A CASE OF INCIPIENT BUDDING SPECIATION IN THE CALIFORNIA FLORISTIC PROVINCE, INFRASPECIFIC DIVERGENCE IN *ABRONIA VILLOSA*

By

Eli Allen

A Thesis Presented to

The Faculty of California State Polytechnic University, Humboldt

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

Committee Membership

Dr. Oscar Mauricio Vargas-Hernandez, Committee Chair

Dr. Erik Jules, Committee Member

Dr. Eric LoPresti Committee Member

Dr. Jeffrey White, Committee Member

Dr. Paul Bourdeau, Program Graduate Coordinator

May 2024

ABSTRACT

A CASE OF INCIPIENT BUDDING SPECIATION IN THE CALIFORNIA FLORISTIC PROVINCE, INFRASPECIFIC DIVERGENCE IN *ABRONIA VILLOSA*

Eli Allen

Physical barriers to gene flow are the traditional evidence for species divergence. Conversely, there has been increasing acknowledgment of speciation in the face of gene flow as an evolutionary process. Budding speciation involves peripheral populations adapting to local ecological conditions, thereby budding off from a widespread progenitor species. Budding speciation is distinguished by ecological divergence and is generally evidenced by asymmetrical range size and nested phylogenetic relationships of sister species. The narrow endemic *Abronia villosa* var. *aurita* is adapted to montane sandy washes adjacent to its widespread sister variety, the desert dwelling var. *villosa*. Here, I tested the hypothesis that *A. villosa* var. *aurita* is derived from its sister variety via budding divergence. My investigation employed phylogenomic and genetic structure analyses of a clade comprised of *A. villosa* and its close allies, *A.umbellata* and *A. gracilis*. My results reveal that *Abronia villosa* var. *aurita* is a recently budded taxon, phylogenetically nested within its sister variety yet still showing unique genetic structure. Unexpectedly, I found evidence that var. *aurita* comprises two reciprocally monophyletic subclades, separated by an elevational gradient. I also confirmed Tillet's 1967 hypothesis that *A. villosa* would hybridize with the coastal *A. umbellata* in the localities where they

are sympatric. Ultimately, my study shows a recent but complex history of divergence and subsequent post-divergence processes, illustrating the importance of capturing nuanced snapshots of microevolutionary mechanisms as they occur.

ACKNOWLEDGEMENTS

I would like to thank my friends and family for their incredible support throughout my academic journey, especially Cozy Giusti for assisting in all my field collections, doing all the driving in heavy Southern California traffic, and of course for being an amazing partner and friend. I am grateful to the entire Cal Poly Humboldt Plant Tax team as well as Vargas Lab for helping me grow as a botanist and instructor: Oscar Vargas, Stefani Brandt, Sarah Norvell Conway, Molly Smith Metok, Kale McNeill, Emma Casselman, Cameron Jones, Ashley Dickinson, and Heather Davis. I would like to extend a special thank you to Dr. Oscar Vargas, who has taught me so much and given me the confidence to continue my journey in academia.

I extend thanks to Eric LoPresti for always sharing his deep *Abronia* lore with me and for reading my impromptu lengthy emails about the taxonomic history of *Abronia pinetorum*. I am incredibly thankful to Matt Johnson and Sherese Price for providing me with bioinformatics advice, an *Abronia* and *Mirabilis* reference file, and their own *Abronia* reads. I thank Jon Rebman and Layla Aerne Hains at The San Diego Natural History Museum for providing me with both collection permits and leaf samples to extract DNA from. I also thank Tom Chester for always keeping me updated on *Abronia* blooms and providing me with so many useful contacts in Southern California.

I would also like to thank my graduate committee members for all their support and advice. Jeffrey White for sharing his knowledge of biogeography and plant taxonomy, for always giving me the thorough edits that I needed, and for always making

the time to meet and discuss thesis matters in person instead of over email. Eric Jules for joining my committee even though my thesis was outside of his area of focus, and for the kind words that always went along with his advice.

I am eternally grateful to Susan Wright for giving me my first job at Humboldt, always ensuring I had access to the equipment necessary for my research, and being the one person who always asks how my dad is doing. Your support of our biology graduate students and the department at large deserves the highest praise possible; thank you for being the pillar of our community.

I would like to thank the numerous institutions and individuals who helped me with my field work: Abby Hanlen at Camp Pendleton, Zach Kantor-Anaya and Miranda Kennedy at the Santa Margarita River Trail Preserve, and Lauren Jonker and Jonathan Reinig at the Regional Conservation Authority of Western Riverside County.

I thank the International Association for Plant Taxonomy, Cal Poly Humboldt, the Biology Graduate Student Association, the CSU Research Competition, and the family of Gregory Mark Jennings for funding my thesis research and travel to conferences to disseminate said research.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Speciation and the maintenance of species boundaries between closely related taxa has been a contentious but integral component to understanding diversification across the tree of life (Rieseberg and Brouillet, 1994, Gottleib, 2004, Kay, 2006, Butlin et al., 2008, Hörandl and Stuessy, 2010, Bock, 2023). Allopatric speciation, divergence due to geographic barriers preventing gene flow between populations, has been considered the most common driver of species divergence in sexual organisms (Mayr, 1942, 1970, Dobzhansky, 1970, Coyn and Orr, 2004), making physical barriers that restrict gene flow the traditional evidence for speciation. The rationale for allopatric speciation is straightforward; if gene exchange between populations is blocked for sufficient time, recombination is prevented and species divergence occurs (Grant, 1981, Coyn & Orr, 2004). By this same reasoning, it was long held that sympatric speciation (divergence within overlapping ranges lacking spatial barriers) was highly unlikely, as gene flow would prevent the process of reproductive isolation to the point where divergence was impossible (Jordan, 1905, 1908, Mayr, 1942, 1959, 1963). However, increasing evidence that speciation with ongoing gene flow occurs has led to a rethinking of how species, particularly plant species, diverge across the landscape (Crawford, 2010, Otero et al., 2022, Ivey et al., 2023, Bock et al., 2023). Comparative investigations of plants, fungi and animals indicate that sympatric speciation has a significant role in the genesis of biodiversity (Fitzpatrick et al., 2008, 2009, Hernández-Hernández, et al., 2021). Sympatric speciation events due to polyploidization and hybridization are known to be

widespread in plants, but recently, instances of sympatric speciation with gene flow have been increasingly gaining attention (Bock et al., 2023). In the same vein, parapatric speciation (divergence of adjacent populations along environmental gradients), which is the intermediate between allopatric and sympatric speciation, is highly common in plants (Otero et al., 2022, Harrison 2012).

In sympatric and parapatric speciation, testing for ecological (vs. geographic) divergence has been reemphasized as a major goal of evolutionary biology (Gottlieb, 2004, Crawford, 2010, Bock et al., 2023). Hence, the language of species divergence has transitioned in the 21st century, with a departure from emphasis on the classic triumvirate (allopatric, parapatric, and sympatric) models of speciation, in favor of "ecological speciation" (Rundle and Nosil, 2005). Ecological speciation is distinguished by its focus on ecologically driven divergent natural selection, paired with continued gene flow during, or even after, divergence (Schluter, 2009, Harrison, 2010). A recent study of *Mimulus glaucescens* (Phrymaceae) showed that while ecologically and morphologically distinct from its sister species, *M. guttatus*, they maintain extensive gene flow, especially in sympatry (Ivey et al., 2023). Evidence of a particular type of ecological speciation, "budding speciation," has been shown to be common in vertebrates, invertebrates, and especially plants (Barraclough and Vogler, 2000, Malay and Paulay, 2010, Claremont et al., 2012, Anacker and Strauss, 2014, Grossenbacher, et al., 2014). In budding speciation small populations within or on the periphery of the range of a larger "progenitor" species adapts, locally producing a "derivative" species (Stebbins and Major, 1965, Rieseberg and Brouillet, 1994, Baldwin, 2005, Schluter, 2009, Crawford, 2010, Sianta and Kay,

2022). Studies on budding speciation in plants have focused on comparing sister pair taxa, searching for highly asymmetrical range sizes and divergence in ecological traits (Harrison, 2012, Anacker and Strauss, 2014, Grossenbacher, et al., 2014, Otero et al., 2022, Ivey et al., 2023). While early studies on budding speciation were macroevolutionary, often sampling only a single individual per taxon (Fitzpatrick and Turelli, 2006, Anacker and Strauss, 2014, Grossenbacher, et al., 2014), recent microevolutionary studies are providing nuanced insight into the ecological and genetic mechanisms underlying the process of "budding off" (Vargas et al., 2020, Otero et al., 2022, Sianta and Kay, 2022, Ivey et al., 2023). These studies not only confirm the presence of budding speciation, but also capture an evolutionary snapshot of how budded sister pairs continue to evolve.

Budding speciation may be an important process that leads to species richness in biodiversity hotspots. A strong signal of budding speciation in plants has been found in the California Floristic Province (CFP), one of only two biodiversity hotspots in the U.S. (Gottleib, 2004, Baldwin 2005, Grossenbacher, et al., 2014, Harrison and Noss, 2017, Ivey et al., 2023). A phylogenetic comparative meta-analysis of 71 CFP sister pairs from 12 families found signatures of the "progenitor – derivative" relationship in 80% of the sister pairs analyzed (Anacker and Strauss, 2014). This high signal for budding speciation could be attributed at the CFP's climatic, geologic, topographic, and soil heterogeneity, potentially promoting ample microhabitats ideal for local ecological specialization, resulting in derivate neo-endemic species from widespread progenitor species (Raven and Axelrod, 1978, Thorne et al., 2009, Kraft et al., 2010). The high level of CFP plant

endemism – 42% (Burge et al., 2016) match the expected proportion of narrow endemics: if 80% of sister pairs diverged via budding speciation (Anacker and Strauss, 2014), then half of this percentage, 40%, would be derivative species. Understanding the linkage between budding speciation and endemism will reveal much about the evolution and assemblage of flora in biodiverse hotspots.

Abronia villosa var. *aurita* (Abrams) Jeps. is a Californian narrow endemic that shows signs of being a recently budded taxon derived from its sister variety, the widespread *A. villosa* var. *villosa* S. Watson (American Society of Naturalists, 1883, Abrams, 1905, Jepson, 1914). The two varieties show strong range asymmetry, with the rare var. *aurita* having a much smaller range on the periphery of the more common var. *villosa* (Fig. 1A, 1B). Where their ranges abut, the two varieties are separated by stark elevational differences, their only range overlap being a long mountain canyon connecting their adjacent populations (Fig. 1D). The two varieties have also long been thought to differ ecologically, with var. *aurita* being considered a montane variety (200-2000 m) compared to var. *villosa* which is mostly found on the low desert floor (0-1000 m) (Curtis, 1977, Galloway, 2003). However, towards the coast – farther west than var. *villosa* occurs in CA – var. *aurita* is found in sandy washes and river-valleys at increasingly lower elevations as it moves westward, with one of these populations reaching the Pacific Ocean (Fig. 1C). Range asymmetry and ecological divergence between sister pair taxa are both indicators of budding speciation (Anacker and Strauss, 2014, Sianta and Kay, 2022). Since both are taxonomically ranked as varieties of the same species, this case could be hypothesized as a case of budding speciation in an early

phase (hereafter “budding divergence”). In Nyctaginaceae, as with many North American plant families, the rank of variety is used entirely in lieu of subspecies even though variety is taxonomically nested within subspecies. (Hamilton and Reichard, 1992). The two varieties of *A. villosa* offer an ideal system to test and study budding divergence at a microevolutionary scale.

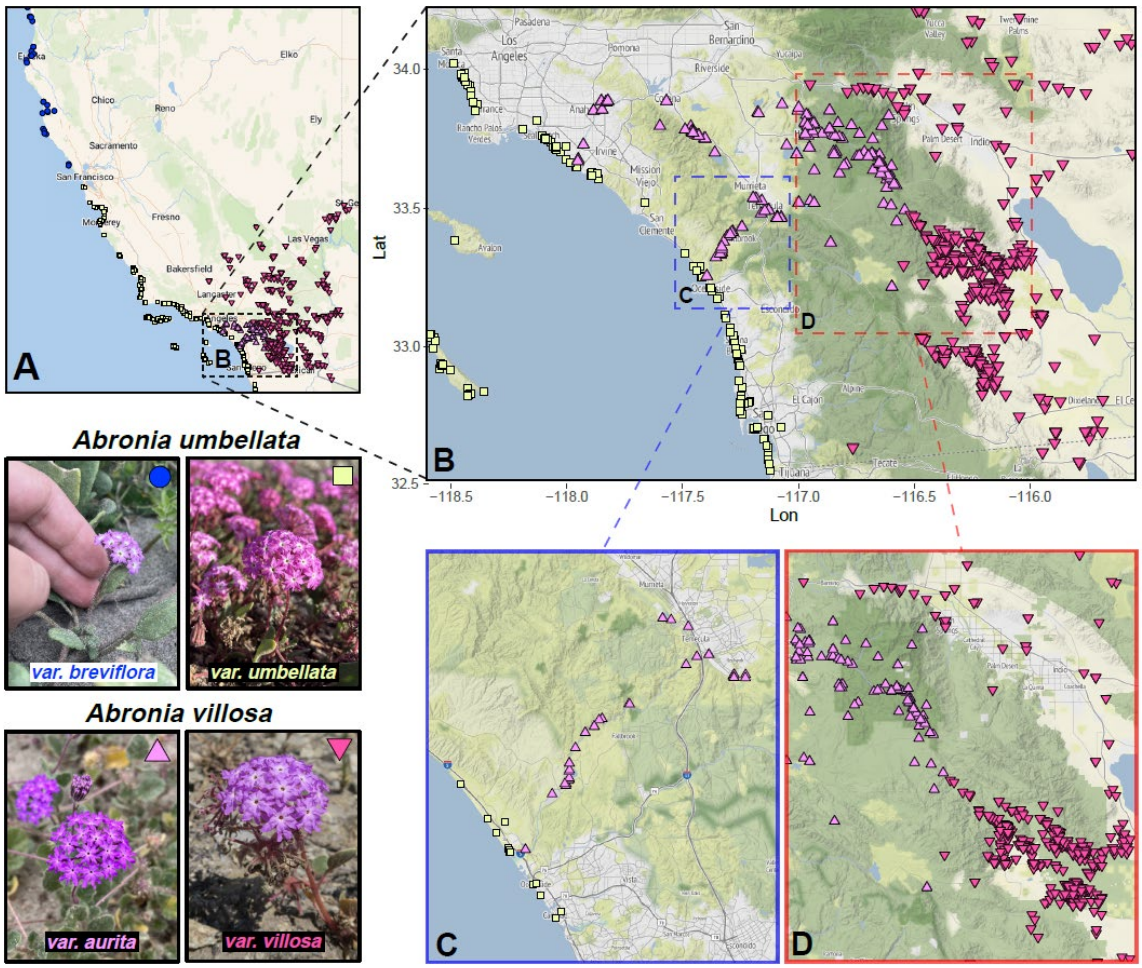


FIGURE 1 Geographic distributions of *Abronia villosa*, *Abronia umbellata*, and their varieties (a) across the California, (b) Southern California, (c) the Santa Margarita River Valley, and (d) Coyote Canyon connecting Borrego Springs to the Peninsular Ranges. Mapped using occurrence data from GBIF and CCH2 repositories in R using GGPLOT.

Although range asymmetry and ecological divergence between sister pair taxa are indicators of budding divergence, they are not conclusive evidence of it (Sianta and Kay, 2023). Progenitor-derivative sister pair relationships are most definitively detected with a phylogenetic analysis (Crawford, 2010), and evidence of budding divergence will result

in a nested phylogenetic relationship, with the derivative species being monophyletic and nested within the progenitor, causing the progenitor to be paraphyletic (Rieseberg and Brouillet, 1994, Baldwin, 2005). The goal of this study is to test for a signal of budding divergence within *Abronia villosa*, with the hypothesis that *A. villosa* var. *aurita* is derivative from of *A. villosa* var. *villosa*. I predict that *A. villosa* var. *aurita* is phylogenetically nested within *A. villosa* var. *villosa* and I expect to detect signals of ecological divergence between the two.

METHODS

Study System

The genus *Abronia* Juss., commonly known as sand verbenas, comprises 20 species of xerophytic plants adapted to coastal dunes and arid, sandy environments that spread across western North America (Jussieu, 1789, Wilson, 1972, 1975, Galloway, 1975, ITIS.gov). There are eight species native to California, five of which are considered rare or contain rare varieties (Wilson, 1972, Jepson Eflora, 2024). *A. villosa* var. *aurita*, a rare variety with a complicated taxonomic history (see Discussion), is one of only two *Abronia* taxa endemic to California (Wilson, 1972, Murdock, 2012). The two varieties of *Abronia villosa* are distinct ecotypes that differ in both elevational range and morphology (Curtis, 1977, Murdock, 2012). Curtis (1977) delineated the ecotypes of var. *villosa* and var. *aurita* as respectively desert and non-desert and distinguished the morphology of var. *aurita* as having distinctly larger floral characters (perianth tube and limb), more flowers per umbel, and a significantly longer peduncle than its sister variety. *A. villosa* var. *villosa* has a range spanning southern CA, at least two other states in the southwestern United States (NZ, AZ, and minimally in UT), as well as parts of Mexico and Baja California (Galloway, 1975), and is found in most arid parts of the Sonoran, Colorado and Mojave deserts (Fig. 1A) (Wilson, 1972, 1976). *Abronia villosa* var. *aurita* is endemic to Riverside and San Diego Counties and is a mostly montane species found in the San Jacinto Mountains, however, part of its range has spread eastward to the

Pacific Ocean in annually flooded sandy washes of the Santa Margarita River Valley (Fig. 1B) (Curtis, 1977, Murdock, 2012). Although Curtis thought var. *aurita* to be geographically isolated from nearby populations of var. *villosa* by the San Jacinto and Santa Rosa Mountain ranges (Peninsular Ranges), he did not inspect Coyote Canyon, a canyon network connecting the desert floor of the Anza-Borrego to the mountain town of Anza (Fig. 1D). Coyote Canyon has an elevational gain of almost 1000 m and contains the populations of var. *villosa* geographically closest to var. *aurita*. This series of canyons may have been the avenue of range expansion for *Abronia villosa* to reach montane elevations where it began diverging and it may still be facilitating gene flow between the two varieties where their ranges abut. Gene flow could also be maintained by long distance pollinators like the generalist hawkmoth, *Hyles lineata* (Sphingidae), a known pollinator for *A. villosa* that abundantly visits both varieties (Tillet, 1967, E.F. LoPresti, T. Chester, personal communication, E.J. Allen, personal observation).

A range extension of *A. villosa* var. *aurita* along the Santa Margarita River Valley – which stretches from Temecula, CA to Oceanside, CA – includes the only populations of var. *aurita* that reach the Pacific Ocean (Fig. 1C). The Santa Margarita populations are separated from the rest of the range of var. *aurita* both geographically and elevationally, with populations reaching the coast at the outlet of the Santa Margarita River. At this outlet, var. *aurita* has significant range overlap with its coastal congener *Abronia umbellata* var. *umbellata* Lam., which has been found to hybridize anytime it occurs sympatrically with a congener (Lamarck, 1791, Tillett 1967, Van Natto, 2020). Morphological similarities have led to the hypothesis that *A. villosa* and *A. umbellata* are

closely related, likely comprising a monophyletic group with the inclusion of the Baja California species *Abronia gracilis* Benth. (Bentham, 1844, Tillet, 1967, Galloway, 1975). Multiple unpublished phylogenies of *Abronia* have supported the hypothesis that *A. villosa*, *A. umbellata*, and *A. gracilis* form a monophyletic group (Nosratinia, 2013, E.F Lopresti et. al, S. Price et al., personal communication).

Taxon Sampling

I used a total of 50 individual samples (Appendix A) of *A. villosa* var. *aurita*, its allies (*A. villosa* var. *villosa*, *A. umbellata*, and *A. gracilis*), and *Abronia latifolia* Eschsch. as an outgroup (Memoirs of the Imperial Academy of Sciences of St. Petersburg, 1826). Three of my *A. umbellata* samples belong to the autogamous variety *A. umbellata* var. *breviflora* (Standl.) L.A. Galloway, while the rest belong to the outcrossing var. *umbellata* (Spellenberg and Poole, 2003, Samis & Eckert 2007, Darling 2008). Two samples were collected from the potential hybrid zone between *A. villosa* var. *aurita* and *A. umbellata* var. *umbellata* (Fig. 1B). Most samples were collected in the field and dried in silica gel for DNA extraction, however, two were grown from field-collected seed in a greenhouse (E.L. Lopresti), four were from herbarium specimens (J. Rebman, San Diego Natural History Museum), and three were obtained as raw reads from collaborators who used the same target sequencing baits (S. Price and M. Johnson). Specimen vouchers for every locality visited in the field were placed in the HSC herbarium. A record for each sample collected was uploaded to the iNaturalist platform

(www.inaturalist.org), and all 47 individuals that were sequenced by me personally will have their raw reads uploaded to GenBank (Appendix A).

DNA Extraction and Sequencing

DNA was extracted from dried plant tissue of field, greenhouse, and herbarium samples using the NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol, with 5 µL proteinase K (20 mg/mL) added to the digestion step. DNA extractions were quantified with Qubit Fluorometric Quantification (Thermo-Fisher), and DNA quality was visualized via gel electrophoresis. Library preparation and sequencing was performed by Rapid Genomics (Gainesville, FL) using the universal Angiosperms353 target capture probe set (Johnson et al., 2019).

Alignment with CAPTUS

Raw reads were cleaned, assembled, extracted, and aligned with the CAPTUS v0.9.89 pipeline (Ortiz et al., 2023). Trimming and quality filtering of raw reads were performed using the CAPTUS "clean" function with default settings, utilizing BBDUK from the BBTOOLS suite (Bushnell et al., 2017). *De novo* assembly of paired reads into contigs was performed using the CAPTUS "assemble" function with the default settings, via MEGAHIT v1.2.9 (Li et al., 2015). Target genes were extracted using a custom reference dataset for *Abronia* and *Mirabilis* (M. Johnson) compatible with the Angiosperms353 probe set (Johnson et. al, 2019). Targets were extracted using the CAPTUS "extract" function with a minimum nuclear identity threshold of 90% (--nuc_min_identity 90), and

Abronia latifolia as the outgroup (--out), matching the assembled contigs to the *Abronia* and *Mirabilis* custom references (-n angiosperms353.abronia.fasta), via SCIPIO v1.4 (Keller et al., 2008). Extracted markers were aligned using the CAPTUS "align" function, with flanking regions included (-f ALL), via MAFFT v7.505 (Katoh et al., 2002) and then trimmed via CLIPKIT v1.3.0 (Steenwyk et al., 2020) with CAPTUS default settings.

Trimming and Subsetting

The alignments from CAPTUS were further trimmed to remove gaps with TRIMAL v1.2 (Capella-Gutiérrez et al., 2009), removing positions from alignments when 50% or more of sequences contain a gap (TRIMAL '-gt 0.5'). Visual inspection of the matrices revealed several problematics regions in the alignments along with outlier sequences. For further trimming and filtering, alignments were categorized into three groups based on a visual assessment of the problematic regions: bad = matrices with multiple problematic regions, questionable = matrices with few problematic regions, and good = matrices with no problematic regions. 218 were marked as good, 61 as questionable, and 66 as bad. Good alignments were not trimmed further. Questionable and bad alignments were further trimmed using the TRIMAL option -automated1. Bad alignments were again further trimmed by removing positions with a similarity score lower than 0.005 the (TRIMAL '-st 0.005'), removing columns in the alignments with doubtful homology among samples. On both questionable and bad alignments, the extra trimming steps caused some samples (rows) to become entirely gaps; these samples were fully excluded from those alignments (TRIMAL '-resoverlap 0.20 -seqoverlap 20').

Phylogenetic Analysis

Species trees were inferred using both concatenated and coalescent-consistent methods. Gene trees were produced for each trimmed alignment using IQ-TREE v2.2.2.6 (Nguyen et al., 2015) with an independent GTR+G model and 1000 ultrafast bootstraps. Outlier long branches were identified and trimmed from resulting gene trees using TREESHRINK v1.3.9 (Mai and Mirarab, 2018), with the alpha setting '-q 0.30'. Four gene trees (from alignments that recovered very few taxa) contained only long branches, indicating outlier samples. Long branch taxa identified with TREESHRINK were then removed from the original Captus alignments using a script modified from remove_long_branched_taxa_from_fasta_005.py (Vargas et al. 2020). Alignments with long branch samples removed were then used to infer a concatenated tree using IQ-TREE v2.2.2.6 (Nguyen et al., 2015), using the "-p" option. For my coalescent-consistent approach, new gene trees were generated from alignments with long branch taxa removed (again with a GTR+G model and 1000 ultrafast bootstraps), and the output trees were used to calculate a coalescent-consistent tree in ASTRAL v.5.7.1(Mirarab et al., 2014).

Genetic Structure Analysis

To explore the genetic structure of *Abronia villosa* var. *aurita* and its allies, we created a matrix of single nucleotide polymorphisms (SNPs) using BWA v0.7.17 (Li and Durbin, 2009), PICARD TOOLKIT v3.1.1 (Broad Institute, 2019), GENOME ANALYSIS TOOLKIT (GATK) v4.4.0.0 (McKenna et al., 2010), and PLINK (Purcell et al., 2007). Two

taxa were excluded from this analysis: *A. latifolia* because it is an outgroup, and *Abronia gracilis* due to low sampling and isolation from focal taxa by geographic distance. First, a custom reference assembly of 345 target loci with a total of 477,308 bp was created from the longest *Abronia villosa* var. *aurita* sequence in each of my 345 trimmed CAPTUS alignments (S1). My cleaned paired reads (CAPTUS 'clean') were mapped to the custom reference with BWA 'mem,' duplicate reads were marked and removed with PICARD TOOLS. SNPs were then called for each sample using GATK 'HaplotypeCaller.' SNPs for all samples were combined into a single dataset using GATK 'CombineGVCFs,' from which variants were jointly genotyped using GATK 'GenotypeGVCFs.' Genotyped variants were selected to include SNPs and exclude any non-variants using GATK 'SelectVariants.' PLINK was then used to filter SNPs, removing individuals missing genotype data for more than 20% of their genotyped markers, removing variants with an allele frequency less than 10%, and accounting for linkage equilibrium using a window size of 50 kb, a variant count of 5, and a linkage threshold of 0.1, finalizing the SNP matrix for downstream analyses.

Relationships and genetic structure were assessed with FASTSTRUCTURE v1.0 (Raj and Pritchard, 2014), with STRUCTURE_THREADER v1.3.11 (Pina-Martins et al., 2017) used to automate and parallelize different runs of K. Taxa were grouped into five groups using STRUCTURE_THREADER '--ind'; this was aesthetic and did not affect clustering. The most likely number of genetic clusters (K) was determined after assessing values of K ranging from 1 to 10 with the FASTSTRUCTURE function, chooseK.py. Plots were visualized using STRUCTURE_THREADER. To further visualize genetic distance between

species and varieties, a principal component analysis (PCA) was calculated. PLINK was used to prune the GATK SNP dataset accounting for linkage disequilibrium with a window size of 50 kb, a variant count of 10, and a linkage threshold of 0.1, and calculate a PCA from the pruned SNP dataset. The PCA was visualized in R v4.3.2 (R Core Team, 2021) using the TIDYVERSE package (Wickham et al., 2019).

RESULTS

Sequencing and Alignment

My raw sequences yielded roughly 206 million reads consisting of 31 million kbp, and after trimming and filtering via CAPTUS 'clean' there were 197 million reads. *De novo* assembly via CAPTUS 'assemble' produced a total of 245,182 contigs consisting of 126 million bp. A total of 16,301 target regions were extracted from contigs with CAPTUS 'extract', averaging 326 target regions per sample. CAPTUS 'align' produced a total of 345 alignments from extracted target regions. After subsetting and extensive trimming of with TRIMAL, alignments had a remaining 14,840 target regions (averaging 297 per sample) consisting of 21.1 million bp.

Phylogenetic Analysis

The concatenated tree shows 100% bootstrap support for each species of *Abronia* being monophyletic (Fig. 2). The coalescent-consistent tree likewise shows strong posterior probability support (1) for monophyly of each species (Appendix B). At the variety level there is 100% bootstrap support and strong posterior probability (1.0) for monophyly of both *A. villosa* var. *aurita* and *A. umbellata* var. *breviflora*. In both trees *A. villosa* var. *aurita* is phylogenetically nested with *A. villosa* var. *villosa*, making var. *villosa* a paraphyletic group – strong evidence that var. *aurita* is a derivate of var. *villosa*. *Abronia villosa* var. *aurita* also comprises two reciprocally monophyletic subclades that

align with the respective geographic isolation of the localities of samples in each subclade; one subclade represents higher elevation montane populations (475 - 1500 m, San Jacinto Mountains and Hemet) and the other subclade consists of lower elevation coastal populations (6 - 350 m, Santa Margarita River Valley) (Fig. 1B, 1C). The auotgamous variety *A. umbellata* var. *breviflora* is likewise phylogenetically nested within *A. umbellata* var. *umbellata*, producing a similar pattern of paraphyly for the latter variety. One of the samples from the hypothesized hybrid zone between *A. villosa* var. aurita and *A. umbellata* var. *umbellata* had conflicting placement in both trees, either being sister to *A. villosa* or a clade comprising *A. umbellata* and *A. gracilis*.

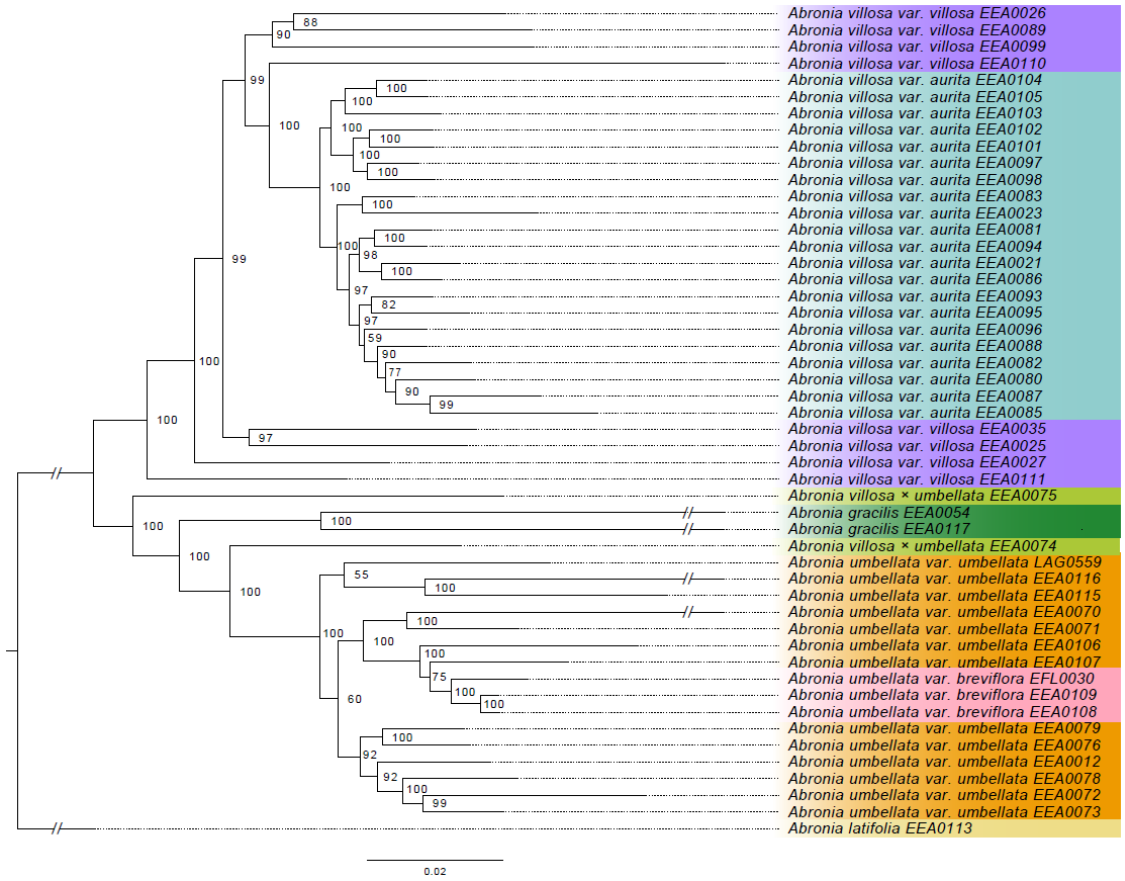


FIGURE 2 Phylogeny inferred from concatenated supermatrix of 345 genes for *Abronia villosa*, *Abronia umbellata*, their varieties, *Abronia gracilis*, and *Abronia latifolia* as an outgroup. Node values indicate bootstrap support. Branch lengths that were shortened to save space are indicated with double slash marks, '//.'Terminal clades are color coded by taxon.

Genetic Structure Analysis

The SNP matrix initially recovered 59,542 variant sites from 340 loci in 47 samples (after excluding two samples of *A. gracilis* and the outgroup *A. latifolia*), and after filtering with Plink 559 SNP variants remained. The genetic structure of *A. villosa* and *A. umbellata* inferred by FASTSTRUCTURE that best explained the data was three genetic clusters (K = 3), at this value of K, the varieties of *A. villosa* show as two distinct genetic clusters, with *A. umbellata* making up the third (Fig. 3). Filtering for PCA with PLINK produced 707 variant SNPS from the same initial SNP Matrix and shows similar clustering to the FASTSTRUCTURE analysis (Fig. 4). Both PCA and FASTSTRUCTURE show evidence of hybridization of *A. villosa* var. *aurita* and *A. umbellata* var. *umbellata* for two samples thought to be part of a hybrid zone.

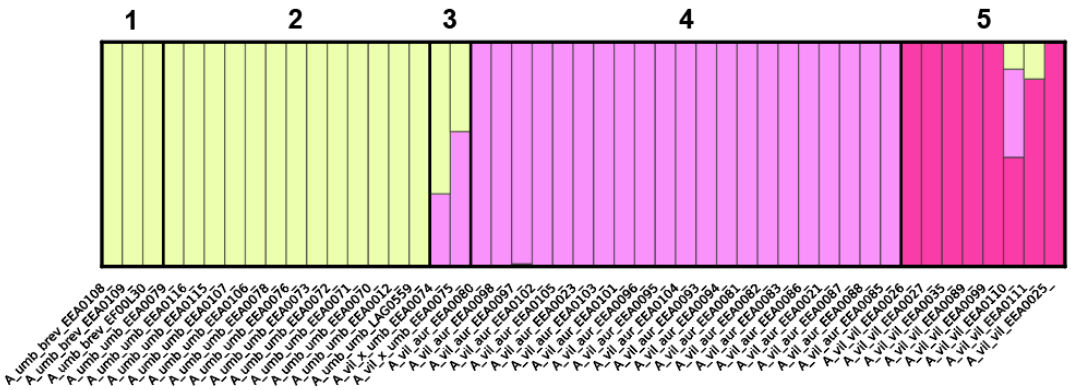


FIGURE 3 Genetic structure (K = 3) of all samples excluding *Abronia gracilis* and *Abronia latifolia*. Bar colors indicate ancestral clustering of individual samples inferred by FASTSTRUCTURE from 559 SNP variants. Samples are each labeled with a unique taxon code. Numbers indicate taxon groups: (1) *A. umbellata* var. *breviflora*, (2) *A. umbellata* var. *umbellata*, (3) *A. villosa* × *umbellata*, (4) *A. villosa* var. *aurita*, (5) *A. villosa* var. *aurita*.

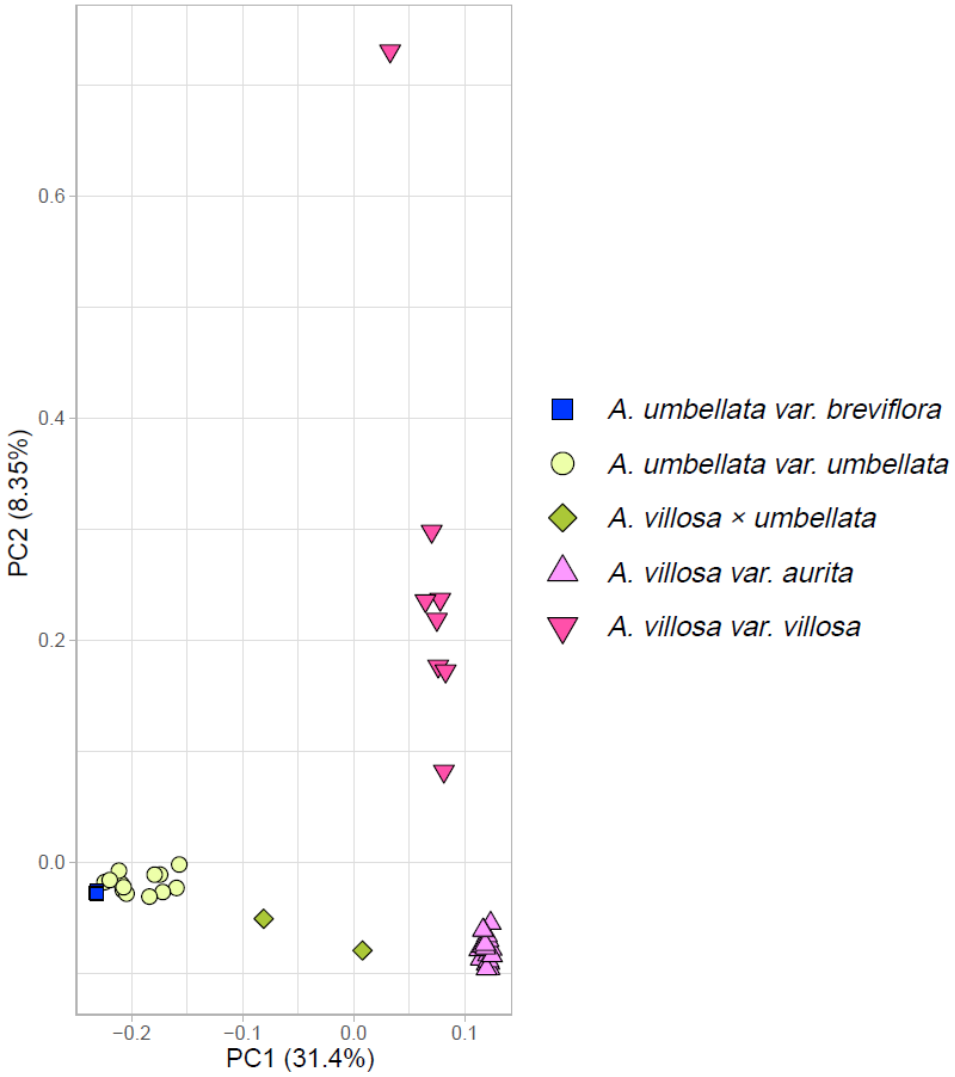


FIGURE 4 First two principal components of 707 SNP variants called across all samples excluding *Abronia gracilis* and *Abronia latifolia*. SNP data was filtered and used to generate eigenvectors and eigenvalues with PLINK and visualized in R with GGPLOT.

DISCUSSION

Budding divergence produces a paraphyletic pattern in *Abronia villosa*

My study found strong signals of budding divergence within the species *Abronia villosa*, with evidence supporting the hypothesis that var. *aurita* is a derivate of var. *villosa*. My topology shows *A. villosa* var. *aurita* to be monophyletic and nested within *A. villosa* var. *villosa*, causing the latter to be paraphyletic (Fig 2, Fig S1). This nested pattern is a well-documented signal for budding divergence, as is the range asymmetry and the geographic peripherality of var. *aurita* to var. *villosa* (Rieseberg and Brouillet, 1994, Baldwin, 2005, Anacker and Strauss, 2014). These findings suggests that var. *aurita* budded off into montane elevations from populations of the widespread desert var. *villosa* as it expanded up canyon washes. It is highly unlikely that this divergence was instead caused by the uplift of the San Jacinto mountains, as its upheaval predates the diversification of *Abronia* by at least 10 million years (Spotila et al., 2020, Cuna Neto et al., 2022). Budding speciation results in progenitor-derivative sister pairs, a phenomenon thought to be common in plants (Rieseberg and Brouillet, 1994; Baldwin, 2005, Crawford, 2010; Anacker and Strauss, 2014; Grossenbacher et al., 2014). While most studies have focused on budding speciation as a macroevolutionary process, there has been increasing interest at microevolutionary scales (Vargas et al., 2020, Sianta and Kay, 2022, Ivey et al., 2023), however, few studies have been at infraspecific levels (Otero et

al., 2022). This study adds more evidence to the hypothesis that budding speciation might be pivotal in the assemblage of biodiverse hotspots.

My genetic structure SNP analysis showed both var. aurita and var. *villosa* cluster separately from each other (Fig. 3, Fig. 4), evidence of divergence and support for their classification as distinct varieties (Abram, 1905, Jepson, 1914, Curtis, 1977). The implications of having one sister variety phylogenetically nested within another, while also having enough genetic structure to cluster separately, is highly relevant in the context of budding speciation. There has not been enough intravarietal gene flow to erase the paraphyletic signal (Rieseberg and Brouillet, 1994, Baldwin, 2005,), yet enough lineage sorting has occurred to differentiate the varieties structurally. This supports the increasing evidence that speciation in the face of gene flow is not just possible, but highly probable in plants, and that the process of budding speciation through ecological divergence contributes to the genesis of neo-endemics and species diversity in California (Gottleib, 2004, Baldwin 2005, Anacker and Strauss, 2014, Grossenbacher, et al., 2014, Ivey et al., 2023). Nested phylogenetic patterns will disappear on evolutionary time scales, eventually becoming reciprocally monophyletic (Rieseberg and Brouillet, 1994, Hörandl and Stuessy, 2010), but taxa in the process of diverging provide a unique opportunity to understand how microevolutionary processes generate biodiversity.

In the case of *A. villosa*, some gene flow may be maintained through the proximity of var. *villosa* populations in Coyote Canyon to nearby montane populations of var. *aurita*, but overall, gene flow is likely facilitated by long range nocturnal pollinators. Like most Nyctaginaceae species, many *Abronia* have nocturnal moths as their primary

pollinator (Grant and Grant, 1983, Williamson et al., 1994, Williamson and Werth, 1999, Saunders and Sipes, 2006), including *Abronia villosa* and its close relatives, *Abronia umbellata* and *Abronia gracilis* (Tillet, 1967, Doubleday et al. 2013). A particularly prolific, long range, and generalist hawkmoth, *Hyles lineata* (Sphingidae) is known to frequently visit *Abronia villosa* and is commonly seen pollinating both the desert and montane varieties (Tillet, 1967, E.F. LoPresti, T. Chester, E.J. Allen, personal observation). Moth pollinators have been shown to travel incredibly long distances with pollen loads, potentially contributing to gene flow (Schmitt, 1980, Courtney, 1982, Barthelmess et al., 2006), and *Hyles lineata* is known to pollinate the narrow mountain endemic, *Abronia alpina* at 8800-9000m in the Sierra Nevada Mountains (Jabis et al., 2011).

Mountains are known generators of biodiversity and species richness in plants (Madriñán et al., 2013, Wen et al., 2014, Bouchenak-Khelladi et al., 2015, Vargas et al., 2023), and upslope divergences leading to the emergence of sister taxa are common (Nürk et al., 2018, Vargas et al., 2020, Juárez et al., 2023, Carruthers et al., 2024). Mountains are known drivers of allopatric divergence through strong geographic isolation, yet also induce ecological divergences due to habitat heterogeneity. However, even in allopatric divergences there is evidence of gene flow across montane geographic barriers at inter- and infraspecific levels (Wu et al., 2022). A study in the Loess Plateau of China found evidence of subspecific divergence with gene flow between populations of *Buddleja alternifolia* (Scrophulariaceae) on either side of a mountain barrier (Ma et al., 2021). Widespread lowland taxa are known to ecologically diverge into montane

environments (Ma et al., 2022), often with attributes that are consistent with budding speciation (Juárez et al., 2023). Progenitor-derivative relationships often start with a widespread progenitor, as they have stable enough population structure to maintain their ancestral ecology in the face of gene flow with ecologically diverged sister taxa (Baldwin, 2005, Hörandl and Stuessy, 2010, Kisel and Barraclough, 2010, Sianta and Kay 2022). Gene flow between widespread progenitors and montane derivates can even persist along evolutionary time scales. In the Qinghai–Tibet Plateau, the montane species *Populus rotundifolia* (Salicaceae) was found to be derivate of the widespread lowland *Populus davidiana*, with continued gene flow and hybridization between adjacent populations since their divergence in the Pleistocene (Li et al., 2021).

On the reciprocal monophyly of *A. villosa* var. *aurita* subclades

Although firmly nested within *Abronia villosa* var. *villosa*, finding that var. *aurita* comprises two reciprocally monophyletic clades was a novel discovery (Fig. 2, Appendix B). These two clades show a degree of geographic and ecological isolation, with the more montane populations (475 - 1500 m) from the San Jacinto Mountains and Hemet, CA forming one clade (Fig. 1B), and the lower elevation populations (6 - 350 m) found in sandy washes along the Santa Margarita River Valley comprising a second clade (Fig. 1C). While not shown in the best K (K= 3) of the FASTSTRUCTURE analysis (Fig. 3), all values of K from 4 to 10 showed these two clades clustering separately to some degree (Appendix C). The expansion of var. *aurita* into the Peninsular Ranges may have facilitated access to lower elevation niches on the western side of the mountains, a large

geographic area that var. *villosa* does not occupy. *Abronia villosa* seeds are known to be transported long distances by flash floods (Curtis, 1977, Wilson, 1976, Murdock, 2012), therefore expansion into lower elevations was likely via downstream seed transportation along seasonally flooding sandy washes. Downstream dispersal is a common long-distance mechanism for plant migration and has been shown to increase species richness in riparian communities (Honnay et al., 2001, Jansson et al., 2005).

The monophyly and structural clustering of lower elevation populations of var. *aurita* may be evidence of early divergence due to downstream migration. Elevational gradients can strongly affect the timing of flowering, and differences in reproductive phenology are known to drive divergent selection (Savolainen, 2006, Givnish, 2010, Frei eta al., 2014). Snowpack can further emphasize elevational phenology gradients (Asam et al., 2018), and snow has been observed delaying the emergence and bloom time of mountain populations of *A villosa* var. *aurita* (T. Chester, personal communication), which may have played a role in divergence in recent evolutionary time.

Topographic complexity in mountain associated environments can lead to infraspecific divergences in geographically close populations (Leles et al., 2015). The topographic heterogeneity of California is known to drive the genesis of new species (Raven and Axelrod, 1978, Thorne et al., 2009, Kraft et al., 2010), and the lower elevational niches occupied by var. *aurita* may be ecologically different enough to eventually lead to divergence. Another contributor to this potential divergence may be anthropogenic disturbance, which is responsible for rampant habitat fragmentation in Southern CA (Soulé eat al., 1992, Schwartz et al., 2006, Girvetz, 2008, Loarie, 2008).

Loss of sandy riparian areas may have disrupted habitat connectivity, limiting genetic exchange between montane and low elevation populations of var. *aurita*. On the other hand, anthropogenic dispersal may also be at play and disentangling the past anthropogenic and environmental influences on endemic plants can be difficult (Fois et al., 2017). However, there is evidence of human disruption of sandy habitats negatively affecting var. *aurita* in the recent past. Populations of var. *aurita* once occurred along the Santa Ana River in Orange County, but its conversion from a sandy river to a series of manmade concrete channels has led to its extirpation, with no observations in Orange County since 1935 (CCH2, 2024). Further research is needed to identify whether the two subclades of var. *aurita* are in the process of divergence, and to understand the mechanisms driving their reciprocal monophyly and unique genetic clustering since the derivation of var. *aurita* from var. *villosa*.

Hybrid zone between *Abronia villosa* and *Abronia umbellata* at Camp Pendleton

Abronia umbellata is a coastal species with a nearly one-dimensional geographic range along the West Coast of North America that has been found to hybridize anytime it occurs sympatrically with a congener – albeit in low percentages within populations (Tillet, 1967, Greer, 2016, Van Natto, 2020, Van Natto & Eckert, 2022). Tillet (1967) hypothesized that where *Abronia villosa* reached the Pacific Ocean via sandy riverways, it hybridizes with its coastal congener, *A. umbellata*, anytime they would overlap in range. Although Tillet specifically mentioned the Santa Ana River – where *A. villosa* var. *aurita* is likely locally extinct – this study has found multiple lines of evidence of a

hybrid zone between *A. villosa* var. *aurita* and *A. umbellata* var. *umbellata* at the outlet of the Santa Margarita River at Camp Pendleton, CA (Fig. 1C). Two samples were collected from a locality containing 19 individuals showing intermediate morphology between *A. villosa* and *A. umbellata*. One of the hybrid samples (EEA0075) had conflicting placements in the concatenated and coalescent-consistent trees (Fig. 2, Appendix B), alternatively being sister to all *Abronia villosa* or a clade comprising *A. umbellata* and *A. gracilis*. The other hybrid sample (EEA0074) was consistently sister to all of *A. umbellata*. Their conflicting placements and tendency to be sister to their parent clades is an indication that they are hybrid individuals. Evidence of hybridization is further supported by the genetic structure SNP analysis. In the FASTSTRUCTURE analysis the hybrid samples show mixed ancestry of *A. umbellata* and *A. villosa* var. *aurita* (Fig. 3), and in my PCA the hybrid samples are distributed intermediately between the clusters for *A. umbellata* var. *umbellata* and *A. villosa* var. *aurita* along the PC1 axis (Fig. 4).

On the taxonomic history of *Abronia villosa* and its varieties

The taxonomic history of *A. villosa* and its two varieties is complicated, leaving many errata in herbaria and occurrence data. *A. villosa* was first collected in Arizona during the G.M. Wheeler Survey (U.S. Army Corps of Engineers) and the type specimen was described by Watson in 1873 (Wheeler and Watson, 1874). In 1905 Abrams named two new Californian *Abronia* from herbarium specimens: *A. aurita* and *A. pinetorum* – collected by S.B. Parish (1896) and H.M. Hall (1901) respectively. Jepson (1914) then chose to lump *A. pinetorum* with *A. aurita* and rename them both as a variety of *A.*

villosa, making *A. aurita* the type specimen for *A. villosa* var. *aurita*. The locality for the type of *A. pinetorum* was Thomas Valley in the San Jacinto Mountains, where today there is still robust populations of its synonym, *A. villosa* var. *aurita*. However, Parish's locality for *A. aurita* has caused some problems. It was described as "Palm Springs, (Agua Caliente), desert base of San Jacinto Mountain, alt. 500-700 feet," with a date range of April 4th to April 13th, 1896. Parish collected many specimens during this 10-day expedition, all listing the exact same locality – an indication of its vagueness. Agua Caliente refers to the local indigenous tribe's reservation, a checkerboard of property rights assigned in alternating 1-mile square blocks to indigenous and non-indigenous owners. This 'checkerboard' reservation encompasses much of Palm Springs and the eastern side of the San Jacinto Mountains, so the exact locality of the *A. aurita* type specimen is highly uncertain. Due to this early taxonomic confusion, most *A. villosa* collections in the greater metropolitan area of Palm Springs have been identified as var. *aurita*. This has proven problematic, as the current range of *A. villosa* var. *aurita* does not include Palm Springs, yet it does include Thomas Valley (also called Garner Valley), where Hall's *A. pinetorum* was found.

CONCLUSIONS

Taken together, my results indicate a strong signal of budding divergence consistent with the hypothesis that *A. villosa* var. *aurita* is derivate of *A. villosa* var. *villosa*, adding to the continually mounting evidence of the relative importance of budding speciation in the assemblage of biodiversity hotspots. The montane range of var. *aurita* is asymmetrically smaller and peripheral to the widespread desert range of var. *villosa*, suggesting an upslope ecological divergence into the Peninsular Ranges. I show that microevolutionary studies are necessary to properly test speciation dynamics, as macroevolutionary studies are often insufficiently granular to elucidate species divergences beyond strictly bifurcating events where a common ancestor neatly splits into two separate lineages. My study documents the attributes of microevolutionary patterns at play during the divergence process, showing the existence of paraphyletic taxa (Rieseberg and Brouillet, 1994, Gottleib, 2004, Hörandl and Stuessy) and highlighting alternative processes to allopatric speciation (i.e., budding speciation) as major players in diversification (Baldwin 2005, Gottleib, 2004, Malay and Paulay, 2010, Claremont et al., 2012, Parins-Fukuchi, 2021, Otero et al., 2022). My study exemplifies how the confluence of phylogenomics, genetic structural analyses, and geographic data can provide nuance to the mechanisms of divergence with gene flow, which often start at infraspecific levels. Further works like this are warranted to shed light on paraphyletic species as natural taxonomic units in the process of diverging and show their relevance for capturing the complex microevolutionary history of species divergence.

REFERENCES

Abrams, L. (1905). Studies on flora of southern California. *Bulletin of the Torrey Botanical Club, 32*(537). Retrieved from https://www.biodiversitylibrary.org/page/13413948#page/634/mode/1up

American Society of Naturalists, & University of Chicago. Press. (1883). *The American Naturalist* (Vol. 7, No. 73-84). American Society of Naturalists by the University of Chicago Press.

Anacker, B. L., & Strauss, S. Y. (2014). The geography and ecology of plant speciation: Range overlap and niche divergence in sister species. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1778), 20132980. https://doi.org/10.1098/rspb.2013.2980

Asam, S., Callegari, M., Matiu, M., Fiore, G., De Gregorio, L., Jacob, A., Menzel, A., Zebisch, M., & Notarnicola, C. (2018). Relationship between Spatiotemporal Variations of Climate, Snow Cover and Plant Phenology over the Alps—An Earth Observation-Based Analysis. *Remote Sensing*, *10*(11), Article 11. https://doi.org/10.3390/rs10111757

Baldwin, B. G. (2005). Origin of the serpentine-endemic herb Layia discoidea from the widespread L. landulosa (Compositae). *Evolution*, *59*(11), 2473-2479.

Baldwin, B. G. (2014). Origins of Plant Diversity in the California Floristic Province. *Annual Review of Ecology, Evolution, and Systematics*, *45*(1), 347–369. https://doi.org/10.1146/annurev-ecolsys-110512-135847

Barraclough, T. G., & Vogler, A. P. (2000). Detecting the geographical pattern of speciation from species-level phylogenies. *The American Naturalist*, *155*(4), 419-434.

Barthelmess, E. L., Richards, C. M., & McCauley, D. E. (2006). Relative effects of nocturnal vs diurnal pollinators and distance on gene flow in small Silene alba populations. *New Phytologist*, *169*(4), 689–698. https://doi.org/10.1111/j.1469-8137.2005.01580.x

Bentham, G. (1844). *The Botany of the Voyage of HMS Sulphur, Under the Command of Captain Sir Edward Belcher During the Years 1836-42*. Smith, Elder.

Bock, D. G., Cai, Z., Elphinstone, C., González-Segovia, E., Hirabayashi, K., Huang, K., ... & Rieseberg, L. H. (2023). Genomics of plant speciation. *Plant Communications*. https://doi.org/10.1016/j.xplc.2023.100599

Bouchenak-Khelladi, Y., Onstein, R. E., Xing, Y., Schwery, O., & Linder, H. P. (2015). On the complexity of triggering evolutionary radiations. *New Phytologist*, *207*(2), 313–326. https://doi.org/10.1111/nph.13331

Burge, D. O., Thorne, J. H., Harrison, S. P., O'Brien, B. C., Rebman, J. P., Shevock, J. R., ... & Barry, T. (2016). *Plant diversity and endemism in the California Floristic Province. Madroño, 63*(2), 3-206. https://doi.org/10.3120/madr-63-02-3-206.1

Bushnell, B., Rood, J., & Singer, E. (2017). BBMerge–accurate paired shotgun read merging via overlap. *PloS one*, *12*(10), e0185056. https://doi.org/10.1371/journal.pone.0185056

Butlin, R. K., Galindo, J., & Grahame, J. W. (2008). Sympatric, parapatric or allopatric: the most important way to classify speciation?. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1506), 2997-3007. https://doi.org/10.1098/rstb.2008.0076

Capella-Gutiérrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, *25*(15), 1972-1973. https://doi.org/10.1093/bioinformatics/btp348

Carruthers, T., Moerland, M. S., Ebersbach, J., Favre, A., Folk, R. A., Hawkins, J. A., Muellner-Riehl, A. N., Röser, M., Soltis, D. E., Tkach, N., Baker, W. J., de Vos, J. M., & Eiserhardt, W. L. (2024). Repeated upslope biome shifts in Saxifraga during late-Cenozoic climate cooling. *Nature Communications*, *15*(1), 1100. https://doi.org/10.1038/s41467-024-45289-w

CCH2. (2022). Specimen Records. Consortium of California Herbaria (CCH2). https://ucjeps.berkeley.edu/consortium/

Claremont, M., Reid, D. G., & Williams, S. T. (2012). Speciation and dietary specialization in Drupa, a genus of predatory marine snails (Gastropoda: Muricidae). *Zoologica scripta*, *41*(2), 137-149.

Courtney, S. P., Hill, C. J., & Westerman, A. (1982). Pollen Carried for Long Periods by Butterflies. *Oikos*, *38*(2), 260–263. https://doi.org/10.2307/3544030

Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sinauer.

Crawford, D. J. (2010). Progenitor-derivative species pairs and plant speciation. *TAXON*, *59*(5), 1413–1423. https://doi.org/10.1002/tax.595008

Cunha Neto, I. L., Pace, M. R., Hernández-Gutiérrez, R., & Angyalossy, V. (2022). Linking the evolution of development of stem vascular system in Nyctaginaceae and its correlation to habit and species diversification. *EvoDevo*, *13*(1), 4. https://doi.org/10.1186/s13227-021-00190-1

Curtis, N. R. (1977). Biosystematics of the Abronia villosa complex from Southern California. *UC Santa Barbara Theses Digitization Project, 64*.

Dobzhansky, T. (1970). *Genetics of the evolutionary process* (Vol. 139). Columbia University Press.

Doubleday, L. A. D., Raguso, R. A., & Eckert, C. G. (2013). Dramatic vestigialization of floral fragrance across a transition from outcrossing to selfing in *Abronia umbellata* (Nyctaginaceae). *American Journal of Botany*, *100*(11), 2280–2292. https://doi.org/10.3732/ajb.1300159

Fitzpatrick, B. M., Fordyce, J. A., & Gavrilets, S. (2008). What, if anything, is sympatric speciation? *Journal of Evolutionary Biology*, 21(6), 1452–1459. https://doi.org/10.1111/j.1420-9101.2008.01611.x

Fitzpatrick, B. M., & Turelli, M. (2006). The geography of mammalian speciation: mixed signals from phylogenies and range maps. *Evolution*, *60*(3), 601-615. https://doi.org/10.1111/j.0014-3820.2006.tb01140.x

Fitzpatrick, B. M., Fordyce, J. A., & Gavrilets, S. (2009). Pattern, process and geographic modes of speciation. *Journal of Evolutionary Biology*, 22(11), 2342–2347. https://doi.org/10.1111/j.1420-9101.2009.01833.x

Fois, M., Fenu, G., Cañadas, E. M., & Bacchetta, G. (2017). Disentangling the influence of environmental and anthropogenic factors on the distribution of endemic vascular plants in Sardinia. *PLOS ONE*, *12*(8), e0182539. https://doi.org/10.1371/journal.pone.0182539

Frei, E. R., Hahn, T., Ghazoul, J., & Pluess, A. R. (2014). Divergent selection in low and high elevation populations of a perennial herb in the Swiss Alps. *Alpine Botany*, *124*(2), 131–142. https://doi.org/10.1007/s00035-014-0131-1

Galloway, L. A. (1975). Systematics of the North American Desert Species of Abronia and Tripterocalyx (Nyctaginaceae). *Brittonia*, 27(4), 328. https://doi.org/10.2307/2805512

Galloway, L. A. (2003). *Abronia villosa*. In Flora of North America Editorial Committee eds. 1993+, *Flora of North America* (Vol. 4) (pp. 66-67). Oxford University Press.

Girvetz, E. H., Thorne, J. H., Berry, A. M., & Jaeger, J. A. G. (2008). Integration of landscape fragmentation analysis into regional planning: A statewide multi-scale case study from California, USA. *Landscape and Urban Planning*, *86*(3), 205–218. https://doi.org/10.1016/j.landurbplan.2008.02.007

Givnish, T. J. (2010). Ecology of plant speciation. *TAXON*, *59*(5), 1326–1366. https://doi.org/10.1002/tax.595003

Gottlieb, L. D. (2004). Rethinking classic examples of recent speciation in plants. *New Phytologist*, 161(1), 71–82. https://doi.org/10.1046/j.1469-8137.2003.00922.x

Grant, V. (1981). Plant speciation. *Columbia university press*.

Grant, V., & Grant, K. A. (1983). Hawkmoth pollination of Mirabilis longiflora (Nyctaginaceae). *Proceedings of the National Academy of Sciences*, *80*(5), 1298–1299. https://doi.org/10.1073/pnas.80.5.1298

Greer, S. U. (2016). *Genomic consequences of mating system evolution in the Pacific coastal dune endemic, Abronia umbellata (Nyctaginaceae)* (Doctoral dissertation, Queen's University (Canada)).

Hamilton, C. W., & Reichard, S. H. (1992). Current practice in the use of subspecies, variety, and forma in the classification of wild plants. *Taxon*, 41(3), 485-498.

Harrison, R. G. (2012). The Language of Speciation. *Evolution*, 66(12), 3643–3657. https://doi.org/10.1111/j.1558-5646.2012.01785.x

Harrison, S., & Noss, R. (2017). Endemism hotspots are linked to stable climatic refugia. *Annals of Botany*, *119*(2), 207–214. https://doi.org/10.1093/aob/mcw248

Hernández-Hernández, T., Miller, E. C., Román-Palacios, C., & Wiens, J. J. (2021). Speciation across the Tree of Life. *Biological Reviews*, *96*(4), 1205–1242. https://doi.org/10.1111/brv.12698

Honnay, O., Verhaeghe, W., & Hermy, M. (2001). Plant Community Assembly Along Dendritic Networks of Small Forest Streams. *Ecology*, *82*(6), 1691–1702. https://doi.org/10.1890/0012-9658(2001)082[1691:PCAADN]2.0.CO;2

Hörandl, E., & Stuessy, T. F. (2010). Paraphyletic groups as natural units of biological classification. *Taxon*, *59*(6), 1641-1653. https://doi.org/10.1002/tax.596001

ITIS.gov (21 November 2021) *Integrated Taxonomic Information System (ITIS) - USGS.GOV*. ITIS.gov. https://doi.org/10.5066/F7KH0KBK

Ivey, C. T., Habecker, N. M., Bergmann, J. P., Ewald, J., Frayer, M. E., & Coughlan, J. M. (2023). Weak reproductive isolation and extensive gene flow between Mimulus glaucescens and M. guttatus in northern California. *Evolution*, 77(5), 1245–1261. https://doi.org/10.1093/evolut/qpad044

Jabis, M. D., Ayers, T. J., & Allan, G. J. (2011). Pollinator-mediated gene flow fosters genetic variability in a narrow alpine endemic, Abronia alpina (Nyctaginaceae). *American Journal of Botany*, *98*(10), 1583–1594. https://doi.org/10.3732/ajb.1000515

Jaeger, S., Girvin, C., Demarest, N., & LoPresti, E. (2023). Secondary pollinators contribute to reproductive success of a pink-flowered sand verbena population. *Ecology*, *104*(4), e3977. https://doi.org/10.1002/ecy.3977

Jansson, R., Zinko, U., Merritt, D. M., & Nilsson, C. (2005). Hydrochory increases riparian plant species richness: A comparison between a free-flowing and a

regulated river. *Journal of Ecology*, *93*(6), 1094–1103. https://doi.org/10.1111/j.1365-2745.2005.01057.x

Jepson, W. L. (1914). *Flora of California* (p. 455). San Francisco, CA: Cunningham, Curtis & Welch.

Jepson Eflora. (2 February 2024). *California flora, Jepson Eflora main page.* UC Berkeley. https://ucjeps.berkeley.edu/eflora/

Jordan, D. S. (1905). The origin of species through isolation. *Science*, *22*(566), 545-562. https://www.jstor.org/stable/1632439

Jordan, D. S. (1908). The law of geminate species. *The American Naturalist*, *42*(494), 73-80. https://doi.org/10.1086/278905

Juárez, P., Vargas, O. M., & Kay, K. M. (2023). Ecological and Evolutionary Origin of Costus flammulus (Costaceae): A New Species from the Montane Cloud Forests of the Volcanic Cordilleras in Northern Costa Rica. *Systematic Botany*, *48*(1), 145–157. https://doi.org/10.1600/036364423X16758877666039

Jussieu, A. L. (1789). *Genera plantarum secundum ordines naturales disposita juxta methodum in horto regio parisiensi exaratam, anno 1774*. veuve Herissant.

Kay, K. M. (2006). Reproductive isolation between two closely related hummingbird pollinated neotropical gingers. *Evolution*, *60*(3), 538-552. https://doi.org/10.1111/j.0014-3820.2006.tb01135.x

Keller, O., Odronitz, F., Stanke, M., Kollmar, M., & Waack, S. (2008). Scipio: using protein sequences to determine the precise exon/intron structures of genes and their orthologs in closely related species. *BMC bioinformatics*, *9*(1), 1-12. https://doi.org/10.1186/1471-2105-9-278

Kisel, Y., & Barraclough, T. G. (2010). Speciation Has a Spatial Scale That Depends on Levels of Gene Flow. *The American Naturalist*, *175*(3), 316–334. https://doi.org/10.1086/650369

Kraft NJB, Baldwin BG, Ackerly DD. Range size, taxon age and hotspots of neoendemism in the California flora. *Divers Distrib*. 2010;16:403–13. http://doi.wiley.com/10.1111/j.1472-4642.2010.00640.x

Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, *30*(14), 3059-3066. https://doi.org/10.1093/nar/gkf436

Lamarck, J. B. D. M. (1791). *Tableau encyclopédique et methodique des trois règnes de la nature: Botanique.* (Vol. 1). chez Panckoucke libraire.

Lancaster, L. T., & Kay, K. M. (2013). Origin and diversification of the California flora: re-examining classic hypotheses with molecular phylogenies. *Evolution: International Journal of Organic Evolution*, 67(4), 1041-1054. https://doi.org/10.1111/evo.12016

Larkin, K., Igler, D. B., & Mitchell, L. J. (2015). The Transformation of the Santa Ana River: A Story of Changing Demographics, Values, and Landscapes. *The UCI undergraduate research journal.*

Leles, B., Chaves, A. V., Russo, P., Batista, J. A. N., & Lovato, M. B. (2015). Genetic Structure Is Associated with Phenotypic Divergence in Floral Traits and Reproductive Investment in a High-Altitude Orchid from the Iron Quadrangle, Southeastern Brazil. *PLOS ONE*, *10*(3), e0120645. https://doi.org/10.1371/journal.pone.0120645

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *bioinformatics*, *25*(14), 1754-1760.

Li, D., Liu, C. M., Luo, R., Sadakane, K., & Lam, T. W. (2015). MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*, *31*(10), 1674-1676. https://doi.org/10.1093/bioinformatics/btv033

Li, J.-L., Zhong, L.-L., Wang, J., Ma, T., Mao, K.-S., & Zhang, L. (2021). Genomic insights into speciation history and local adaptation of an alpine aspen in the Qinghai–Tibet Plateau and adjacent highlands. *Journal of Systematics and Evolution*, *59*(6), 1220–1231. https://doi.org/10.1111/jse.12665

Loarie, S. R., Carter, B. E., Hayhoe, K., McMahon, S., Moe, R., Knight, C. A., & Ackerly, D. D. (2008). Climate Change and the Future of California's Endemic Flora. *PLoS ONE*, *3*(6), e2502. https://doi.org/10.1371/journal.pone.0002502

Ma, Y.-P., Wariss, H. M., Liao, R.-L., Zhang, R.-G., Yun, Q.-Z., Olmstead, R. G., Chau, J. H., Milne, R. I., Van de Peer, Y., & Sun, W.-B. (2021). Genome-wide analysis of butterfly bush (Buddleja alternifolia) in three uplands provides insights into biogeography, demography and speciation. *New Phytologist*, *232*(3), 1463–1476. https://doi.org/10.1111/nph.17637

Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics*, *4*. https://doi.org/10.3389/fgene.2013.00192

Mai, U., & Mirarab, S. (2018). TreeShrink: fast and accurate detection of outlier long branches in collections of phylogenetic trees. *BMC genomics*, *19*(5), 23-40. https://doi.org/10.1186/s12864-018-4620-2

Malay, M. C. M. D., & Paulay, G. (2010). Peripatric speciation drives diversification and distributional pattern of reef hermit crabs (Decapoda: Diogenidae: Calcinus). *Evolution*, *64*(3), 634-662.

Mayr, E. (1942). *Systematics and the origin of species*. Columbia Univ. Press, New York.

Mayr, E. (1959). Isolation as an evolutionary factor. *Proceedings of the American Philosophical Society*, *103*(2), 221-230. https://www.jstor.org/stable/985150

Mayr, E. (1963). *Animal species and evolution*. Harvard Univ. Press, Cambridge, MA.

Mayr, E. (1970). *Populations, species, and evolution*. Cambridge: Harvard Univ. Press.

Memoirs of the Imperial Academy of Sciences of St. Petersburg. (1826). *Mém. Acad. Imp. Sci. St. Pétersbourg Hist. Acad.* (Vol. 10). St. Petersburg, Russia.

Mirarab, S., Reaz, R., Bayzid, M. S., Zimmermann, T., Swenson, M. S., & Warnow, T. (2014). ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics*, 30(17), i541-i548. https://doi.org/10.1093/bioinformatics/btu462

Mishler, B. D., Guralnick, R., Soltis, P. S., Smith, S. A., Soltis, D. E., Barve, N., Allen, J. M., & Laffan, S. W. (2020). Spatial phylogenetics of the North American flora.

Journal of Systematics and Evolution, 58(4), 393–405. https://doi.org/10.1111/jse.12590

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Research, 20(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

Mittermeier, R. A., Turner, W. R., Larsen, F. W., Brooks, T. M., & Gascon, C. (2011). Global Biodiversity Conservation: The Critical Role of Hotspots. In F. E. Zachos & J. C. Habel (Eds.), *Biodiversity Hotspots* (pp. 3–22). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-20992-5_1

Murdock, A. (2012). Abronia. In Jepson Flora Project (Eds.), *Jepson eFlora*. Retrieved from https://ucjeps.berkeley.edu/eflora/eflora_display.php?tid=8780 on February 17, 2024.

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular biology and evolution*, *32*(1), 268-274. https://doi.org/10.1093/molbev/msu300

Nosratinia, S. (2013). Evolutionary relationships in sand verbenas: insights from DNA sequence data (Unpublished master's thesis). San Francisco State University, San Francisco, California.

Nürk, N. M., Michling, F., & Linder, H. P. (2018). Are the radiations of temperate lineages in tropical alpine ecosystems pre-adapted? *Global Ecology and Biogeography*, *27*(3), 334–345. https://doi.org/10.1111/geb.12699

Otero, A., Vargas, P., Fernández-Mazuecos, M., Jiménez-Mejías, P., Valcárcel, V., Villa-Machío, I., & Hipp, A. L. (2022). A snapshot of progenitor–derivative speciation in Iberodes (Boraginaceae). *Molecular Ecology*, 31(11), 3192–3209. https://doi.org/10.1111/mec.16459

Ortiz, E. M., Hoewener, A., Shigita, G., Raza, M., Maurin, O., Zuntini, A., ... & Schaefer, H. (2023). A novel phylogenomics pipeline reveals complex pattern of reticulate evolution in Cucurbitales. *bioRxiv*, 2023-10. https://doi.org/10.1101/2023.10.27.564367

Parins-Fukuchi, C. (2021). Morphological and phylogeographic evidence for budding speciation: An example in hominins. *Biology Letters*, *17*(1), 20200754. https://doi.org/10.1098/rsbl.2020.0754

"Picard Toolkit." (2019). Broad Institute. Retrieved from https://broadinstitute.github.io/picard/

Pina-Martins, F., Silva, D. N., Fino, J., & Paulo, O. S. (2017). Structure_threader: an improved method for automation and parallelization of programs structure, fastStructure and MavericK on multicore CPU systems. *Molecular ecology resources*, *17*(6), e268-e274. https://doi.org/10.1111/1755-0998.12702

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., ... & Sham, P. C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American journal of human genetics*, *81*(3), 559-575. https://doi.org/10.1086/519795

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/

Raj, A., Stephens, M., & Pritchard, J. K. (2014). fastSTRUCTURE: variational inference of population structure in large SNP data sets. *Genetics*, *197*(2), 573-589. https://doi.org/10.1534/genetics.114.164350

Raven, P. H., & Axelrod, D. I. (1978). Origin and relationships of the California flora (Vol. 72). *Univ of California Press*.

Rieseberg, L. H., & Brouillet, L. (1994). Are many plant species paraphyletic?. *Taxon*, *43*(1), 21-32. https://doi.org/10.2307/1223457

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology letters*, *8*(3), 336-352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Saunders, N. E., & Sipes, S. D. (2006). Reproductive biology and pollination ecology of the rare Yellowstone Park endemic Abronia ammophila (Nyctaginaceae). *Plant Species Biology*, *21*(2), 75–84. https://doi.org/10.1111/j.1442-1984.2006.00153.x

Savolainen, V., Anstett, M.-C., Lexer, C., Hutton, I., Clarkson, J. J., Norup, M. V., Powell, M. P., Springate, D., Salamin, N., & Baker, W. J. (2006). Sympatric speciation in palms on an oceanic island. *Nature*, *441*(7090), 210–213. https://doi.org/10.1038/nature04566

Schmitt, J. (1980). Pollinator Foraging Behavior and Gene Dispersal in Senecio (Compositae). *Evolution*, *34*(5), 934–943. https://doi.org/10.2307/2407999

Schluter, D. (2009). Evidence for ecological speciation and its alternative. *Science*, *323*(5915), 737-741. DOI: 10.1126/science.1160006

Schwartz, M. W., Thorne, J. H., & Viers, J. H. (2006). Biotic homogenization of the California flora in urban and urbanizing regions. *Biological Conservation*, *127*(3), 282–291. https://doi.org/10.1016/j.biocon.2005.05.017

Sianta, S. A., & Kay, K. M. (2022). Phylogenomic analysis does not support a classic but controversial hypothesis of progenitor-derivative origins for the serpentine endemic *Clarkia franciscana*. *Evolution*, *76*(6), 1246–1259. https://doi.org/10.1111/evo.14484

Smith, S. A., Brown, J. W., & Walker, J. F. (2018). So many genes, so little time: a practical approach to divergence-time estimation in the genomic era. *PloS one*, *13*(5), e0197433. https://doi.org/10.1371/journal.pone.0197433

Soulé, M. E., Alberts, A. C., & Bolger, D. T. (1992). The Effects of Habitat Fragmentation on Chaparral Plants and Vertebrates. *Oikos*, *63*(1), 39–47. https://doi.org/10.2307/3545514

Spellenberg, R., & Poole, J. M. (2003). Nomenclatural adjustments and comments in Abronia and Acleisanthes (Nyctaginaceae). *SIDA, Contributions to Botany*, 885-889. https://www.jstor.org/stable/41968118

Spotila, J. A., Mason, C. C., Valentino, J. D., & Cochran, W. J. (2020). Constraints on rock uplift in the eastern Transverse Ranges and northern Peninsular Ranges and

implications for kinematics of the San Andreas fault in the Coachella Valley, California, USA. *Geosphere*, *16*(3), 723–750. https://doi.org/10.1130/GES02160.1

Stebbins Jr, G. L. (1952). Aridity as a stimulus to plant evolution. *The American Naturalist*, 86(826), 33-44. https://doi.org/10.1086/281699

Stebbins, G. L., & Major, J. (1965). Endemism and speciation in the California flora. *Ecological Monographs*, *35*(1), 2-35. https://doi.org/10.2307/1942216

Steenwyk, J. L., Buida III, T. J., Li, Y., Shen, X. X., & Rokas, A. (2020). ClipKIT: a multiple sequence alignment trimming software for accurate phylogenomic inference. *PLoS biology*, *18*(12), e3001007. https://doi.org/10.1371/journal.pbio.3001007

Thorne, J. H., Viers, J. H., Price, J., & Stoms, D. M. (2009). Spatial patterns of endemic plants in California. *Natural Areas Journal*, 29(4), 344-366. https://doi.org/10.3375/043.029.0402

Thornhill, A. H., Baldwin, B. G., Freyman, W. A., Nosratinia, S., Kling, M. M., Morueta-Holme, N., ... & Mishler, B. D. (2017). Spatial phylogenetics of the native California flora. *BMC biology*, 15, 1-18. https://doi.org/10.1186/s12915-017-0435-x

Tillett, S. S. (1967). The Maritime Species of Abronia (Nyctaginaceae). *Brittonia*, *19*(4), 299. https://doi.org/10.2307/2805531

Van Natto, A. C. (2020). *Effects of Peripherality and Hybridization on Range-Wide Genetic Structure of the Endangered Coastal Dune Plant Abronia umbellata (Nyctaginaceae)* (Doctoral dissertation, Queen's University (Canada)).

Van Natto, A. C., & Eckert, C. G. (2022). Genetic and conservation significance of populations at the polar vs. Equatorial range limits of the Pacific coastal dune endemic Abronia umbellata (Nyctaginaceae). *Conservation Genetics*, *23*(2), 255–269. https://doi.org/10.1007/s10592-021-01409-3

Vargas, O. M., Goldston, B., Grossenbacher, D. L., & Kay, K. M. (2020). Patterns of speciation are similar across mountainous and lowland regions for a Neotropical

plant radiation (Costaceae: Costus). *Evolution*, *74*(12), 2644-2661. https://doi.org/10.1111/evo.14108

Vargas, O. M., Madriñán, S., & Simpson, B. (2023). Allopatric speciation is more prevalent than parapatric ecological divergence in a recent high-Andean diversification (Linochilus: Asteraceae). *PeerJ*, *11*, e15479. https://doi.org/10.7717/peerj.15479

Wen, J., Zhang, J., Nie, Z.-L., Zhong, Y., & Sun, H. (2014). Evolutionary diversifications of plants on the Qinghai-Tibetan Plateau. *Frontiers in Genetics*, *5*. https://doi.org/10.3389/fgene.2014.00004

Wheeler, G. M., & Watson, S. (1874). *Catalogue of plants collected in the years 1871, 1872, and 1873, with description of new species*. Government Printing Office. https://doi.org/10.3133/70042883

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D. A., François, R., ... & Yutani, H. (2019). Welcome to the Tidyverse. *Journal of open source software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Williamson, P. S., Muliani, L., & Janssen, G. K. (1994). Pollination Biology of Abronia macrocarpa (Nyctaginaceae), An Endangered Texas Species. *The Southwestern Naturalist*, *39*(4), 336–341. https://www.jstor.org/stable/30054368

Williamson, P. S., & Werth, C. R. (1999). Levels and patterns of genetic variation in the endangered species *Abronia macrocarpa* (Nyctaginaceae). *American Journal of Botany*, *86*(2), 293–301. https://doi.org/10.2307/2656946

Wilson, R. (1972). Abronia: I. Distribution, Ecology and Habit of Nine Species of Abronia Found in California. *Aliso*, *7*(4), 421–437. https://doi.org/10.5642/aliso.19720704.07

Wilson, R. (1976). Abronia: IV. Anthocarp Dispersibility and its Ecological Implications for Nine Species of Abronia. *Aliso*, *8*(4), 493–506. https://doi.org/10.5642/aliso.19760804.11

Wu, S., Wang, Y., Wang, Z., Shrestha, N., & Liu, J. (2022). Species divergence with gene flow and hybrid speciation on the Qinghai–Tibet Plateau. *New Phytologist*, *234*(2), 392–404. https://doi.org/10.1111/nph.17956

APPENDICES

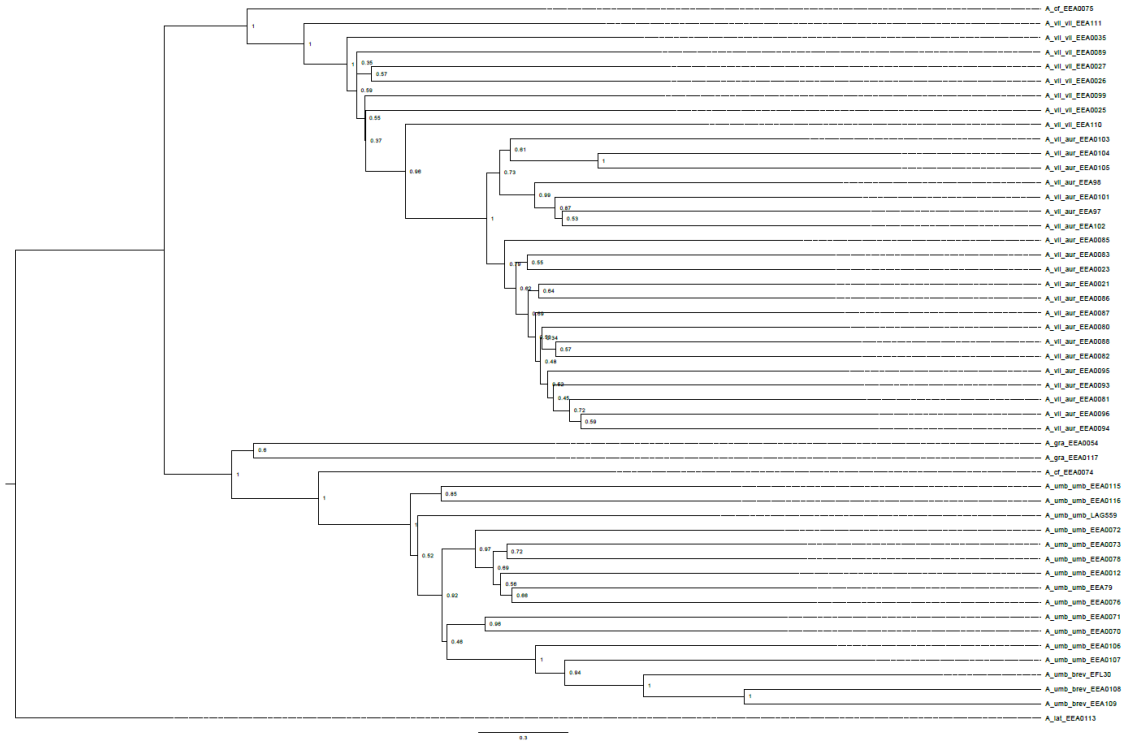
Appendix A: Table of samples extracted for target sequencing, including two donated sets of raw reads extracted by the lab of Matt Johsnon (Eric F. Lopresti s.n, Leo A. Galloway 559). HSC: Cal Poly Humboldt Vascular Plant Herbarium, SD: SD Herbarium San Diego Natural History Museum, TTC: Texas Tech University, E. L. Reed Herbarium.

Species	**Voucher**	**Collection**	**iNaturalist**	**Code**
Abronia gracilis Benth.	Rebman 18418	SD	-	EEA-0117
Abronia gracilis Benth.	Riley 230	SD	-	EEA-0054
Abronia latifolia Eschsch.	Eli Allen 55	HSC (sample only)	-	EEA-0113
Abronia umbellata var. *breviflora* (Standl.) L.A.Galloway	Eli Allen 51	HSC	123637204	EEA-0108
Abronia umbellata var. *breviflora* (Standl.) L.A.Galloway	Eli Allen 53	HSC	199763393	EEA-0109
Abronia umbellata var. *breviflora* (Standl.) L.A.Galloway	Eric F. Lopresti 30	OC	-	EFL0030
Abronia umbellata var. *umbellata* Lam.	Eli Allen 2	HSC	118615246	EEA-0070
Abronia umbellata var. *umbellata* Lam.	Eli Allen 3	HSC	118615247	EEA-0071
Abronia umbellata var. *umbellata* Lam.	Eli Allen 5	HSC	118883977	EEA-0072
Abronia umbellata var. *umbellata* Lam.	Eli Allen 6	HSC	118883978	EEA-0073
Abronia umbellata var. *umbellata* Lam.	Eli Allen 9	HSC	118883982	EEA-0076
Abronia umbellata var. *umbellata* Lam.	Eli Allen 10	HSC (sample only)	119022374	EEA-0012
Abronia umbellata var. *umbellata* Lam.	Eli Allen 11	HSC	118883983	EEA-0078
Abronia umbellata var. *umbellata* Lam.	Eli Allen 12	HSC	118883986	EEA-0079

Species	Voucher	Collection	iNaturalist	Code
Abronia umbellata var. *umbellata* Lam.	Eli Allen 49	HSC	123633008	EEA-0106
Abronia umbellata var. *umbellata* Lam.	Eli Allen 50	HSC	123633010	EEA-0107
Abronia umbellata var. *umbellata* Lam.	Gregory 3448	SD	-	EEA-0116
Abronia umbellata var. *umbellata* Lam.	Leo A. Galloway 559	TTC	-	LAG-0559
Abronia umbellata var. *umbellata* Lam.	Rebman 19264	SD	-	EEA-0115
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 13	HSC	118883987	EEA-0080
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 14	HSC	118883989	EEA-0081
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 15	HSC	118883992	EEA-0082
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 16	HSC	118881959	EEA-0083
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 17	HSC	118881965	EEA-0023
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 18	HSC	118883993	EEA-0085
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 19	HSC	119019952	EEA-0086
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 21	HSC	119019954	EEA-0087
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 24	HSC	119019958	EEA-0088
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 29	HSC	122068884	EEA-0093
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 30	HSC	119633892	EEA-0094
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 31	HSC	119633893	EEA-0095
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 32	HSC (sample only)	119633905	EEA-0096
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 33	HSC	119633917	EEA-0097

Species	Voucher	Collection	iNaturalist	Code
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 34	HSC	119633925	EEA-0098
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 41	HSC	120610615	EEA-0101
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 42	HSC	120610610	EEA-0102
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 45	HSC	122066401	EEA-0103
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 46	HSC	122066404	EEA-0104
Abronia villosa var. *aurita* (Abrams) Jeps.	Eli Allen 47	HSC	122066407	EEA-0105
Abronia villosa var. *villosa* S. Watson	Eli Allen 25	HSC	119017579	EEA-0089
Abronia villosa var. *villosa* S. Watson	Eli Allen 26	HSC	119017581	EEA-0025
Abronia villosa var. *villosa* S. Watson	Eli Allen 27	HSC	119017587	EEA-0026
Abronia villosa var. *villosa* S. Watson	Eli Allen 28	HSC	119017589	EEA-0027
Abronia villosa var. *villosa* S. Watson	Eli Allen 37	HSC	120610585	EEA-0099
Abronia villosa var. *villosa* S. Watson	Eli Allen 38	HSC	120610586	EEA-0035
Abronia villosa var. *villosa* S. Watson	Sierra Jaeger s.n.	Grown from collected seed	-	EEA-0110
Abronia villosa var. *villosa* S. Watson	Sierra Jaeger s.n.	Grown from collected seed	-	EEA-0111
Abronia villosa var. *aurita* (Abrams) Jeps. × *umbellata* var. *umbellata* Lam.	Eli Allen 7	HSC	118883979	EEA-0074
Abronia villosa var. *aurita* (Abrams) Jeps. × *umbellata* var. *umbellata* Lam.	Eli Allen 8	HSC	118883981	EEA-0075

Appendix B: ASTRAL coalescent-consistent tree, values indicate posterior probability. Samples are each labeled with a unique taxon code.



Appendix C All other values of K, besides the best K (K = 3), of all samples excluding *Abronia gracilis*. Bar colors indicate ancestral clustering of individual samples inferred by FASTSTRUCTURE from 559 SNP variants. Samples are each labeled with a unique taxon code.