (Supplemental Materials 17). Branches leading to outgroup samples are in black. Branches of the small and large clades are blue and orange respectively. Nodes marked with a green circle have ≥ 95 UFBootstrap support. Color-coded tip labels indicate taxonomic support (based on monophyly of replicate samples). Narrow endemic taxa with strong support are in bright green, well-supported widespread taxa are in magenta. Narrow endemics and widespread taxa that are not fully supported are in cyan and yellow, respectively. Unsupported taxa are in Red, and outgroups, and unreplicated taxa for which support can not be evaluated are in gray. Removal of tetraploids significantly influences the topology of the tree, with multiple species resolving in different positions, including shifting to the opposite clade (for example, *A. klamathensis*). Clade labels indicate the overall structure of geographic association between related species within the major clades (the names were selected by the authors).

the phylogeny. *Arctostaphylos viscida* and *A. klamathensis* indicate hybridization between the two major *Arctostaphylos* clades (the small and large clades) because both taxa resolve at the base of the large clade in the “all samples” trees and all samples of *A. klamathensis* and a single sample of *A. viscida* migrate to the base of the small clade in the “diploids only” tree. Most samples of *A. rudis*, a confirmed complex of hybrids between the two clades, resolve in the small clade forming a grade at the base of their nearest relative, *A. nipumu*, which segregates from them via a long branch (Supplemental Materials 18, 19), and one sample of *A. rudis* forms a clade with the samples of *A. purissima* in the large clade. Samples from most populations of the widespread species, *A. pungens*, resolve in a single clade in the small clade with striking exceptions from a suspected hybrid zone for the species in Monterey and San Benito Counties, California (labeled *A. pungens** in Figure 3.2) which resolve in multiple places, typically as part of a grade along with *A. parryana* at the base of the small clade. Lastly, there are several examples of apparent cryptic hybrid samples. Notable examples include: Morrison 461, a perfect morphological match for *A. pungens* collected in a mixed population with *A. pringlei* in Arizona, clusters with *A. pringlei* in all maximum likelihood phylogenies, one sample of *A. morroensis* revolves with *A. silvicola* in all maximum likelihood phylogenies, and a sample of *A. penchoensis* along with a sample of *A. obispoensis* are found in a grade at the base of the small clade in the “all samples” trees but resolve intermediate to the clades that include the remaining *A. pechoensis* and *A. obispoensis* samples in the “diploids only” tree.

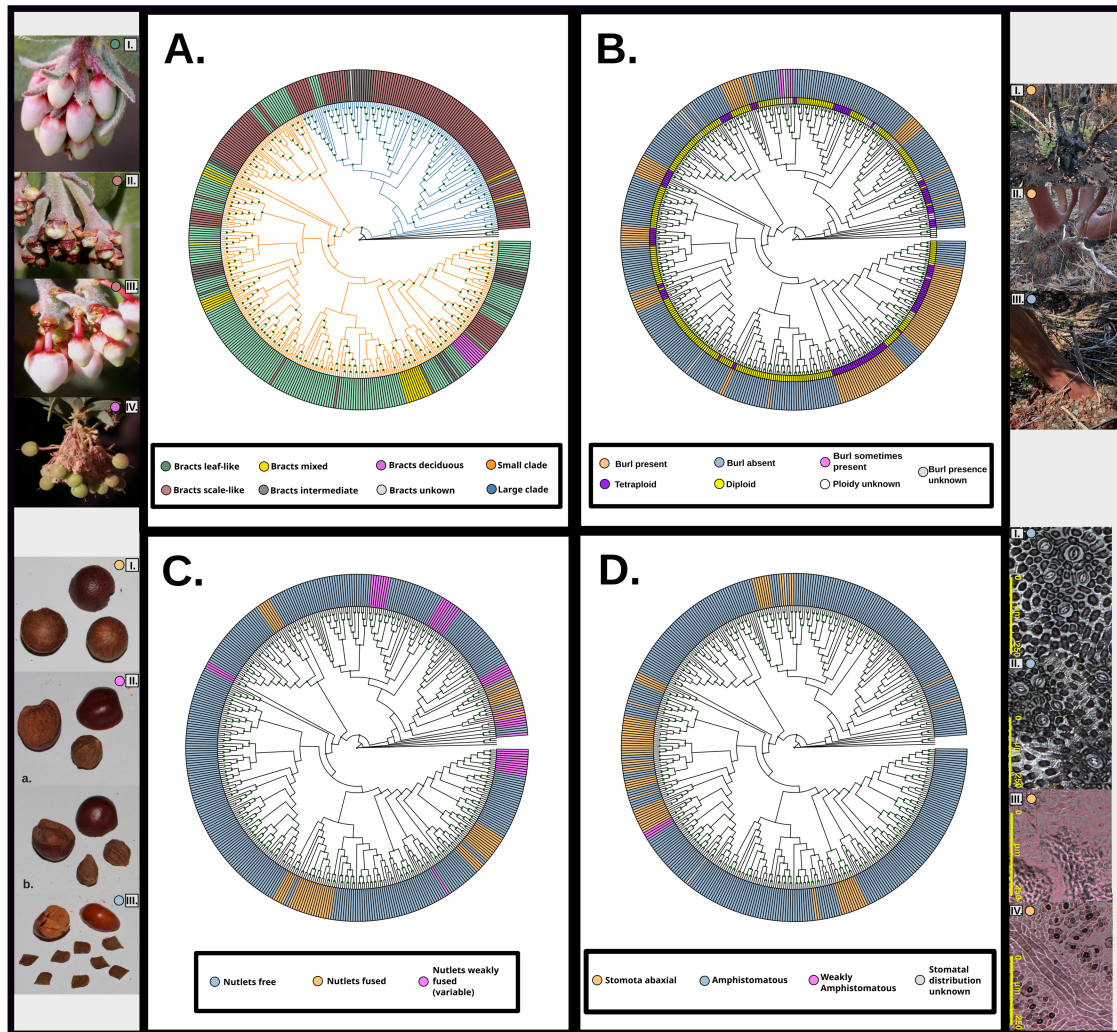


Figure 3.4: Phylogenetic distribution of taxonomically informative characters in *Arctostaphylos* using the phylogeny from Figure 3.1. For each panel, characters possessed by samples are represented as color-coded bars explained in legend below the tree. **Panel A** shows the phylogenetic distribution of bract types. Accompanying images: **I** shows leaf-like bracts, **II** and **III** show scale-like bracts, and **IV** shows an example of deciduous bracts. **Panel B** shows the phylogenetic distribution presence or absence of a basal burl which is often associated with the ploidy of taxa. Accompanying images: **I** shows fire resprouting from a burl, **II** shows a close up view of a burl, and **III** shows the trunk of a nonburl-forming *Arctostaphylos* species. **Panel C** shows the phylogenetic distribution of nutlet types (fruit). Accompanying images: **I** shows an example of fused nutlets, **II a** and **b** show examples of weakly fused nutlets, and **III** shows an example of free nutlets. **Panel D** shows the phylogenetic distribution of stomatal characters. Accompanying images: **I** and **II** show the adaxial and abaxial sides respectively of an amphistomatous leaf blade, and **III** and **IV** show the adaxial and abaxial sides respectively of leaf that only has stomata on the abaxial surface. Stomata images by Tito Abbo and Anna Rothfusz; all other photographs by Morgan A. Stickrod.

DIAGNOSTIC CHARACTERS FOR *ARCTOSTAPHYLOS* IDENTIFICATION SHOW
WIDESPREAD HOMOPLASY BUT INDICATE HOMOLOGY OR
AUTAPOMORPHY IN SOME CASES

Figure 3.4 and Supplemental Materials 21 depict the distribution of important taxonomic characters in *Arctostaphylos* throughout the “all samples 2MA” maximum likelihood phylogeny; collectively the distribution of these characters is more commonly associated with factors other than shared evolutionary history. The most prominent exceptions are bracts, for all members of the small clade have either scale-like or intermediate bracts, and these conditions are rare in the large clade which is composed of an overwhelming majority of leaf-like-bracted taxa (Figure 3.4 A). Other exceptional characters include the traits of *A. pringlei*, well known for uniquely lacking inflorescence dormancy and having deciduous bracts, and the two species that have four-merous corollas, *A. sensitiva* and *A. nummularia*; these two species monophyletic, indicating a single origin for this trait (Figure 3.4 A; Supplemental Materials 21 D). In contrast, traits such as gray shredding bark (Supplemental Materials 21 C), having stomata only on the abaxial leaf surface (Figure 3.4 D), fused nutlets (Figure 3.4 C), and prostrate habit (Supplemental Materials 21 B) arise sporadically and more or less randomly throughout the phylogeny. However, the prevalence of fused nutlets at the base of the small clade, a grade occupied by several apparent hybrid samples and the presence of a cryptic hybrid sample of *A. edmundsii* among prostrate members of the small clade suggest that these characters may have been acquired via hybridization (Supplemental Materials 21 B; Figure 3.4 C). Figure 3.4 B

indicates that the formation of a basal burl has arisen multiple times in the phylogeny, seemingly independently, but is more commonly observed in tetraploid taxa than in diploid taxa. We can not determine whether there is phylogenetic signal for auriculate leaves (Supplemental Materials 21 A) because we can not rule out a potential single origin with repeated reversals; auriculate taxa are relatively closely related phylogenetically and the trait is geographically associated with the central coast, and these two factors are entangled.

THERE IS HIGH UNCERTAINTY IN THE DIVERGENCE TIMES OF *ARCTOSTAPHYLOS* SPECIES, WHICH POTENTIALLY DIVERSIFIED RAPIDLY AND IN THE PRESENCE OF GENEFLOW

Two of the three replicate MCMC chains for the Bayesian time-calibrated phylogeny converged relatively quickly to the same posterior distribution for all parameters which all displayed effective sample sizes significantly above 200 in TRACER. The other of the three replicate chains failed to converge because it escaped several local optima throughout its run but eventually was sampling from the same posterior distribution as the other two chains by the end of the run (at ca. 83 million iterations). This suggested an overall consensus that the two replicates that converged quickly were sampling from the correct posterior distribution and we used the first of the two to build the maximum credibility tree shown in Figure 3.5. The posterior mean birth rate was 2.113×10^{-2} births/My, and the mean clock rate was 1.175×10^{-3} μ /My with a mean standard

deviation 0.195 (showing only weak deviations from a strict molecular clock).

BMODELTEST selected a TVM+G+I substitution model. The high birth rate is indicative of a rapid radiation but the wide 95% highest probability density intervals at all nodes show massive uncertainty regarding relative divergence times. Mean divergence times for all nodes are within the mid to late Miocene. All nodes have a value of one for their posterior probabilities showing strong support for the topology which largely matched that of the “diploids only” maximum likelihood tree with two exceptions.

Arctostaphylos klamathensis resolved in the same clade as *A. patula* and *A. nummularia* instead of as sibling to the small clade as in “diploids only” maximum likelihood tree or near the base of the large clade in the “all samples” maximum likelihood trees, and *A.*

auriculata resolved as sibling to *A. catalinae*, similar to its position in the “all samples 4MA” maximum likelihood tree. Taxa with inconsistent topologies across trees, *A.*

klamathensis, *A. auriculata*, and *A. viscida* (one sample) and the sibling taxa they associate with, have the highest clock rates in the maximum credibility tree indicating that the clock rates could be inflated due to a history of hybridization in these samples.

This along with other indications of hybridization seen throughout the results suggest that divergence in *Arctostaphylos* took place in the presence of gene flow.

DISCUSSION

This investigation marks a significant milestone in understanding the diversification and diversity in *Arctostaphylos* and by extension broadens our understanding of

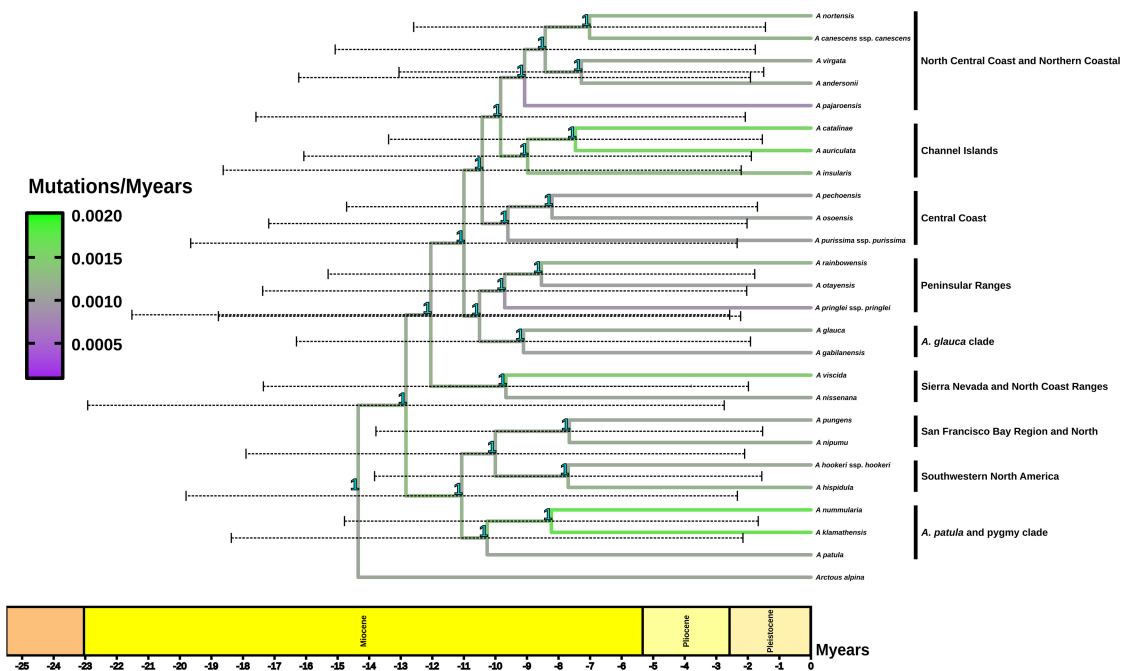


Figure 3.5: A time-calibrated tree of consensus sequences for a subset of diploid *Arctostaphylos* (“time calibration” dataset). Branch-lengths are proportional to time as represented by the scale bar at the bottom which shows geological epochs along an interval of 0 to 25 million years before the present. The tree was calibrated at the MRCA of *Arctostaphylos* based on limited fossil evidence using a lognormal relaxed clock model. The dataset for the tree was generated by including 2-6 taxa from each of the geographic clades in the maximum likelihood phylogeny (Figure 3.3), relative to the size of the geographic clade to which the taxa belongs. Consensus sequences for represented species were derived by pooling the sequences of replicate samples. Numbers at the nodes are posterior probabilities (0-1), and the dashed lines at nodes denote the 95% highest probability density intervals for divergence time of their respective nodes. Branch colors correspond to molecular clock rate at that specific branch in mutations / million years (bright green branches have higher clock rates, and bright purple branches have lower clock rates relative to the tree-wide mean).

diversification in the CFP and for woody plants at large. Woody plants face a unique challenge relative to other biological organisms in that they must tolerate potentially harsh abiotic conditions for long time scales in order to successfully reproduce and typically evolve slowly making them potentially vulnerable in changing environments (Haukioja and Koricheva, 2000; Smith and Donoghue, 2008; Pausas and Kelley, 2014; Pavanetto, et al. 2023; Dyderski, et al. 2025). The counterintuitive mystery of *Arctostaphylos* is how these slow growing woody plants could diversify so remarkably,

and while this study falls short of solving this mystery, it suggests that hybridization is a key element in the evolution of *Arctostaphylos*. This element, however, also complicates the interpretation of the genus in terms of strict Linnaean taxonomy. The morphological distribution in taxonomically informative characters fails to neatly recapitulate the species relationships in *Arctostaphylos*, which are instead best predicted by spatial/geographic patterns. The genus likely diversified in a rapid radiation in the presence of gene flow, potentially originating in the southern Sierra Nevada, quickly giving rise to two major lineages, one that produced numerous narrow endemic species and another that gave rise to fewer species but several widespread species that successfully expanded their ranges outside of the CFP. The genus seems to be currently characterized by numerous diploid species that occasionally hybridize and do not always show the clearest species boundaries but are nonetheless readily discernible from each other at their core, but it also includes several putative tetraploid species that are much more complex and much less discernible from each other genetically.

THE CURRENT TAXONOMIC TREATMENT OF *ARCTOSTAPHYLOS* IS LARGELY SUPPORTED AND THE GENUS DOES NOT APPEAR TO BE OVERSPLIT

Our aim is to provide a broad preliminary molecular evaluation of the majority of currently recognized *Arctostaphylos* species that can serve as the basis for future studies of evolution and conservation. Although we identify areas of the genus which warrant

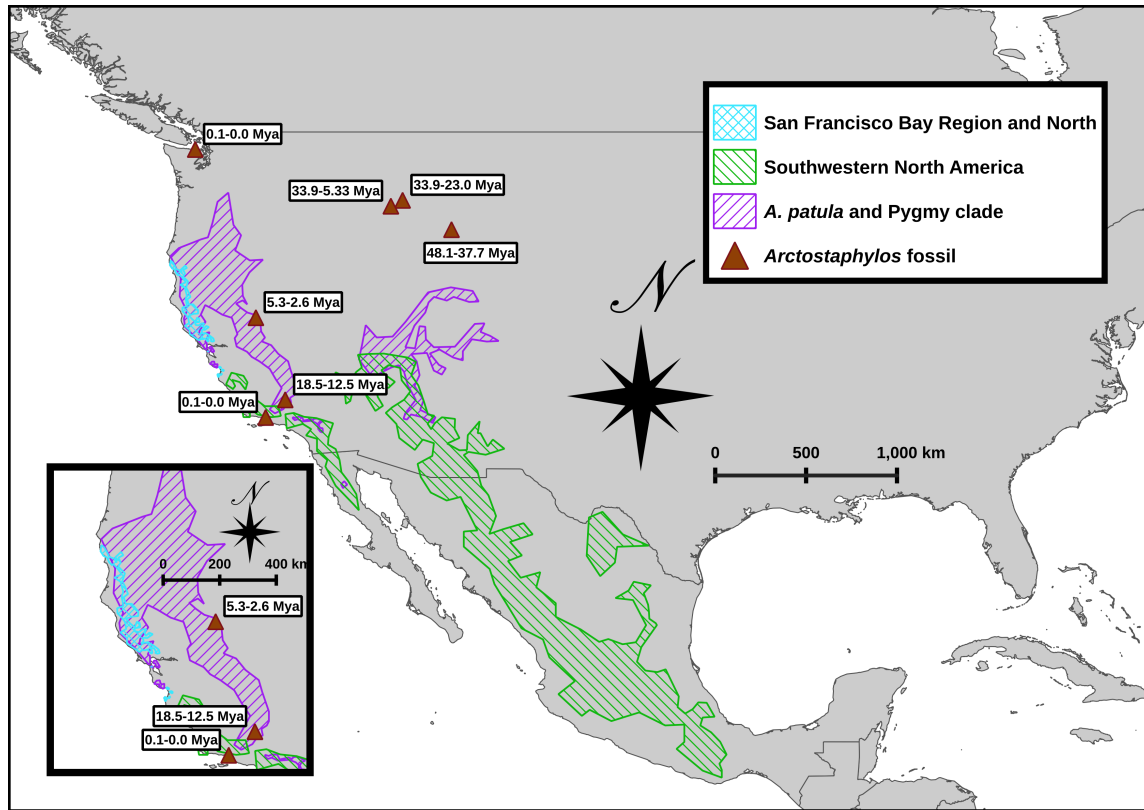


Figure 3.6: A map showing the ranges of the three geographic clades that make up the small clade of *Arctostaphylos*. The San Francisco Bay region and North geographic clade is marked in a light blue diamond pattern, the *A. patula* and Pygmy geographic clade is marked by purple diagonal lines, and the Southwestern North America geographic clade is indicated by green diagonal lines. Locations where reported fossils of *Arctostaphylos* have been found are indicated by brown triangles, with the estimated age of the fossil included (fossils older than 20 million years are generally thought to be misidentified). Fossil locations and ages were sourced from the Paleo Biology Database.

taxonomic reconsideration, this manuscript is not intended to be the sole basis for a taxonomic revision and rather should serve as a guide for what areas of the genus require specific targeted studies and which areas of the genus are taxonomically well understood. Questions about whether taxa are closely related enough to be lumped into single species or whether taxa are better treated at the specific or infraspecific rank are beyond our scope, which is to show which taxa can be readily distinguished from one another

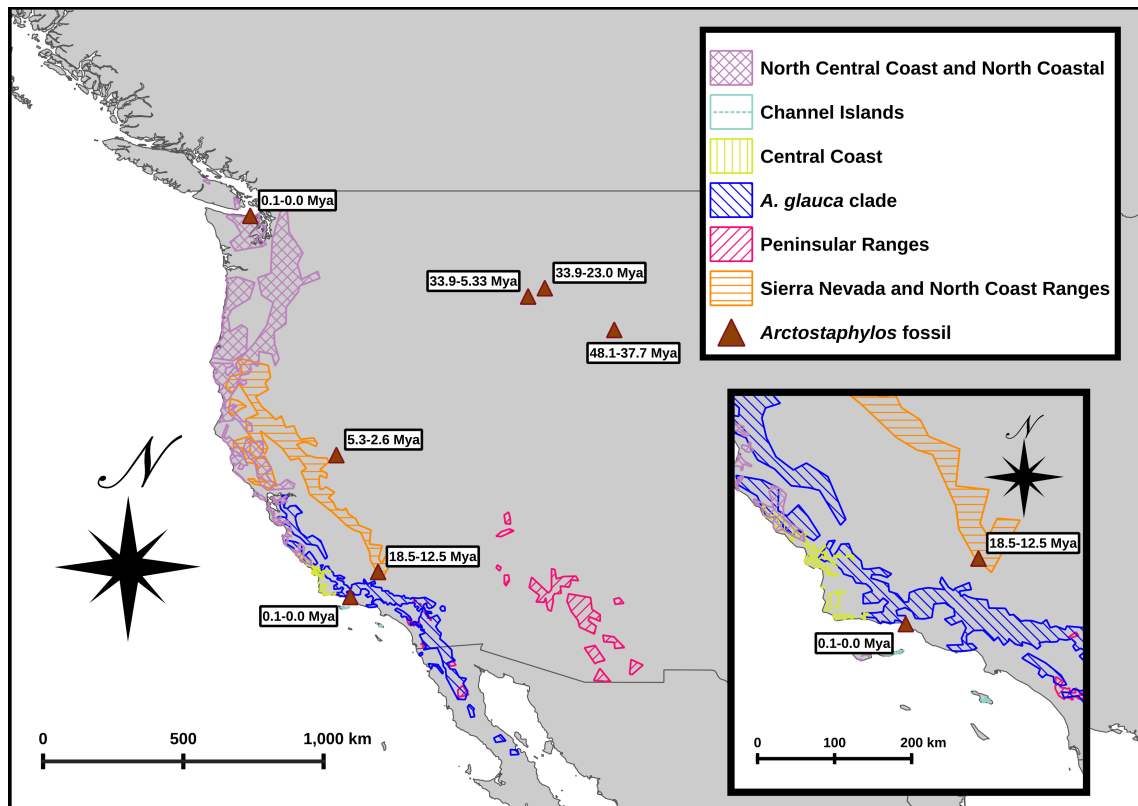


Figure 3.7: A map showing the ranges of the six geographic clades that comprise the large clade of *Arctostaphylos*. Each clade is denoted by a unique color and pattern combination. The North Central Coast and North Coastal clade is marked by the light lavender diamond pattern, The Sierra Nevada and North Coast Ranges geographic clade is marked by orange-colored horizontal lines, and the *A. glauca* clade is marked by blue diagonal lines. The central coast clade is indicated by chartreuse vertical lines, the Channel Islands geographic clade is marked by light blue dashes, and the Peninsular Ranges clade is indicated by pink diagonal lines. Locations where reported fossils of *Arctostaphylos* have been found are indicated by brown triangles, with the estimated age of the fossil included (fossils older than 20 million years are generally thought to be misidentified). Fossil locations and ages were sourced from the Paleo Biology Database.

molecularly using phylogenetic methods. This is not a high bar and one should reasonably expect that similar looking individuals from a small population to be more closely related to each other than other such populations. Nevertheless, narrow endemic *Arctostaphylos* often have long histories of recognition and are distinguishable morphologically so their monophyly should be sufficient to consider them supported. It is also important to note that several widespread species, sampled from diverse populations,

such as *A. glauca*, *A. nevadensis*, *A. patula*, and *A. pringlei* all group together in the phylogenies instead of forming islands of paraphyletic clusters (Figures 3.2, 3.3). Equally, *Arctostaphylos* species, including narrow endemics, do not exclusively occur in isolation from one another but rather are frequently found in mixed populations and appear to have limited barriers of geneflow between them (Parker et al., 2020; Abbo et al., 2025). Despite this propensity for hybridization we find at least partial support for the overwhelming majority of taxa included in this investigation (Supplemental Materials 16).

Although many taxa do not meet our criteria for full support which we established here, they remain largely coherent and distinguishable in the absence of violations. For example, *A. klamathensis*, *A. nevadensis*, and *A. nissenana* are all recovered as monophyletic but are nested within the samples of *A. viscida*. The issues with those four taxa are more likely associated with *A. viscida*, which has a history of documented hybridization with *A. patula* (Schierenbeck, et al., 1992), and our dataset includes putative hybrids between *A. viscida* and *A. patula* (Figure 3.2; Supplemental Materials 15), so we suspect that *A. viscida* samples may resolve in different parts of the Sierra Nevada and North Coast Ranges clade depending on their level of introgression. Other examples such as *A. insularis*, *A. obispoensis*, *A. pechoensis*, *A. pringlei* ssp. *drupacea*, *A. silvicola*, and *A. viridissima* include a single sample that is an apparent cryptic hybrid or include a single apparent cryptic hybrid in their respective clades but otherwise cluster

in the phylogeny with their respective replicate samples. *Arctostaphylos pungens* is a complicated case where, with the exception of two samples, all replicates from the US Southwest (including Southern California, Arizona, and New Mexico), Baja California and mainland Mexico form a single clade. The two exceptions are both from Arizona: Morrison 417, and 461; the former occurs in a mixed population with *A. pringlei* and resolves with samples of *A. pringlei* suggesting it is a cryptic hybrid with *A. pringlei*, and the latter resolves with a clade of *A. pungens* samples from San Benito County, California. The samples of *A. pungens* from San Benito and Monterey Counties are paraphyletic, appearing in multiple areas throughout the small clade, and although their association with the single sample of *A. pungens* from Arizona indicates an overall alliance with *A. pungens*, their chaotic phylogenetic distribution suggests the area may be a hybrid zone requiring future investigation. Overall, the genus should be considered a group of separate evolutionary entities which occasionally to frequently hybridize causing exceptional individuals, often from specific regions, to exhibit different genomic backgrounds compared to their orderly and distinguishable counterparts. Land managers should therefore note, especially when dealing with mixed populations, that morphological identifications on their own will not always be reliable for determining the genomic background of *Arctostaphylos* individuals. Burge et al. (2019) observed a similar phenomenon in a hybrid zone of *Quercus dumosa* and *Q. berberidifolia* in San Diego County where hybrid individuals were frequently cryptic, perfectly matching one

parental species morphologically but matching the other parental species by up to 90% genomically.

Even though it is not the norm, our study did reveal cases that merit future investigation for taxonomic reconsideration. The most notable examples are the widespread tetraploid species *A. glandulosa* and *A. crustacea*, and to a lesser degree *A. tomentosa*, *A. manzanita*, and *A. parryana*. These appear in multiple locations throughout the phylogeny (Figure 3.2, Supplemental Materials 18, 19) sometimes with a clear pattern, such as in the case of *A. parryana* where samples always occur as part of a grade at the base of the small clade, but other times are devoid of obvious explanation. One intriguing observation is that the phylogeny of *Ceanothus*, considered a case of convergent evolution with *Arctostaphylos* (Parker 2007), consists of two early diverging clades which do not appear to hybridize but within the two clades nearly all taxa are polyphyletic, exhibiting chaotic topology as observed in these five tetraploid *Arctostaphylos* species (Burge et al., 2011). In contrast, *Arctostaphylos* is composed of two early diverging clades which can hybridize, at least to a limited degree (Abbo et al., 2025), and taxa within the two clades resolve in a phylogenetically ordered manner minus specific exceptions. Given that widespread tetraploid *Arctostaphylos* samples resolve in multiple clades, it is also likely an invalid assumption that the few chromosome counts, used to determine their ploidy (Supplemental Materials 17; Abbo et al., in prep) apply to all individuals, highlighting the need for a highly replicated cytological survey of the

genus across its range. Little is known about polyploidy in *Arctostaphylos*. Are these tetraploids, allotetraploids and if so, is their similar morphology due to repeated crosses between the same or closely related parental individuals or is it incidental? A good starting point for investigating this question would be to study *A. rudis*, the product of repeated hybridization between the small and large clade, to test whether hybridization between distant lineages of *Arctostaphylos* yields meiotic incompatibilities in the progeny. This investigation would also benefit from a reference genome from a member of the small clade to compare with the current reference (*A. glauca*) to see if there are structural variations between the two genomes that could lead to reduced recombination and imperfect chromosomal pairing in hybrids (Chawla et al., 2020; Liu et al., 2022; Nibau, et al., 2024). A reference genome from a polyploid would further be beneficial for comparison. We also identified a likely hybrid between *A. nevadensis* and *A. klamathensis* during this investigation suggesting that crosses between taxa of different ploidies can occur, so this cross is an avenue for future investigation to assess whether hybridization occurs freely between ploidies or whether hybrids are sterile triploids, which to our knowledge have not been documented in the genus (although the second chromosome count for *A. nevadensis*, from an individual from outside of the CFP, suggested diploidy [see Supplemental Materials 17]) . Lastly, *A. densiflora* and *A. columbiana* are diploid taxa that were unsupported in this study making them priorities for future taxonomic reconsideration studies. Such studies should have broad sampling and include representatives of co-occurring and closely related taxa as was recently

demonstrated in the case of *A. glandulosa* ssp. *crassifolia*, which has complicated species relationships with other narrow endemics from the Peninsular Ranges that likely share a history of hybridization leading to a history of conflicting evidence regarding its genetic distinctiveness in studies with lower individual and taxon sampling (Abbo et al., in prep). Our sampling of *A. columbiana* is unreasonably small (only two individuals, all from California), given the wide range of this species, to draw meaningful conclusions about this species, which like *A. pungens* may prove taxonomically coherent in certain regions of its range. However, *A. columbiana* is known to hybridize extensively throughout its entire range with *A. uva-ursi* producing the named hybrid *A. Xmedia*, which suggests that *A. columbiana* may be unstable (Kruckeberg, 1977; Wells 1988). *Arctostaphylos Xmedia* is also an example of hybridization between the small and large clades and its future investigation would complement that proposed for *A. rudis*.

SCALE-LIKE INFLORESCENCE BRACTS ARE A POTENTIAL SYNAPOMORPHY FOR THE SMALL CLADE

The limited resolution of the previous phylogenetic study in *Arctostaphylos* with high taxon sampling precluded a detailed evaluation of the phylogenetic distribution of taxonomic characters in *Arctostaphylos* (Wahlert et al., 2009). With the current phylogenetic resolution we are able to show that the majority of these characters arose multiple times independently or were transferred horizontally between taxa. The most prominent exception to this is inflorescence bracts. Figure 4.1 A suggests that scale-like

bracts are a synapomorphy for the small clade and that leaf-like bracts arose early in the evolution of the large clade and are typically diagnostic of large clade membership; this could have been deduced using the memberships of taxa within the two clades (provided in Boykin et al., 2005, and Wahlert et al., 2009) but to our knowledge has not been mentioned previously in the literature. *Arctostaphylos viscida* is one of only two taxa with scale-like bracts in the large clade and its position near the base of this clade suggest that this is a retained ancestral trait; conversely, the repeated indication of hybridization between *A. viscida* and *A. patula* suggests horizontal transfer of this character.

Arctostaphylos viscida is part of a clade of leafy bracted species one of which, *A. klamathensis*, is nested within *A. viscida* in the “all samples” phylogenies (Figure 3.2; Supplemental Materials 19), is at the base of the small clade in the “diploids only” maximum likelihood phylogeny (Figure 3.3), and is a member of the *A. patula* and Pygmy Clade in the maximum credibility tree (Figure 3.5). Furthermore, *A. nissenana*, another species nested within *A. viscida*, shares important traits with pygmy manzanitas (*A. myrtifolia*, *A. nummularia*, and *A. sensitiva*) mainly shredding gray bark (Supplemental Materials 21 C) and highly reduced leaves. All these factors together suggest a history of hybridization between the members of the “*A. patula* and Pygmy Clade” and the “Sierra Nevada and North Coast Ranges” clade; the distributions of the “Sierra Nevada and North Coast” clade is almost perfectly situated within that of the “*A. patula* and Pygmy Clade” (Supplemental Materials 22) providing ample opportunity for gene exchange. Other characters that were potentially transferred via hybridization

include fused nutlets into putative hybrid individuals at the base of the small clade (Figure 3.4 C) and the prostate habit of *A. pumila* and *A. edmundsii* (Supplemental Materials 21 B). In order to test these hypotheses one would need a much greater understanding of the underlying genes that control phenotype in *Arctostaphylos*. Future studies could use whole genome sequencing to look for genome wide associations of these characters (Chhetri, et al., 2019; Li et al. 2022).

CFP NARROW ENDEMICS ARE LIKELY NEOENDEMICS THAT AROSE AS PART OF A RAPID RADIATION

The estimated mean divergence times (Figure 3.5) allege that *Arctostaphylos* diversified in a pulse during the late Miocene that lasted around 5 million years with peak diversification occurring in the large clade over the span of 2 million years (10-8 Mya). The mean age of the MRCA of *Arctostaphylos* estimated at ca. 12.8 million years is consistent with that estimated for *Ceanothus* (12.9 million years), but the MRCAs for the two clades of *Ceanothus* were estimated to ca. 5-6.5 million years younger than the MRCAs for the two major *Arctostaphylos* clades estimated herein (Burge et al., 2011). The *Ceanothus* study had similarly wide 95% highest probability density intervals as our study, but a more complete fossil record facilitated a low margin of error estimation of the diversification of *Quercus* section *Lobatae* in the CFP to also have occurred during the late Miocene (Hauser et al., 2017). Despite the uncertainties in the estimated divergence times, *Arctostaphylos* most likely diversified as part of a rapid radiation. The

MRCA of the large clade is estimated to be older than that of the small clade but this seems mainly due to the “Sierra Nevada and North Coast” clade while the core large clade is younger. With the evidence of hybridization between the *A. patula* and Pygmy Clade, and the Sierra Nevada and North Coast clade further enhanced by the high clock rates of *A. klamathensis*, *A. nummularia*, and *A. viscida*, the *A. patula* and Pygmy Clade and Sierra Nevada and North Coast clade likely exchanged genes throughout their divergence. Both lineages are abundant throughout the Sierra Nevada and their shared distributions (Supplemental Materials 22) suggest they arose near the *A. mohavensis* fossil in the southern Sierra Nevada moving north along the east side of the Central Valley eventually migrating towards the coast extending south up to the San Francisco Bay Region (for similar distribution and migration patterns in other CFP taxa, see: Axelrod 1981, Hernández et al. 2022, Karimi et al. 2024). Significant genetic differentiation between lineages of *A. glandulosa*, and later for multiple large clade taxa, were observed for samples collected south of the Transverse Ranges compared to those collected along and north of the Transverse Ranges (Huang et al., 2020; Abbo et al., in prep). Together the topology of the maximum credibility tree and the extant distribution patterns (Figure 3.7) suggest that the “Peninsular Ranges Clade” became isolated by the Transverse Ranges but both it and its sibling lineage the “*A. glauca* Clade” likely diverged before the uplift of these ranges during the mid Pliocene (Nicholson et al., 1994) allowing sufficient time for *A. glauca* to expand north. The remaining diversification in the large clade appears to proceed south to north, giving rise to narrow neoendemics in

situ as lineages made their way through the Central Coast. Further diversification in the small clade (Figure 3.6) appears to follow a parallel trajectory within the CFP. We note as an aside that the burl forming lineages *A. patula* and *A. rainbowensis* do not have substantially reduced clock rates compared to obligate seeders, preliminary suggesting that resprouting life history does not noticeably reduce rates of evolution or perhaps the effect is counteracted by hybridization.

SPECIAL CASES OF INTEREST

The study reveals numerous intriguing anecdotes that would be too cumbersome to cover exhaustively so we instead focus on two specific examples. The first is the observation that Channel Islands species resolve as more closely related to lineages from the north Central Coast and north thereof than to the nearest neighboring species on the mainland (Figures 3.2, 3.3, 3.5, 3.7). Moreover, *A. confertiflora* consistently resolves in the “North Central Coast and Northern Coastal” clade, and *A. auriculata*, endemic to Mount Diablo in the San Francisco Bay Region, either resolves in the “North Central Coast and Northern Coastal” or in the “Channel Islands” clade depending on the dataset and method for building the phylogeny (Figures 3.2, 3.3, 3.5; Supplemental Materials 19). This suggests that Channel Islands endemics were in contact with northern mainland taxa in the past or perhaps long-distance dispersal occurred between the two regions.

The second observation is that the samples that resolve in the paraphyletic grade of samples at the base of the small clade in the “all samples” trees (Figure 3.2, Supplemental Materials 19) all have similar morphologies to *A. pungens*. *Arctostaphylos parryana* and *A. montana* were formerly treated as subspecies of *A. pungens* (Roof, 1975; Wells, 1988; Markos et al., 1998), and plants from San Benito and Monterey Counties currently treated as *A. pungens* were treated by Roof as three separate species *A. benitoensis*, *A. chaloneorum* and *A. pseudopungens* (Roof, 1978). These plants resemble *A. pungens* in that they tend to have shiny green, point-tipped leaves and short, scaly-bracted inflorescences but tend to differ from *A. pungens* in variable ways; frequently, they have shorter stems, fused nutlets and tolerate serpentine soils. The grade also includes samples of *A. glandulosa* ssp. *gabrielensis*, which often resembles *A. parryana* (Wells, 2000). Future studies should investigate the hypothesis that *A. pungens* experienced a bifurcation following the formation of the Transverse Ranges and the isolated individuals in the north experienced genetic differentiation possibly accelerated via hybridization with species found throughout the Coast Ranges. As a final note, the Roof designations of *A. benitoensis*, *A. chaloneorum* and *A. pseudopungens* do not appear tenable as ‘*A. pungens*’ samples from these localities do not cleanly resolve in monophyletic clades based on populations and our preliminary investigations do not show clear morphological separation between them (unpublished). This suggests that they are hybrids with unequal levels of introgression similar to *A. rudis* (Abbo et al., 2025). Wells (1988) reached this same conclusion and proposed treating them under the rank *A. Xbenitoensis* to refer to

them concisely as a unit but also recognize their non-species-like character which is more akin to similar-looking individuals, likely derived from repeated hybridization from unknown sources (Wells suggested *A. glauca*). This seems a far less cumbersome solution than continuously referencing these individuals as *A. pungens* from San Benito and Monterey Counties as we resorted to herein.

CONCLUSION

Although many challenges and unanswered questions remain, we have established molecular support for the majority of species in *Arctostaphylos* and in doing so provide support for the hypothesis that *Arctostaphylos* is among other prominent woody genera that radiated in the CFP during the late Tertiary era (*Ceanothus* [Burge et al., 2011]; *Quercus* [Hauser et al., 2017]). A continuously growing consensus of molecular data suggests that the pattern of middle Miocene diversification, which is before a Mediterranean-type climate fully established but when aridification began to increase, is the norm for plants in the CFP (Baldwin, 2014). These conditions, which forced most woody plants to retreat to stable refugio, were exploitable by *Arctostaphylos* allowing it to give rise to an astounding number of species. The most burning question is why? *Arctostaphylos* species are considered difficult to identify because of how similar they appear and while morphological traits such as having a basal burl or having stomata on one or two leaf surfaces have clear ecological implications, the vast majority of *Arctostaphylos* species appear to be ecologically equivalent at least at a coarse scale. This

would suggest that the radiation is largely nonadaptive, but morphological characters generally lack strong phylogenetic signal and therefore arose multiple times independently. While inferred indirectly herein, hybridization is clearly an important feature in the genus. Future studies should investigate whether these morphological characters have strong genetic and environmental associations which will help determine if these characters were acquired horizontally and whether or not that transfer was accidental or has some adaptive significance. It is also profoundly mysterious why the majority of individuals cluster into clearly discernible genetic groups despite occasional hybridization, while others resolve chaotically without a clear pattern. The chaotic individuals appear to be most commonly widespread tetraploid taxa, but their ploidy on its own does not offer a direct explanation for their difference in behavior. As mentioned in Abbo et al. (in prep) ploidy is generally understudied in *Arctostaphylos*, and future research should combine broad scale cytological studies to better understand the distribution of ploidy across the landscape with high quality whole genome data to better understand the differences in genomic architecture between ploidy levels in *Arctostaphylos*. Ultimately these should help reveal the conditions which lead to polyploidization in the genus. Such information is of broad interest for understanding molecular evolution, genomics, molecular ecology, and more. We sincerely hope that this phylogenetic study is not seen as the culmination of a long-term project but rather as a foundation used to further our understanding of speciation and evolution in this captivating genus.

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AUTHOR CONTRIBUTIONS

T.A. and A.B.: Primary analysis, writing manuscript.

M.A.S., G.R.M., Y.H., A.M., A.C.S., V.T.P., M. C. V., J. E. K., and A. L.: Assisted with result interpretation and editing manuscript.

T.A., A.B., M.A.S., G.R.M., Y.H., V.T.P., and A.L.: Conceptualizing.

T.A., A.B., M.A.S., G.R.M., Y.H., A.M., A.C.S., V.T.P., M.C.V., J.E.K., and A.L.: Collected or assisted with field collection of specimens.

T.A., A.B., G.R.M., Y.H., A.M., and A.L.: Participated in or assisted with benchwork, and wetlab methods conceptualization and improvement.

M.A.S., V.T.P., M.C.V., and J.E.K.: Provided special expert evaluation of taxonomy and ecological context.

A.C.S.: Managed curation and preparation of voucher specimens.

A.L.: Principal instigator; managed resources and funds, and provided supervision, and mentorship.

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CONCLUSION

Successful conservation of natural biological systems is facilitated by a well-supported taxonomy that reveals where threatened organisms occur in the landscape but also requires cognizance of hybridization, an important element of biological systems.

Hybridization may have positive or negative effects depending on context but generally introduces complexities to the orderly boundaries that taxonomy attempts to enforce.

Conversely the studies of taxonomy and hybridization are enabled by the presence of protected wildlands that allow researchers to investigate organisms in their natural state.

The example of *Arctostaphylos rudis* strongly illustrates this point. Prior to the discovery of *A. nipumu*, *A. rudis* was still considered a threatened species and its conservation was directed by the best available information that existed at the time. Some decisions, such as the apparent use of restoration plants in the Nipomo Mesa sourced from Santa Barbara County, were ill advised in light of the present data but this could not have been predicted from what was previously known. In the context of the significant land use change that has occurred in California over the last century, it is probable that *A. nipumu* would not have been discovered had it not been protected under the previous classification. In turn, the greater understanding of the system provided by the current taxonomic and hybridization research enables even better conservation of the system moving forward.

At this population scale of observation in a unique case, hybridization seems to not be a component of the natural history of *A. nipumu* and is likely to have negative effects if facilitated by anthropogenic means. At intermediate scales of observation in the case of Del Mar manzanita (DMM), however, the role of hybridization is not readily clear. It appears to be a factor that is present in the background of the system, introducing uncertainty and requires comprehensive sampling of the manzanitas throughout the subregion to begin to see through it. While little is known about the evolutionary origins of DMM, the study herein was able to find evidence of its distinctiveness while also reconciling the apparently conflicting results of previous studies. At even broader scales of observation, hybridization was still inferred largely indirectly herein but its impact is apparent throughout the manzanita phylogeny and is implicated as a factor that promotes manzanita diversification. Thus, the genuswide phylogenetic scale provides context for the patterns observed at the lower scales in chapters I and II. The species *A. viscida* and *A. klamathensis* were shown to have affinities to both major clades of manzanitas suggesting that the hybridization between these two clades seen in *A. rudis* is a common theme in the evolution of system. The hypothesis that this cross-clade hybridization leads to polyploidy in the genus needs to be tested in future studies. Equally, the abundance of putative cryptic hybrids observed throughout the phylogeny provides a plausible explanation for why two samples of *A. otayensis* clustered among the samples of DMM despite lacking any morphological or geographic indicators of hybridization. The possibility of cryptic hybrids poses a significant challenge for conservation because it

means that morphology will not always be reliable for species identification. These samples should be investigated with whole genome sequencing to try to determine what portions of the genome remain similar to those found in nonhybrids which share morphology, potentially offering an avenue to identify loci that control phenotype in manzanitas. Furthermore, the ubiquitous paraphyly seen in widespread tetraploid species throughout the phylogeny suggests that the paraphyly of *A. glandulosa* seen in chapter II is a persistent feature of the system rather than a phenomenon unique to *A. glandulosa*. These putative tetraploids pose significant challenges for conservation because the phylogeny does not offer clear insight into their origins or relationships making it unclear how they should be categorized and by extension how they should be prioritized for conservation. Understanding these tetraploids is therefore the top priority for future research in manzanitas providing further insight into the interaction between taxonomy, hybridization, and conservation.