High-throughput sequencing reveals a complicated evolutionary history between endangered *Dudleya verityi*, (Crassulaceae) and a widespread congener, *D. lanceolata*

Final Report

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High-throughput sequencing reveals a complicated evolutionary history between endangered *Dudleya verityi*, (Crassulaceae) and a widespread congener, *D. lanceolata*

Final Report

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Abstract Genomic analyses can provide critical information to guide on-the-ground conservation of threatened and endangered species. In this study, a large high-throughput SNP dataset was used to assess the potential interactions between the rare *Dudleya verityi* (Crassulaceae) and its widespread congener, *D. lanceolata*. This study demonstrates considerable genetic differentiation among the sampled populations of *D. verityi*, which we attribute to limited gene flow between the isolated outcrops of Conejo Volcanic rock on which it occurs. Broader analyses of *D. verityi* and *D. lanceolata* support the genetic distinctiveness of *D. verityi*, but reveal a complicated evolutionary history in *D. lanceolata*, which is non-monophyletic in our analyses. A set of analyses focusing on one sampling location, Malibu Ridge, showed that morphological and ecological intermediates between *D. verityi* and *D. lanceolata* were likely the result of hybridization. Finally, a rooted phylogenetic analysis with additional outgroup taxa shed light on earlier analyses that suggested a close relationship between *D. verityi* and Santa Monica Mountains *D. lanceolata*. Collectively these analyses drive our understanding of *D. verityi* forward, answering some questions and revealing others.

Introduction

"*Dudleya* is notoriously difficult taxonomically" - Uhl and Moran (1953)

Dudleya Britton & Rose (Crassulaceae) is a diverse, western North American genus of ca. 46 species and 68 taxa (inclusive of species, subspecies, and varieties) of succulent perennials. Up to an additional 8 new species identified on the basis of morphology are awaiting formal description (McCabe, personal communication). Taxa range from diminutive geophytes with annual above-ground parts to robust, caudex-bearing rosette plants. The genus is centered in coastal southern California and adjacent Baja California, Mexico, although taxa occur from southwestern Oregon to the tip of the Baja California Peninsula, and east to Arizona and northwestern mainland Mexico (Figure 1). In these regions, plants most commonly grow on rock outcrops and other sparsely vegetated habitats, but also occur in sage scrub, coastal bluff communities, and are dominant members of the maritime succulent scrub in northwestern Baja California.

As is typical of diverse genera of the California Floristic Province (Howell, 1957; Burge et al. 2016), many *Dudleya* taxa are narrowly-distributed and 27 are given the highest rarity ranking (List 1B) by the California Native Plant Society (CNPS 2020); 9 of these CNPS listed taxa are also listed as endangered or threatened under the federal Endangered Species Act (FESA; United States Fish and Wildlife Service [USFWS] 1978, 1995, 1997a, b, 1998) and 5 taxa are listed as endangered, threatened, or rare under the California Endangered Species Act (CESA; California Department of Fish and Wildlife [CDFW], in litt. 2017; Table 1).

Dudleya has a suite of natural history features that have complicated its taxonomic study. First, although in aggregate the genus is morphologically diverse with respect to plant habit, caudex size and branching, leaf shape and size, inflorescence architecture, and features of the flower, nearly all of these characters vary continuously. In practice, most members of the genus have been circumscribed using combinations of these continuous characters, and in pairwise comparisons of taxa, individual characters often overlap broadly. The paucity of readily observable, discrete characters has led some experts to describe the genus as having "differentiating or well-marked vegetative and floral characters...largely absent (Jepson, 1936)."

Study of the genus is also affected by the drying process, which can greatly alter the appearance of pressed specimens of *Dudleya*. Reid Moran, a taxonomic expert in *Dudleya* and Crassulaceae in general notes that:

> "One difficulty is a common one in dealing with succulents: flattened and dried dudleyas have little character or personality left, so make very unrevealing specimens, whose interpretation is often hard and sometimes subjective. Except for the herbarium specimens, nothing here is cut and dried (Moran 1995)."

In many succulent plant groups, the shape and size of leaves and flower parts are maintained in large part by turgor pressure. As these often highly three-dimensional structures dry, they can change shape and size dramatically. In *Dudleya*, many taxonomically important characters are essentially lost during plant drying. For *Dudleya* leaves, some characters lost or modified in drying include overall leaf shape, leaf size, leaf abaxial cross-sectional shape, leaf adaxial cross-sectional shape, and leaf longitudinal posture. For *Dudleya* flowers, some characters lost in drying include overall sepal shape, sepal size, petal color, overall petal shape, petal size, and petal orientation and longitudinal posture. Data pertaining to these characters are seldom recorded on specimen labels. As a result, specimens are of limited or reduced utility in later studies of plant morphology (Moran 1951a, Moran 1995).

Taxonomic study of *Dudleya* is made more difficult by weak or absent barriers to gene flow among the species and polyploidy (Moran 1951a; Uhl and Moran 1953; Yost et al. 2013). Botanists have long noted the apparent broad interfertility of *Dudleya* taxa (Moran 1949, 1951a, 1951b, Moran and Uhl 1952, Uhl and Moran 1953, Nakai 1983, McCabe unpublished data), with putative hybrids naturally occurring in the field between a number of pairwise combinations of taxa. Although reportedly uncommon in nature (Uhl and Moran 1953), Moran (1949) made the first

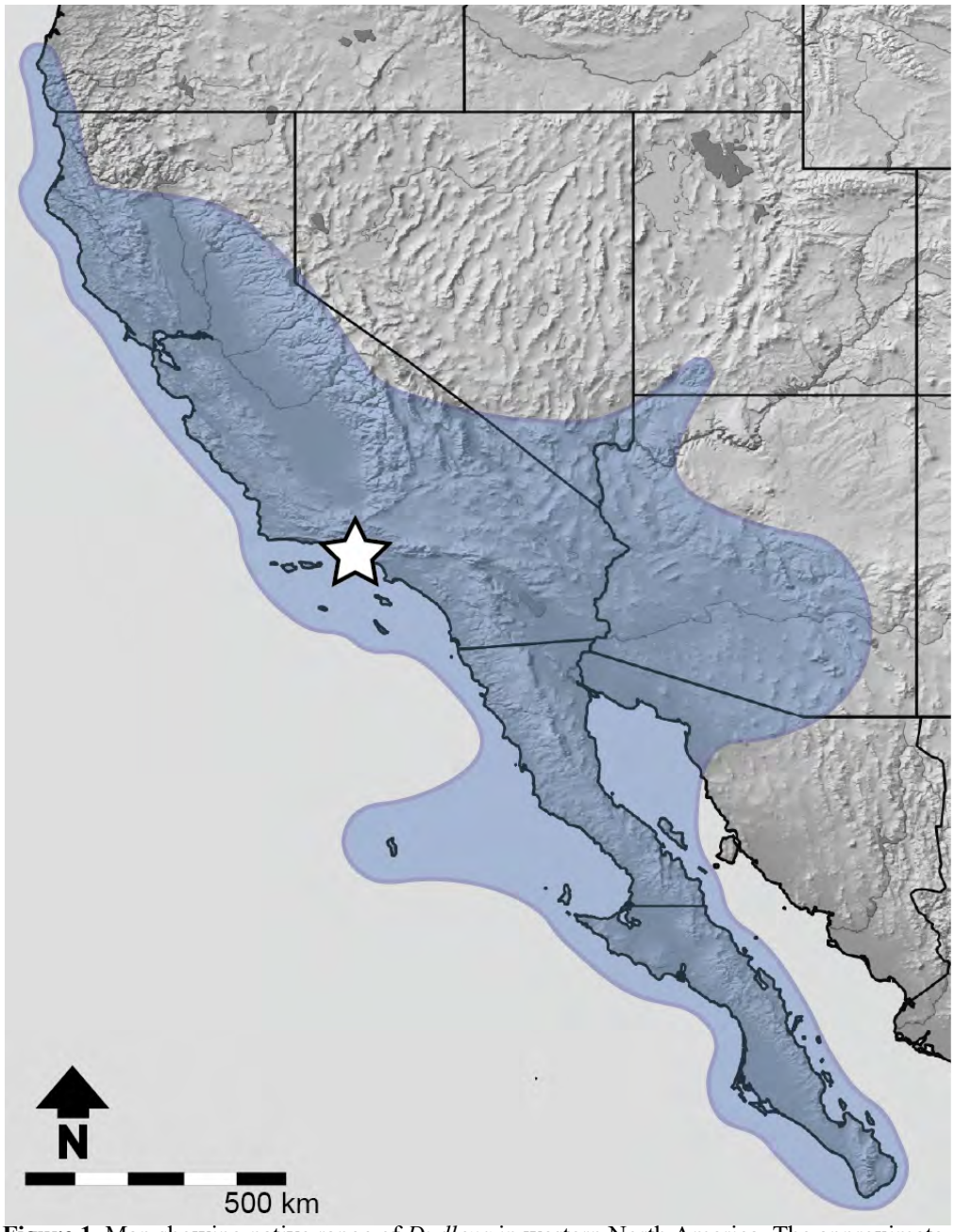

Figure 1. Map showing native range of *Dudleya* in western North America. The approximate location of *D. verityi* is indicated by a star.

Table 1. Federal and state listed *Dudleya* taxa.

Scientific Name	Common Name	CNPS	CESA	FESA
Dudleya abramsii Rose ssp. *setchellii* (Jeps.) Moran	Santa Clara Valley dudleya	1B.1	–	FE
Dudleya brevifolia (Moran) Moran	short-leaved dudleya	1B.1	CE	–
Dudleya cymosa (Lem.) Britton & Rose ssp. *agourensis* K.M. Nakai	Agoura Hills dudleya	1B.2	–	FT
Dudleya cymosa ssp. *marcescens* Moran	marcescent dudleya	1B.2	CR	FT
Dudleya cymosa ssp. *ovatifolia* (Britton) Moran	Santa Monica dudleya	1B.1	–	FT
Dudleya nesiotica (Moran) Moran	Santa Cruz Island dudleya	1B.1	CR	FT
Dudleya parva Rose & Davidson	Conejo dudleya	1B.2	–	FT
Dudleya stolonifera Moran	Laguna Beach dudleya	1B.1	CT	FT
Dudleya traskiae (Rose) Moran	Santa Barbara Island dudleya	1B.2	CE	FE
Dudleya verityi K.M. Nakai	Verity's dudleya	1B.1	–	FT

formal description of a naturally occurring hybrid in the genus, this between *D. edulis* (Nutt.) Moran and *D. stolonifera* Moran. He later (1951b) described three other naturally occurring hybrid combinations, these between: *D. attenuata* (S. Watson) Moran subsp. *orcuttii* (Rose) Moran and *D. variegata* (S. Watson) Moran (therein referred to as *Hasseanthus variegatus* (S. Watson) Rose); *D. edulis* and *D. brevifolia* (Moran) Moran (therein referred to as *H. blochmaniae* (Eastw.) Rose subsp. *brevifolius* Moran); and *D. edulis* and *D. blochmaniae* subsp. *blochmaniae* (therein referred to as *H. blochmaniae* subsp. *blochmaniae*). Moran and Uhl (1952) later described five more natural hybrids between *Dudleya* taxa, including between: *D. anthonyi* Rose and *D. cultrata* Rose; *D. attenuata* subsp. *orcuttii* and *D. brittonii* D.A. Johans. (one putative parental pair involved with the recurrent formation of *D.*×*semiteres*); *D. attenuata* subsp. *orcuttii* and *D. candida* Britton (the other putative parental pair involved with the recurrent formation of *D.*×*semiteres*); *D. brittonii* and *D. formosa* Moran; and *D. attenuata* subsp. *orcuttii* and *D. formosa*. Nakai (1983) described hybrids between *D. blochmaniae* (Eastw.) Moran and *D. verityi* K. Nakai; this hybrid combination has recently been seen at several locations (Guilliams and Hasenstab-Lehman, unpublished data).

Historical hybridization with or without subsequent allopolyploidization has also been suggested to play a key role in the formation of a number of species-rank *Dudleya* taxa. Endemic to Fraser Point on Santa Cruz Island, *D. nesiotica* (Moran) Moran is a tetraploid (n=34) thought to be derived from an ancient hybridization event between

ancestors of diploid (*n*=17) *D. blochmaniae* and ancestors of a diploid member of subgenus *Dudleya*, followed by polyploidization (Moran 1951a). Endemic to Santa Barbara Island, tetraploid (*n*=34) *D. traskiae* (Rose) Moran has been suggested to have arisen through an inter-subgeneric hybridization event between members of subgenus *Dudleya* and subgenus *Stylophyllum*, followed by polyploidization (Moran 1951a). Nakai (1983) suggested that the unique combination of features found in diploid (n=17) *D. verityi* may point to historical hybridization between an ancestor of diploid (n=17) *D. caespitosa* (Haw.) Britton & Rose and an ancestor of diploid (n=17) *D. cymosa* (Lem.) Britton & Rose subsp. *ovatifolia* (Britton) Moran, both of which are presently known from the Santa Monica Mountains region where *D. verityi* occurs.

Experimental work in the greenhouse has also shed light on the role of hybridization in *Dudleya*. Both Verity (Nakai 1983) and McCabe (unpublished data) have made experimental crosses in the greenhouse between many pairwise combinations of taxa, including many that represent inter-subgeneric crosses. Most crosses have been successful, with no apparent loss of fertility in F1 hybrid offspring. This is consistent with the cytological findings of Uhl and Moran (1953), who note that meiosis appears to progress normally even in high polyploidy and hybrid plants. In at least one case, deliberate greenhouse crosses resulted in F1 plants consistent with a recognized, naturally-occurring form in nature (McCabe unpublished data), yielding a potential insight into the hybrid parentage of the natural form. Weak barriers to gene flow have also been noted in closely related western North American *Sedum* taxa (Zika et al. 2018).

There are many potential consequences of lack of barriers to gene flow in *Dudleya*. Hybridization between two taxa could yield a morphologically distinctive population. For taxonomists encountering these plants, it may not be clear whether the distinctive population is the result of long-term evolutionary processes such as isolation and genetic drift or selection and local adaptation, on the one hand, or the chance, relatively transient encounter between two recognized *Dudleya* taxa on the other. The former should be recognized taxonomically. While opinions might vary, most taxonomists would not choose to recognize populations of recent hybrid origin unless barriers to backcrossing -- such as ploidy differences in the case of allopolyploid hybridization -- are thought to exist. This effect of weak barriers to gene flow is expected to be greatest near geographic range edges or local ecotones where otherwise allopatric taxa may overlap spatially. Hybrid swarms in *Dudleya* are typically diagnosed through morphological intermediacy and proximity to putative parent taxa.

Weak barriers to gene flow could have potentially serious consequences for rare *Dudleya* taxa (Levin et al. 1996; Rhymer and Simberloff 1996; Soltis and Gitzendanner 1999; Balao et al. 2015). The CDFW estimates that 90 percent of California's rare plants co-occur with a more widespread congener (CDFW 1989), a statistic that likely includes a number of rare-common pairs in *Dudleya*. Potential outcomes of gene flow between rare and common *Dudleya* taxa range from local hybridization, with or without introgression, to population extirpation or taxon extinction. Local hybridization *without* introgression between a widespread, common *Dudleya* taxon and a narrowly distributed, rare *Dudleya* taxon could result in the formation of hybrid plants that directly complete with the rare taxon for resources (e.g., space, light, pollinators; Levin et al. 1996); the waste of pure pollen or ovules of the rare plant in the production of hybrid seed (Levin et al. 1996; Burgess et al. 2008); and the increase in herbivore or pathogen pressure when hybrids are less resistant (Levin et al. 1996). All of these negative effects on fitness are amplified to the detriment of the rare plant when the common plant is present in larger numbers, which is likely as rare plants typically occur in populations of relatively few individuals (Ellstrand 1991; Rieseberg 1991; Levin et al. 1996; Fant et al. 2010; Beatty et al. 2015; Barmentlo et al. 2018).

Hybridization *with* introgression occurs when alleles are passed between the taxa through backcrossing with the recently formed hybrids. Considering the general case of gene flow between a local and non-local populations of the same species, newly introduced alleles from a non-local population could result in outbreeding depression, defined here as the reduction in fitness observed in plants that result from the cross between genetically dissimilar populations or taxa (Ellstrand 1991; Waser and Price 1994; Hufford and Mazer 2003). Outbreeding depression happens due to the breakup of coadapted gene complexes (e.g., genes with epistatic effects) or disruption of locally adapted parental genotypes (Waser and Price 1994; Hufford and Mazer 2003). If such gene flow occurs between

species rather than populations of one species, as is possible in *Dudleya* through introgressive hybridization, outbreeding depression could result in meaningful reductions in fitness, the effects of which could be amplified by small population sizes in a rare *Dudleya* taxon. Outbreeding depression has been studied and detected in numerous plant groups (e.g., Ellstrand 1991; Waser and Price 1994; Fenster and Galloway 2000; Montalvo and Ellstrand 2001; Crémieux et al. 2010; Goto et al. 2011; Barmentlo et al. 2018). Of course, it should be noted that many would not consider plants that have incorporated non-conspecific alleles through introgressive hybridization as belonging to either the local or non-local taxon, even if they may harbor important and potentially unique local genetic diversity (Rieseberg 1991). By this perspective, establishment of genetically admixed plants through introgressive hybridization replaces potential reproduction and establishment of genetically intact plants of the rare species, an event that effectively removes individuals from the rare plant population. In summary, the long-term effects of hybridization, with or without introgression, could include a range of outcomes, from relatively localized direct competition on the one hand, to a severe reduction of the likelihood of population persistence on the other. These effects can be so severe, that in some cases conservationists and land managers opt to cull individuals of the common plant to prevent hybridization (Rieseberg 1991, Rhymer and Simberloff 1996).

DUDLEYA VERITYI - A RARE SPECIES AT RISK DUE TO POTENTIAL HYBRIDIZATION

Dudleya verityi is a caudex-bearing rosette plant endemic to Ventura County, California, United States. The species is distributed in a narrow, approximately 8 kilometer-long band from Round Mountain in the southwest to Conejo Mountain in the northeast, immediately south of Pleasant Valley and the city of Camarillo (Figure 2). It grows nearly exclusively on cliffs and outcrops of Conejo Volcanic Rock. Eight Element Occurrences (EOs) are listed in the California Natural Diversity Database (California Natural Diversity Database (CNDDB) 2020), although some of these EOs such as Round Mountain may not contain pure *D. verityi* plants. By the CNDDB definition, an EO is a group of plants that is separated from other such groups by 0.25 mi (0.4 km) and not separated by habitat discontinuities. *Dudleya verityi* was listed as threatened under the Federal Endangered Species Act in 1997 and has been given the rare plant ranking 1B.1 by the California Native Plant Society (CNPS, Rare Plant Program 2020).

Plants of *D. verityi* can have only 1 rosette from an unbranched caudex or may have clumps of 2-30 rosettes from moderately branched caudices (Figure 3A-F). Rosettes are usually 2-5 cm wide with 6-10 leaves each (Nakai 1983, McCabe 2012). Leaves are usually gray-glaucous, oblong-lanceolate, 2-5 cm long, 0.4-0.8 cm wide, convex abaxially, and flat to concave adaxially. The peduncle (inflorescence stalk) is usually gray-glaucous, sometimes purple-tinged proximally, erect, 3-15 cm tall, and 0.3-0.6 cm wide. The peduncle usually bears 5-15 cauline leaves (also sometimes called bracts), the largest, proximal-most being 0.8-1 cm long. The inflorescence is 2-3 branched, branches ascending, 2-5 cm long, each simple or sometimes forked. The calyx is 4-5 mm long and 5-7 mm wide, with triangular lobes 3-4 mm long. The corolla is lemon yellow, 8-10 mm long; petals are oblong-lanceolate, midribs can be greenish distally, and apices are usually recurved to 90 degrees or more. Stamens are yellow and about 8 mm long. Carpels are erect in flower, and ascending in fruit.

Hybridization between the rare *D. verityi* and the common *D. lanceolata* (Nutt.) Britton & Rose has been suspected at several of the *D. verityi* EOs (McCabe 2012). *Dudleya lanceolata* is one of the most widespread members of the genus, with a range that extends from southern Santa Cruz County, California, in the north to northwestern Baja California, México, in the south. The species is often found on soil or in rocky areas of gentle to moderate slope (McCabe 2012). Plants of *D. lanceolata* usually have 1-3 basal rosettes that are up to 35 cm wide and usually of 10-25 leaves. Leaves are bright green to green-glaucous, oblong-lanceolate to lanceolate, 5-30 cm long, 1-4 cm wide, usually convex abaxially, and flat to slightly concave adaxially. The peduncle is often pinkish, erect, 15-95 cm tall, and 0.3-1.2 cm wide. The largest, proximal-most cauline leaves are usually 1-3 cm long (Moran, 1951). The inflorescence is 2-3 branched, branches ascending, 2-25 cm long, each simple or forked. The calyx is 4-7 mm long and 5-8 mm wide, with triangular-ovate lobes 3-6 mm long. The corolla is usually orange to red, but can also be yellow, 8-16 mm long; petals are elliptic to oblanceolate, midribs are often white-glaucous, and the apices are usually erect or slightly outcurved. Stamens are yellow. Carpels are erect in flower. *D. lanceolata* is a polyploid, with gametic chromosome counts of n=34 (tetraploid) and 68 (octoploid) (Moran 1951a).

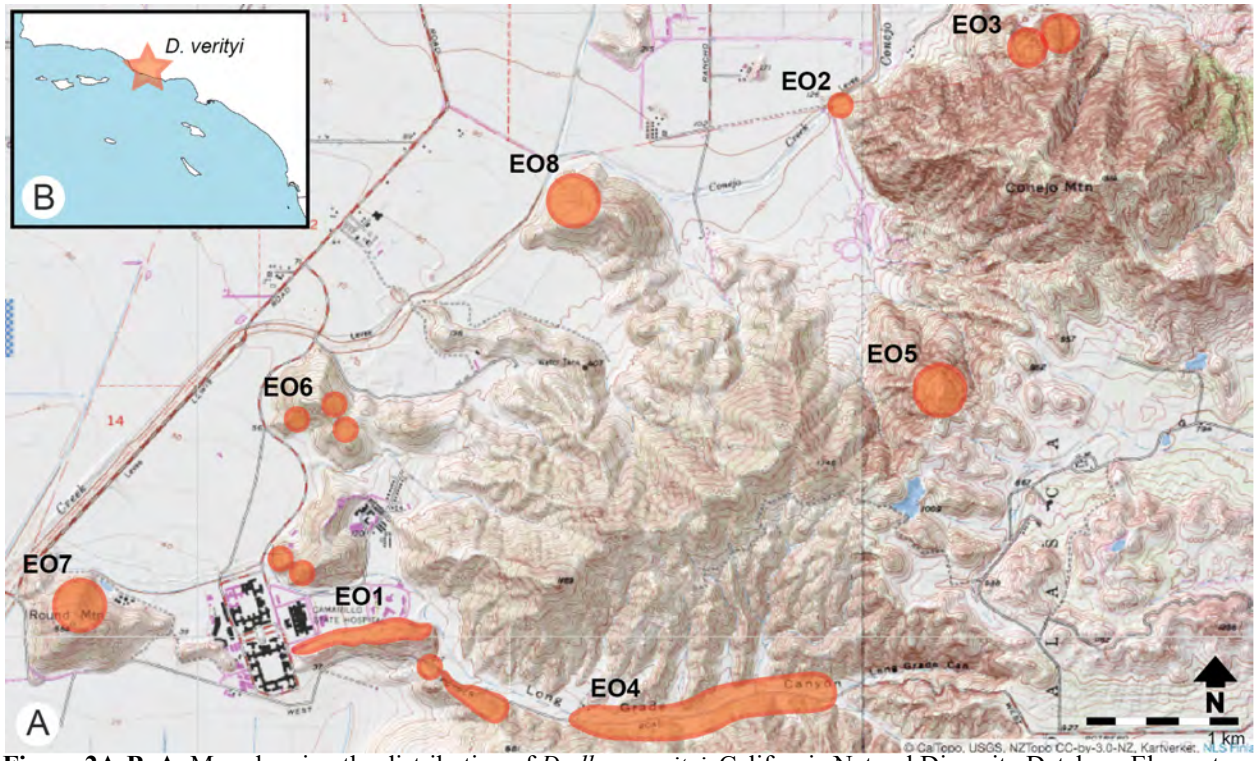

Figure 2A-B. A. Map showing the distribution of *Dudleya verityi.* California Natural Diversity Database Element Occurrence (EO) numbers given for polygon or groups of polygons **B.** Regional map of southern California, with the location of *D. verityi* indicated by a star.

Putative hybrids between *D. verityi* and *D. lanceolata* have been identified in local areas where their ranges overlap on the basis of morphological and ecological intermediacy. The two taxa differ morphologically in several characters, most notably leaf color and length, peduncle length, corolla color, and petal apex posture (Figure 4). The most conspicuous putative hybrids are intermediate for these morphological features. Similarly, putative hybrids occur in areas of ecological intermediacy, at the ecotone between the vertical cliffs and steep rock outcrops of Conejo Volcanic Rock where *D. verityi* occurs, and the adjacent, more gentle earthen slopes where *D. lanceolata* occurs. It is unclear the extent to which this apparent hybridization has impacted or will impact the rare *D. verityi*.

In this study, we gathered a large DNA sequence dataset and subsequently performed a series of related analyses to examine the population genomics of *D. verityi* and to assess the degree of interaction between *D. verityi* and *D. lanceolata*. This study addresses the following questions:

1. To what extent are the *D. verityi* EOs genetically differentiated?
2. Are plants identified as putative hybrids on the basis of morphological and ecological intermediacy naturally occurring hybrids?
3. Is there phylogenetic structure in the widespread taxon *D. lanceolata* and do samples from throughout the range of this taxon form a clade?

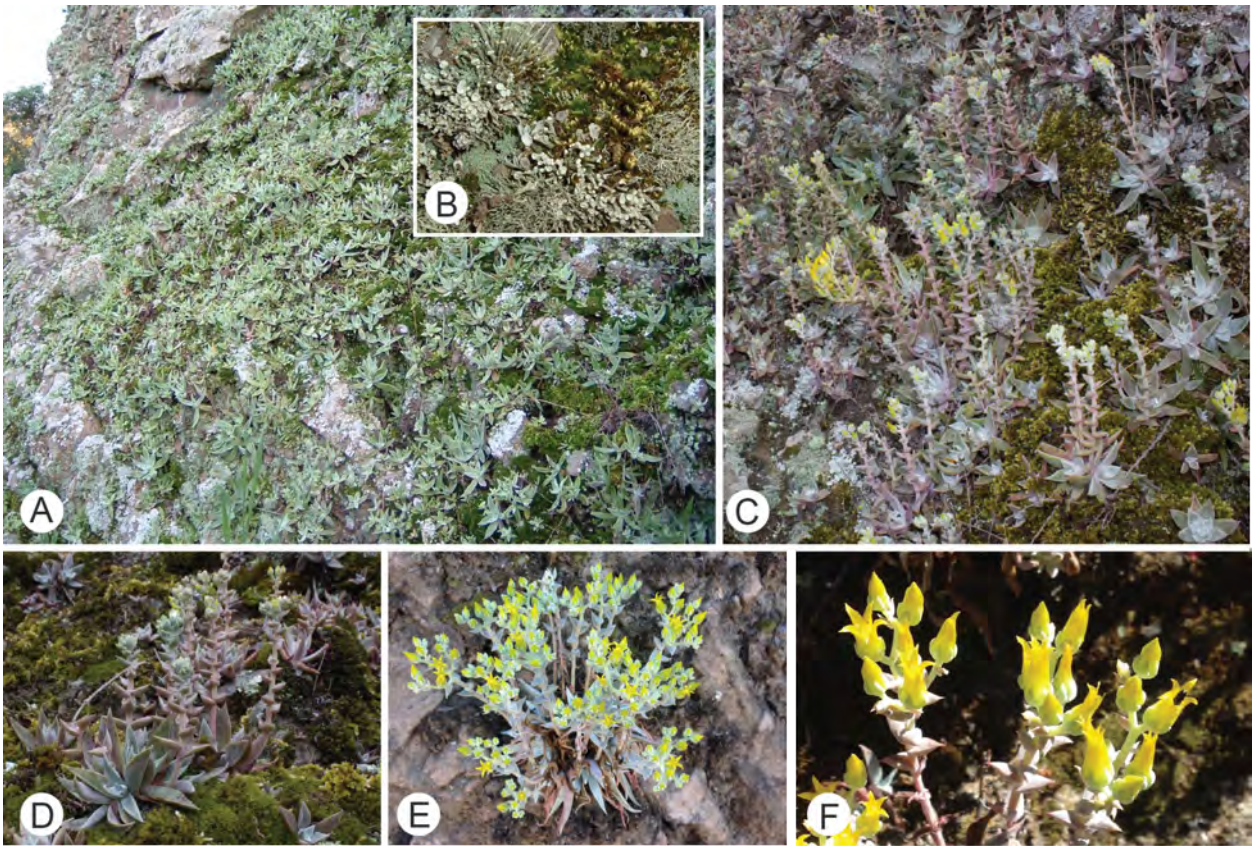

Figure 3A-F. Images of *Dudleya verityi*. **A.** Hundreds of plants growing on steep cliff of Conejo Volcanic rock prior to the 2013 Springs Fire; **B.** Close-up view of lichens and bryophytes that co-occur with *D. verityi*; **C.** Population producing inflorescences; **D.** Plant with multiple rosettes from a branched caudex; **E.** A large plant showing inflorescences; **F.** Flowers.

Materials and Methods

SAMPLING

Sampling for this study focused on *D. verityi*, *D. lanceolata*, and a small number of other taxa in *Dudleya* and *Sedella* that serve as phylogenetic outgroups for certain analyses. Table 2 gives information for all samples included in analyses in this study. The sampling design was limited to three *D. verityi* localities from three different CNDDB EOs, based on accessibility and ownership: Air Field (EO6), Malibu Ridge (EO1), and Treatment Plant (EO2). A total of 25 samples were gathered from each of these localities. To examine if morphological and ecological intermediates between *D. verityi* and *D. lanceolata* represent the products of hybridization between these taxa, an additional 25 samples of putative hybrids were gathered from the Malibu Ridge locality, along with 25 samples of *D. lanceolata* from throughout the Santa Monica Mountains (including from the Malibu Ridge locality). Putative hybrids were selected on the basis of morphological and ecological intermediacy between *D. verityi* and *D. lanceolata*. Morphological intermediates were often intermediate with respect to overall size, leaf glaucescence (waxiness), inflorescence shape, flower morphology, and flower color. Furthermore, morphological intermediates occurred at the ecotone between rock outcrops and the adjacent earthen slopes. We included 25 other samples of *D. lanceolata* from portions of the species' range outside of the Santa Monica Mountains. In total, we included 150 samples of *D. verityi*, *D. lanceolata*, and putative hybrids between the two. As phylogenetic outgroups for certain analyses, we included single exemplars of 10 other *Dudleya* and *Sedella* taxa. As not all samples were retained after filtering, Table 2 is a subset (126 samples) of the idealized sampling for the project.

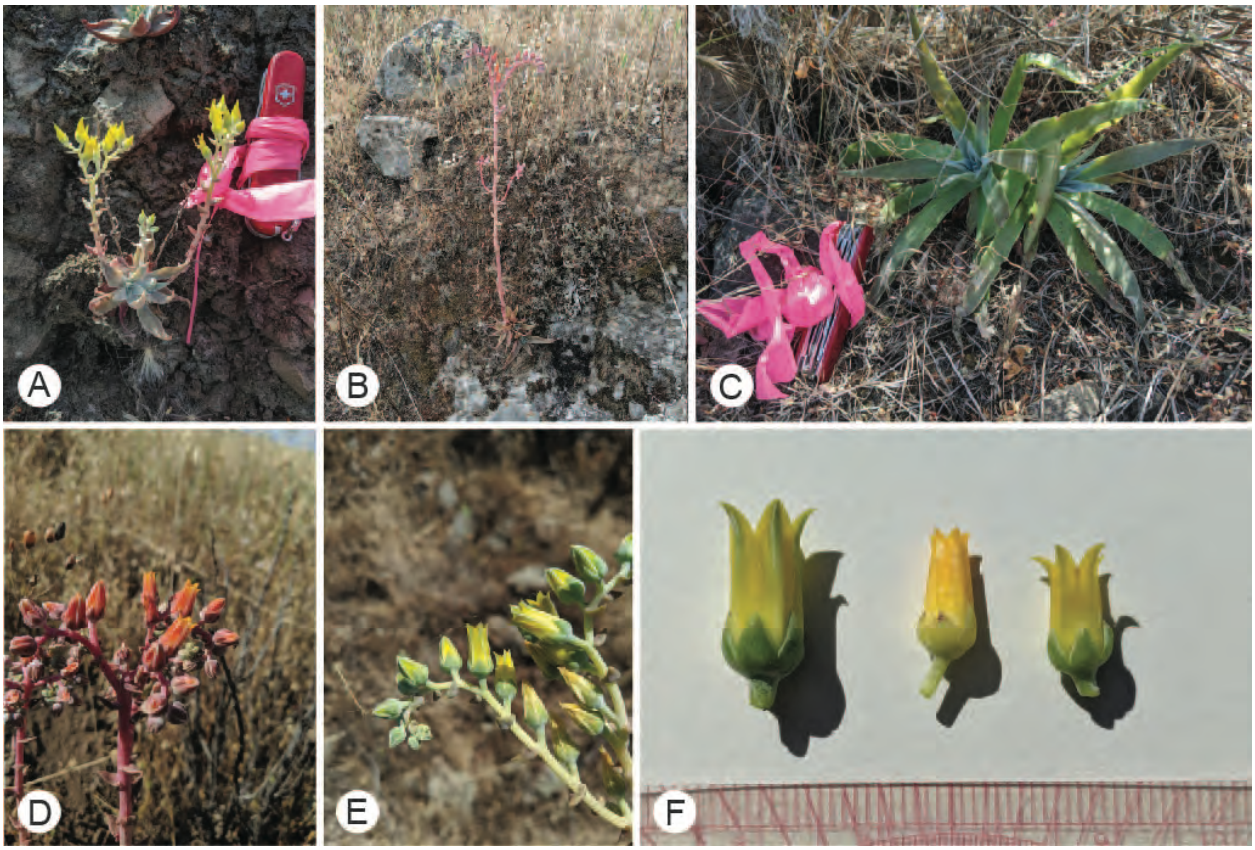

Figure 4A-F. Morphological comparisons between *D. verityi* and *D. lanceolata*. **A.** Habit of *D. verityi*, showing mature rosette and inflorescence, with pocket knife for scale; **B.** Habit of *D. lanceolata*; **C**. Typical rosette of *D. lanceolata*, with pocket knife for scale; **D.** Inflorescence of *D. lanceolata,* orange-red-flowered form; **E.** Inflorescence of *D. lanceolata,* yellow-flowered Santa Monica Mountains form; **F.** Flowers of *D. lanceolata*, yellow-flowered Santa Monica Mountains form (left), *D. cymosa* subsp. *agourensis* K.M. Nakai (middle, not further discussed), and *D. verityi* (right).

Table 2. Project sampling by taxon. Numbers referenced in figures correspond to Santa Barbara Botanic Garden (SBBG) Extraction Numbers (Nos.).

Taxon	SBBG Extraction Nos.	Locality	Collector	Coll No
Dudleya abramsii Rose subsp. *bettinae* (Hoover) Bartel	1987	San Luis Obispo County, California, USA	M. Elvin	9791
Dudleya abramsii Rose subsp. *murina* (Eastw.) Moran	2074	San Luis Obispo County, California, USA	C.M. Guilliams	4805

Dudleya blochmaniae (Eastw.) Moran subsp. *blochmaniae*	1985	Vandenberg Air Force Base, California, USA	S. Khalsa	s.n.
Dudleya brevifolia (Moran) Moran	2909	Torrey Pines State Park, San Diego County, California, USA	C.M. Guilliams	4490B
Dudleya cymosa (Leh.) Britton & Rose subsp. *agourensis* K.M. Nakai	2017	Santa Monica Mountains, California, USA	M. Elvin	9613B
Dudleya cymosa (Leh.) Britton & Rose subsp. *cymosa*	2013	Hidden Valley, Ventura County, California, USA	M. Elvin	9400
Dudleya cymosa (Leh.) Britton & Rose subsp. *paniculata* (Jeps.) K.M. Nakai	2031	King City, Monterey County, California, USA	M. Elvin	9751
Dudleya cymosa (Leh.) Britton & Rose subsp. *pumila* (Rose) K.M. Nakai	2033	Santa Barbara County, California, USA	S. Calloway	1890
Dudleya lanceolata (Nutt.) Britton & Rose	1716	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9276
Dudleya lanceolata (Nutt.) Britton & Rose	1717	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9277
Dudleya lanceolata (Nutt.) Britton & Rose	1718	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9278
Dudleya lanceolata (Nutt.) Britton & Rose	1719	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9279
Dudleya lanceolata (Nutt.) Britton & Rose	1720	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9280

Dudleya lanceolata (Nutt.) Britton & Rose	1721	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9281
Dudleya lanceolata (Nutt.) Britton & Rose	1724	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9284
Dudleya lanceolata (Nutt.) Britton & Rose	1725	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9285
Dudleya lanceolata (Nutt.) Britton & Rose	1726	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9286
Dudleya lanceolata (Nutt.) Britton & Rose	1727	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9287
Dudleya lanceolata (Nutt.) Britton & Rose	1729	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9289
Dudleya lanceolata (Nutt.) Britton & Rose	1730	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9290
Dudleya lanceolata (Nutt.) Britton & Rose	1731	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9301
Dudleya lanceolata (Nutt.) Britton & Rose	1732	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9302
Dudleya lanceolata (Nutt.) Britton & Rose	1733	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9303

Dudleya lanceolata (Nutt.) Britton & Rose	1762	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9229
Dudleya lanceolata (Nutt.) Britton & Rose	1763	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9230
Dudleya lanceolata (Nutt.) Britton & Rose	1800	Round Mountain, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9319
Dudleya lanceolata (Nutt.) Britton & Rose	1854	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9151
Dudleya lanceolata (Nutt.) Britton & Rose	1858	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9155
Dudleya lanceolata (Nutt.) Britton & Rose	1970	Rancho Palos Verdes, California, USA	K. Hasenstab-Lehman	1108
Dudleya lanceolata (Nutt.) Britton & Rose	1971	Orange County, California, USA	C.M. Guilliams	4261
Dudleya lanceolata (Nutt.) Britton & Rose	1972	Santa Barbara County, California, USA	C.M. Guilliams	4524
Dudleya lanceolata (Nutt.) Britton & Rose	1974	Julian, San Diego County, California, USA	S. McCabe	1354
Dudleya lanceolata (Nutt.) Britton & Rose	1975	Thousand Oaks, Ventura County, California, USA	C.M. Guilliams	4504
Dudleya lanceolata (Nutt.) Britton & Rose	1976	Torrey Pines State Park, San Diego County, California, USA	C.M. Guilliams	4492
Dudleya lanceolata (Nutt.) Britton & Rose	1977	Dana Point, Orange County, California, USA	C.M. Guilliams	4259

Dudleya lanceolata (Nutt.) Britton & Rose	1978	San Luis Obispo County, California, USA	Collins	1078
Dudleya lanceolata (Nutt.) Britton & Rose	1979	San Luis Obispo County, California, USA	Collins	102G
Dudleya lanceolata (Nutt.) Britton & Rose	1980	San Luis Obispo County, California, USA	Collins	102E
Dudleya lanceolata (Nutt.) Britton & Rose	1981	Round Mountain, Santa Monica Mountains, Ventura County, California, USA	C.M. Guilliams	4078
Dudleya lanceolata (Nutt.) Britton & Rose	1990	Rancho Palos Verdes, Los Angeles County, California, USA	C.M. Guilliams	3970
Dudleya lanceolata (Nutt.) Britton & Rose	1993	Rattlesnake Canyon, Santa Barbara County, California, USA	K. Hasenstab-Lehman	1300
Dudleya lanceolata (Nutt.) Britton & Rose	1994	Rattlesnake Canyon, Santa Barbara County, California, USA	K. Hasenstab-Lehman	1350
Dudleya lanceolata (Nutt.) Britton & Rose	1996		K. Hasenstab-Lehman	1109
Dudleya lanceolata (Nutt.) Britton & Rose	1997		K. Hasenstab-Lehman	1238
Dudleya lanceolata (Nutt.) Britton & Rose	1998		K. Hasenstab-Lehman	1236
Dudleya lanceolata (Nutt.) Britton & Rose	1999	Moorpark, Santa Monica Mountains, Ventura County, California, USA	C.M. Guilliams	4613
Dudleya parva Rose & Davidson	2069	Moorpark, Ventura County, California, USA	M. Elvin	9745

Dudleya variegata (S. Watson) Moran	2088	Baja California, México	C.M. Guilliams	2743
Dudleya verityi K.M. Nakai	1738	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9205
Dudleya verityi K.M. Nakai	1739	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9206
Dudleya verityi K.M. Nakai	1740	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9207
Dudleya verityi K.M. Nakai	1741	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9208
Dudleya verityi K.M. Nakai	1742	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9209
Dudleya verityi K.M. Nakai	1746	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9213
Dudleya verityi K.M. Nakai	1747	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9214
Dudleya verityi K.M. Nakai	1748	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9215
Dudleya verityi K.M. Nakai	1749	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9216

Dudleya verityi K.M. Nakai	1750	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9217
Dudleya verityi K.M. Nakai	1751	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9218
Dudleya verityi K.M. Nakai	1752	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9219
Dudleya verityi K.M. Nakai	1753	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9220
Dudleya verityi K.M. Nakai	1754	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9221
Dudleya verityi K.M. Nakai	1755	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9222
Dudleya verityi K.M. Nakai	1756	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9223
Dudleya verityi K.M. Nakai	1757	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9224
Dudleya verityi K.M. Nakai	1759	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9226
Dudleya verityi K.M. Nakai	1761	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9227

Dudleya verityi K.M. Nakai	1833	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9101
Dudleya verityi K.M. Nakai	1836	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9104
Dudleya verityi K.M. Nakai	1837	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9105
Dudleya verityi K.M. Nakai	1838	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9106
Dudleya verityi K.M. Nakai	1839	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9107
Dudleya verityi K.M. Nakai	1840	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9108
Dudleya verityi K.M. Nakai	1841	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9109
Dudleya verityi K.M. Nakai	1842	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9110
Dudleya verityi K.M. Nakai	1843	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9111
Dudleya verityi K.M. Nakai	1844	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9112
Dudleya verityi K.M. Nakai	1845	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9113
Dudleya verityi K.M. Nakai	1846	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9114

Dudleya verityi K.M. Nakai	1847	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9115
Dudleya verityi K.M. Nakai	1848	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9116
Dudleya verityi K.M. Nakai	1849	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9117
Dudleya verityi K.M. Nakai	1851	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9118
Dudleya verityi K.M. Nakai	1852	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9119
Dudleya verityi K.M. Nakai	1853	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9120
Dudleya verityi K.M. Nakai	1869	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9131
Dudleya verityi K.M. Nakai	1870	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9132
Dudleya verityi K.M. Nakai	1872	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9134
Dudleya verityi K.M. Nakai	1883	Air Field, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9145
Dudleya verityi K.M. Nakai	1884	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9001
Dudleya verityi K.M. Nakai	1885	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9002

Dudleya verityi K.M. Nakai	1889	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9006
Dudleya verityi K.M. Nakai	1890	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9007
Dudleya verityi K.M. Nakai	1891	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9008
Dudleya verityi K.M. Nakai	1892	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9009
Dudleya verityi K.M. Nakai	1893	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9010
Dudleya verityi K.M. Nakai	1894	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9011
Dudleya verityi K.M. Nakai	1897	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9014
Dudleya verityi K.M. Nakai	1898	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9015
Dudleya verityi K.M. Nakai	1899	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9016
Dudleya verityi K.M. Nakai	1900	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9017

Dudleya verityi K.M. Nakai	1901	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9018
Dudleya verityi K.M. Nakai	1902	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9019
Dudleya verityi K.M. Nakai	1904	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9021
Dudleya verityi K.M. Nakai	1906	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9022
Dudleya verityi K.M. Nakai	1907	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9023
Dudleya verityi K.M. Nakai	1908	Treatment Plant, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9024
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1764	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9231
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1765	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9241
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1766	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9242
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1767	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9243

Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1769	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9245
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1770	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9246
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1773	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9249
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1775	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9251
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1777	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9253
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1778	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9254
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1779	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9255
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1780	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9256
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1781	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9257
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1782	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9258

Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1784	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9260
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1785	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9261
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1786	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9262
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1787	Malibu Ridge, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9263
Dudleya verityi K.M. Nakai x *Dudleya lanceolata* (Nutt.) Britton & Rose	1799	Round Mountain, Santa Monica Mountains, Ventura County, California, USA	M. Elvin	9318
Dudleya virens (Rose) Moran subsp. *insularis* (Rose) Moran	2062	Santa Catalina Island, Los Angeles County, California, USA	P. Dixon	122
Dudleya *virens* (Rose) Moran subsp. *virens*	2091	San Clemente Island, Los Angeles County, California, USA	H. Elias	615
Sedella Britton & Rose	1982	Mendocino County, California, USA	C.M. Guilliams	4820

Tissue samples for this study were gathered in 2017 and 2018. Approximately 4-5 cm^2 of new, fresh, green cauline leaves were removed from plants in the field and placed into individually marked paper coin envelopes. Envelopes were sealed and placed in silica gel to rapidly desiccate the tissue samples and preserve DNA quality. A total of 160 individuals were sampled for this project. Herbarium vouchers were gathered from each locality (Table 2). Due to the rarity of *D. verityi* and the well-publicized cases of poaching of this genus in general (Callahan 2019, McConnell 2019, Robertson 2019), we did not gather whole plants as vouchers. Rather, vouchers comprised 2-3 rosette leaves, carefully removed with a sharp knife near the leaf base, along with 1-2 fresh inflorescences, carefully removed from the caudex. All vouchers for this project have been deposited at the Clifton Smith Herbarium at the Santa Barbara Botanic Garden (SBBG).

DNA EXTRACTION

Silica dried material was ground with a mortar and pestle into a fine powder and extracted using a modified CTAB protocol (Doyle and Doyle 1987) with the following change: incubation in the CTAB extraction buffer with proteinase K at 65 degrees for 3-4 hours. Extractions were quantified on a Qubit fluorometer using the Qubit Double Stranded High Sensitivity Assay Kit (Invitrogen, Carlsbad, CA) to check for suitable genomic DNA quantity. DNA quality was assessed via visualization on an agarose gel following gel electrophoresis.

LIBRARY PREPARATION

Libraries were prepared for high throughput sequencing using a Restriction site Associated DNA sequencing (RADseq) protocol. The RADseq approach is a genomic DNA reduction technique that isolates for sequencing regions of genomic DNA near to a set of restriction enzyme cut sites. The approach is cost-effective and can be repeated in large numbers of samples to produce nearly the same reduced subset of the genome in each individual. After sequencing, the data are re-assembled into loci, anchored by the presence of the restriction enzyme cut site (Baird et al. 2008; Etter et al. 2011) and subsequently SNPs are identified across those loci. Double digestion RADseq (ddRADseq) was selected for its ease of use and cost effective implementation for generating a large SNP dataset from non-model organisms (Parchman et al. 2012; Peterson et al. 2012). In ddRADseq, two restriction enzymes are used to fragment genomic DNA, followed by size selection of the fragments. This results in sequencing libraries with loci randomly distributed throughout the genome of the study system. Representation of the genome in this library is expected to be inversely proportional to deviation from the size-selection target, thus read counts across regions are expected to be correlated between individuals (Peterson et al. 2012). This method has been employed in numerous studies and has typically resulted in hundreds to thousands of loci sufficient to address typical population genetics studies in model and non-model organisms.

ddRADseq libraries were prepared in the genetics laboratory at the Santa Barbara Botanic Garden. Library preparation and barcode design follow Tripp et al. (2017). Total genomic DNA was fragmented using the MseI and EcoRI restriction enzymes. A total of 150-500 ng of genomic DNA was added to a reaction solution consisting of: 8.2 µL molecular grade water, 1.15 µL Tango Buffer (Fisher Scientific, Carlsbad, CA), 0.6 µL 1.0 M NaCl, 0.3 µL (1.0 mg/mL) Bovine Serum Albumin (BSA), 0.28 µL High Fidelity EcoRI (Fisher scientific), and 0.12 µL MseI (Fisher Scientific). Digestion reactions were incubated at 37^{o}C for 15 minutes, followed by an incubation step at 65^{o}C for 45 minutes.

Barcodes, an individual set of nucleotides used as a unique identifier to demultiplex pooled samples after sequencing, and adaptors containing an Illumina PCR priming site and the EcoRI cut site were prepared by Integrated DNA technologies (Coralville, Iowa) and follow the design of Tripp et al. (2017). Each ligation reaction consisted of the entire double restriction digestion reaction containing the fragmented genomic DNA to which we added 1.0 ul of 1.0 µM EcoRI adaptor+barcodes, 0.072 µL water, 0.1 µL 10X T4 buffer, 0.05 µL 1.0 M NaCl, 1.0 mg/mL BSA, 1.0 10 nM MseI adaptor, and 0.165 µL T4 DNA ligase. Reactions were mixed, centrifuged, and incubated for 16 hours at 16^{o}C, then heat inactivated at 65^{o}C for 10 minutes. These restriction-ligation reactions were diluted 1:10, using 0.1X TE buffer. To ameliorate stochastic differences that can be introduced during Polymerase Chain Reaction (PCR) production of fragments in library preparation, two separate 20 µL PCR reactions were performed per restriction-ligation product (Parchman et al. 2012). PCR reactions contained: 8.6 µL molecular grade water, 4.0 µL Phusion High Fidelity Buffer (New England Biolabs, Ipswich, MA), 0.5 µL of 10 µM Illumina primer 1 (IDT; (A*A*TGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCT CTTCCGATCT), 0.5 µL of 10 uM Illumina primer 2 (IDT; C*A*AGCAGAAGACGGCATACGA GCTCTTCCGATCTGTAAG), 1.6 ul 2.5 mM dNTPs, 0.1 ul Phusion High Fidelity DNA polymerase (New England Biolabs, Ipswich, MA) and 5 µL diluted restriction-ligation reaction. Each PCR reaction used the following cycling parameters: 98^{o}C for 60s; 25 cycles of 98^{o}C for 20s, 60^{o}C for 30s, 72^{o}C for 40s; 72^{o}C for 10m; 4^{o}C hold. Gel electrophoresis and imaging was used as a qualitative assay to ensure PCR amplification of fragments at the

desired 300-400 bp range for each sample. Successful PCR amplifications were cleaned with Zymo DNA Clean and Concentrator kits (Zymo Research, Irvine, CA) then pooled with other samples for a total of 96 samples.

SIZE SELECTION, LIBRARY QUANTIFICATION, AND SEQUENCING

The pooled genomic library was sent to the University of California Riverside (UCR) Institute for Integrative Genome Biology Core Instrumentation Facility for size selection. The library was size selected on a Blue pippen 1.5% agarose gel cassette for fragments between 350-550 bp in length. The library was quality checked with a Bioanalyzer 2100 (Agilent, Santa Clara, CA) at UCR to ensure library quality and concentration prior to sequencing on a nextSeq 4000 (Illumina, La Jolla, CA) each as a single lane of 2 x 100 bp paired end reads under the rapid run setting.

DATA PROCESSING

Raw sequence reads were demultiplexed by University of California, Riverside using custom scripts. Read pools were cleaned and quality checked using FastQC (Andrews 2010) and quality filtered using Trimmomatic under default settings (Bolger et al. 2014). To assemble loci and generate files for downstream population genetics analyses, cleaned sequence data were further processed with ipyrad (Eaton and Ree 2013; Eaton 2014) on a DL580 node using 32 core processors in batches of 15-25 individuals on an IMAC PRO with 10 cores for steps 1-3 in the ipyrad pipeline. All samples were merged at step 4, and further processed through the entire ipyrad pipeline.

Step 1 reads the data into the pipeline, step 2 provides additional quality filtering, step 3 builds sequence clusters from the sequence reads within an individual sample and aligns the loci into a matrix. Steps 4 estimates heterozygosity and error rates across reads, while step 5 filters undetermined sites per locus. At step 6, all individual samples were merged into one file to allow clustering and alignment of consensus sequences across individuals, while step 7 generates final output files.

Sequence assembly was performed using the *de novo* assembly setting in ipyrad, under the following parameters: ddrad datatype, phred quality score minimum of 33, parameters clustering threshold of 0.85, mindepth of 10, maximum barcode mismatch of 0, minimum length of sequences after the adaptor trim 45 bps, a maximum of 2 alleles per site in consensus sequences. SNP datasets resulting from step 7 of the ipyrad pipeline were filtered to include only one SNP from each locus to fit the assumptions of independent and unlinked loci required for statistical calculations (Arnold et al. 2013).

Four different datasets were generated in ipyrad to address different components of the study goals. For clarity, these datasets will be referred to as Datasets 1 through 4. Dataset 1 was generated to examine population genetics of *D. verityi*. This dataset was limited to samples of *D. verityi* from the Airfield, Malibu Ridge, and Treatment Plant localities. Dataset 2 was generated to assess overall genetic patterns in the two focal taxa. This dataset contained all samples of *D. verityi* from each locality, all samples of *D. lanceolata* from throughout the range of the taxon, and putative hybrids from the Malibu Ridge sampling locality. Dataset 3 was generated to assess hybridization at the local scale. This dataset contained samples from the Malibu Ridge sampling locality of *D. verityi*, *D. lanceolata*, and putative hybrids between them. Dataset 4 was generated to examine broader phylogenetic patterns in *D. lanceolata*. This dataset contained all samples of *D. lanceolata*, 9 total samples of *D. verityi*, and 10 additional *Dudleya* and *Sedella* taxa.

ANALYSES

Genetic differentiation between pairwise combinations of *D. verityi* EOs were examined in Dataset 1 using Fst. Fst is a common measure of genetic differentiation, with higher values indicating a greater degree of genetic differentiation between populations, and lower values indicating a greater genetic similarity. These values were calculated in R using the StAMPP-package (Pembleton et al. 2013).

Multivariate statistical methods were used to examine patterns in Datasets 1, 2, and 3. These methods do not have strong assumptions about an underlying genetic model, such as the presence of Hardy-Weinberg equilibrium or the absence of linkage disequilibrium (Jombart 2008). The alignments produced by ipyrad were converted into genlight objects using the function fasta2genlight in adegenet version 2.1.0 (Jombart 2008; Jombart and Ahmed 2011), implemented in R (R Development Core Team 2011). A principal coordinates analysis (PCoA) was performed on the genlight matrix in the R package dartR (Gruber et al. 2018). PCoA is a statistical procedure that transforms a large number of variables to fewer composite variables, or PCs. These composite variables can be used to identify possible structure or clusters of genotypes within and among populations of individuals in the dataset, and is particularly informative when visualized as a scatter plot (Jombart 2009).

Rooted and unrooted phylogenetic trees were inferred using maximum likelihood in the program RAxML (Stamatakis 2014) on the CIPRES Science Gateway v3.3 (Miller et al. 2010). Analyses were performed with the RAxML HPC2 on XSEDE tool using default parameters. Topological support was assessed with 100 rapid bootstrap replicates. Unrooted trees were inferred from Datasets 1, 2, and 3. A rooted tree was inferred from Dataset 4, using a sample of *Sedella* as a phylogenetic outgroup. All resulting trees were visualized using the program FigTree v1.4.3.

Phylogenetic networks were constructed for Datasets 2 and 3 using the program SplitsTree4 v.4.15.1 (Huson and Bryant 2006). Because of weak barriers to gene flow in *Dudleya*, it is possible that the genomes of any plant may contain a mixture of genetic material owing to historical interactions between previously separated populations of the same or different taxa. For this reason, a bifurcating phylogenetic tree may not accurately represent the evolutionary history of the genus. Phylogenetic networks are a type of diagram that depict more complicated evolutionary scenarios, such as those involving hybridization and gene duplication. Networks were constructed using the NeighborNet method under default settings.

Bayesian analysis of population structure was performed in the ParallelStructure (Besnier and Glover 2013) implementation of the program STRUCTURE (Pritchard et al. 2000) on the CIPRES Science Gateway v3.3 for Datasets 1, 2, and 3. STRUCTURE identifies genetic subdivisions in the data and then assigns samples to these subdivisions using an admixture model, assuming correlation of allele frequencies, with or without prior knowledge of sample locality. The program was run for different values of K, each run with a 25,000 MCMC burn-in period followed by 50,000 MCMC iterations. STRUCTURE was used with the default settings (admixture; inferred initial a = 1.0, with a uniform prior across populations; correlation of allele frequencies within populations) to identify genetics subdivisions. To obtain the most likely value of K, LnP(K) and Delta K were evaluated under the Evanno Method (Evanno et al. 2005) in STRUCTURE HARVESTER (Earl and vonHoldt 2012). CLUMPAK (Kopelman et al. 2015) was used to visualize the results across STRUCTURE runs for the optimal K value.

Results

DATASET ASSEMBLY

Initial quality filtering based on the quality score (Q) removed 24 individuals from the dataset in the ipyrad pipeline, with 126 samples remaining in the final dataset. The Q score for each nucleotide position is a metric for evaluating high-throughput sequence data based on an algorithm developed from phred scores (Ewing et al. 1998, Ewing and Green 1998). A Q score of 30 or greater corresponds to an error rate of approximately 1/1000 (Illumina 2011). Prior to quality filtering, the average number of reads per individual included in the final dataset was 1,272,377, ranging between 190,940 and 4,523,116. Quality filtering removed an average of 12,278 reads per sample, retaining on average 1,260,098 per sample. See Appendix 1 for comprehensive results for each sample.

After sequencing and filtering, Dataset 1 contained 57 samples: 21 from the Airfield locality, 18 from the Malibu Ridge locality, and 18 from the Treatment Plant locality. The unreduced FASTA matrix for Dataset 1 containing the full sequence for each locus was 240,996 base pairs (bp) in length. It was imported into the R package adegenet v

2.4 (Jombart 2008, 2011) to screen the alignment for polymorphic positions and extract single nucleotide polymorphisms (SNPs). The resulting SNP matrix contained a binary score representing presence/absence for each SNP. For Dataset 1, the SNP matrix for population genetic analysis contained 2,275 SNPs.

After sequencing and filtering, Dataset 2 contained 108 total samples, 57 of *D. verityi,* 31 of *D. lanceolata,* and 20 samples of putative hybrids between *D. verityi* and *D. lanceolata*. The unreduced FASTA matrix containing the full sequence for each locus was 240,996 bp in length.This dataset had a total of 3,505 SNPs.

After sequencing and filtering, Dataset 3 contained 45 samples:14 Malibu Ridge locality samples of *D. verityi,* 14 samples of *D. lanceolata,* and 17 samples of putative hybrids between *D. verityi* and *D. lanceolata*. The unreduced FASTA matrix containing the full sequence for each locus was 516,832 bp in length. This dataset had a total of 7471 SNPs.

After sequencing and filtering, Dataset 4 contained 56 samples: 9 samples of *D. verityi,* 37 samples of *D. lanceolata*, and 10 outgroup *Dudleya* and *Sedella* samples. The unreduced FASTA matrix containing the full sequence for each locus was 329,132 bp in length.

POPULATION GENOMIC ANALYSES

Results of Analyses of Dataset 1 - Fst values are presented in Table 3. Values are moderate to relatively high, ranging from 0.2477 to 0.3660. The smallest Fst value (0.2477) was between the Malibu Ridge locality (EO1) and the Treatment Plant locality (EO2). The largest Fst value (0.3660) was between the Air Field locality (EO6) and the Treatment Plant locality.

Table 3. Pairwise Fst values between sampled locations of *Dudleya verityi*.

	Malibu Ridge (EO1)	**Air Field (EO6)**	**Treatment Plant (EO2)**
Malibu Ridge (EO1)	-	0.3313	0.2477
Air Field (EO6)	0.3313	-	0.3660
Treatment Plant (EO2)	0.2477	0.3660	-

Figure 5A shows a scatterplot of PCoA axis 2 plotted against axis 1. PCoA axis 1 explains 12.2% of variation in the data, while axis 2 accounts for 7.7% of variation. Samples form non-overlapping clusters by sampling location. Samples within a cluster are relatively tightly spaced.

The Evanno Method as implemented in STRUCTURE HARVESTER resulted in a best supported model of genetic subdivisions in the data of K = 4 based on delta K (Figure 5B). Figure 5B shows summary barplots across STRUCTURE runs for K = 3 and K = 4 generated in CLUMPAK. For both values of K, genetic subdivisions are typically assigned to samples of one sampling location. In the K = 4 barplot, the last genetic subdivision is weakly represented across samples from all three sampling locations. The overall pattern is strong congruence between genetic subdivisions and sampling locations.

Figure 5C shows the unrooted maximum likelihood phylogenetic tree, which has patterns that are similar to genetic clusters found in the PCoA (Figure 5A) and the STRUCTURE analysis (Figure 5B). Samples are grouped into clusters by sampling location. The Malibu Ridge locality cluster is supported by a maximum likelihood bootstrap value (ML BS) of 90. The Air Field locality cluster is supported by a ML BS value of 96. The Treatment Plant locality cluster is supported by a ML BS value of 86. Bootstrap values above 70 are considered strong.

Results of Analyses of Dataset 2 - Figure 6A shows a scatterplot of PCoA axis 2 plotted against axis 1. PCoA axis 1 explains 17.8% of variation in the data, while axis 2 accounts for 4.6% of variation. Samples form clusters by sampling location, although some clusters are broadly overlapping. Samples within a cluster are somewhat diffuse to relatively tightly spaced.

The Evanno Method resulted in a best supported model of genetic subdivisions in the data of K = 3 based on delta K, although K = 8 and K = 11 also had relatively high values of delta K (Figure 6B). Figure 6B shows summary barplots across STRUCTURE runs for K = 3, K = 8, and K = 11 generated in CLUMPAK. Barplots for each of these values of K were nearly identical, with only two genetic subdivisions being commonly inferred among the individual samples, here represented by the colors blue and orange. In all three barplots for the values of delta K, samples of *D. verityi* from the three sampling locations were assigned to the same genetic subdivision, shown in blue. At the other extreme and in contrast, individual samples of *D. lanceolata* from non-Santa Monica Mountains locations were often each assigned with equal probability to the blue genetic subdivision and the orange genetic subdivision. Samples of *D. lanceolata* from Santa Monica Mountains locations were also often assigned to the orange genetic subdivision, but with reduced probability. Putative hybrid individuals were largely assigned to the blue genetic subdivision, although some samples were assigned with low probability to the orange genetic subdivision.

Figure 6C shows the unrooted maximum likelihood phylogenetic tree. Samples of *D. verityi* from the Air Field and Treatment plant sampling locations form clusters with strong support (ML BS = 100 and 91, respectively). Samples of *D. verityi* from the Malibu Ridge sampling locality occur in a moderately well-supported cluster with some samples of putative hybrids between *D. verityi* and *D. lanceolata* (ML BS = 79). All remaining putative hybrids form a moderately well-supported group (ML BS = 87) with samples of *D. lanceolata* from throughout its range. Within this group, relatively short branch lengths and clusters samples of putative hybrids and *D. lanceolata* from the Santa Monica Mountains contrasts with the long, well-spaced branches in the nested group of samples of *D. lanceolata* from throughout the remainder of its range. The nested group of *D. lanceolata* samples is supported with an ML BS of 100.

Figure 6D shows the tree diagram from the SplitsTree analysis of Dataset 2. The overall tree network shape is essentially identical to the tree topology of the ML analysis (Figure 6C). However, under this approach that explicitly allows for reticulation, significant potential interaction among samples of *D. verityi*, *D. lanceolata*, and putative hybrids between the two at the Malibu Ridge sampling locality is evident, as indicated by numerous parallel branches among samples.

Results of Analyses of Dataset 3 - Figure 7A shows a scatterplot of PCoA axis 2 plotted against axis 1. PCoA axis 1 explains 13.6% of variation in the data, while axis 2 accounts for 6.1% of variation. Patterns in the scatterplot are not as obvious as in Datasets 1 and 2. While samples by category (*D. verityi*, *D. lanceolata*, and putative hybrids) are broadly overlapping, when outliers are excluded from consideration some clustering by category is evident. Even then, these clusters are overlapping. Overlapping the least are the outlier-excluded clusters of *D. verityi* and *D. lanceolata*. The outlier-excluded cluster of putative hybrids overlaps broadly with the outlier-excluded cluster of *D. lanceolata*, and overlaps somewhat with the outlier-excluded cluster of *D. verityi* samples.

The Evanno Method resulted in a best supported model of genetic subdivisions in the data of K = 3 based on delta K (Figure 7B). Figure 7B shows a summary barplot for the K = 3 STRUCTURE runs generated in CLUMPAK. The summary barplot for K =3 shows that samples are assigned with high likelihood to only two genetic subdivisions, here represented by the colors blue and orange. Samples of *D. verityi* were usually, but not always, assigned to the blue genetic subdivision. In contrast, samples of *D. lanceolata* were usually but not always assigned to the orange genetic subdivision. Samples of putative hybrids were more often assigned to the blue genetic subdivision. The third genetic subdivision, shown in the barplot as dark blue, occurs across *D. lanceolata* samples at low probability. This third genetic subdivision is also present at low probability in the putative hybrids, but is nearly absent in samples of *D. verityi*.

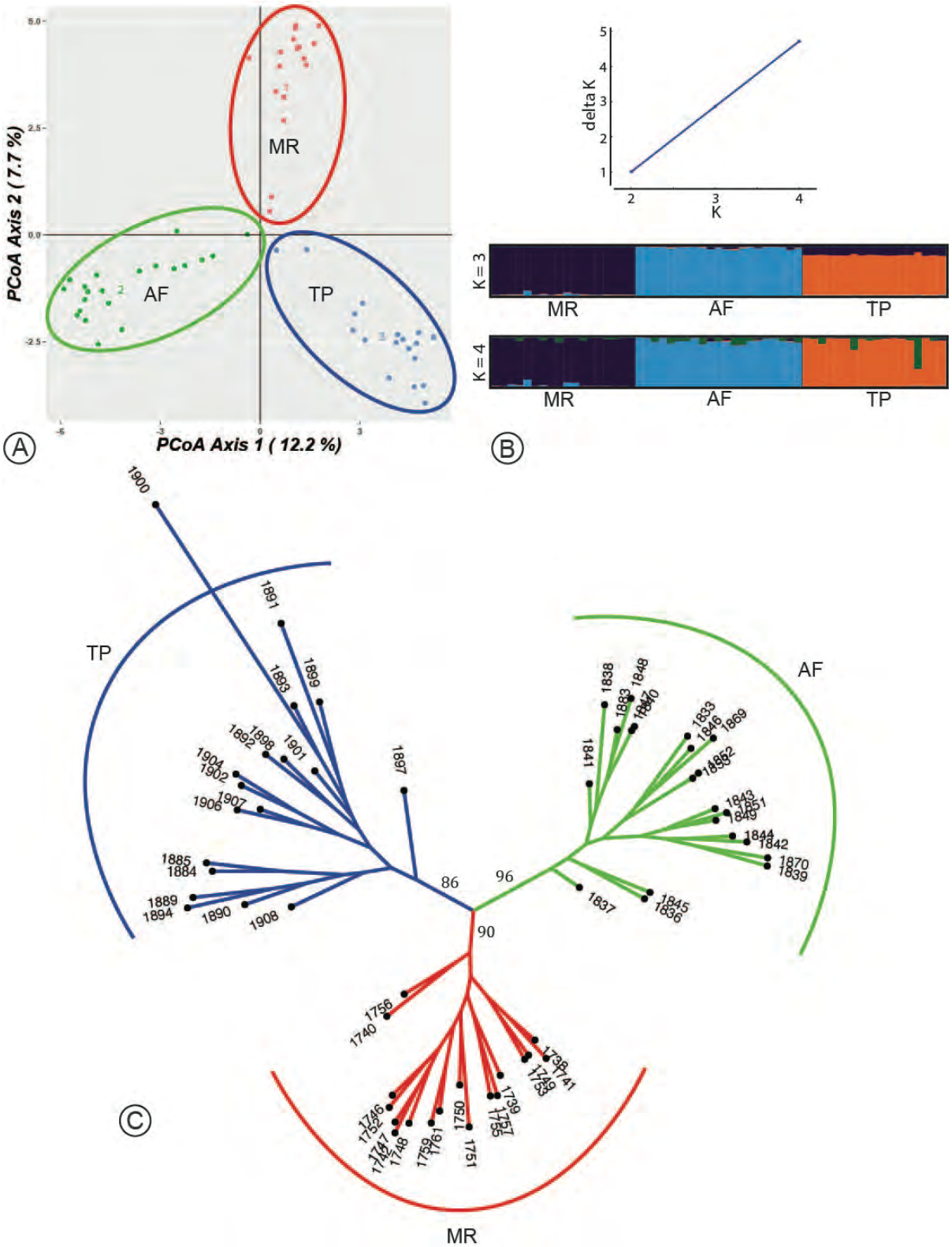

Figure 5A-C. Graphical results from analyses of Dataset 1. **A.** Scatterplot of principal coordinate axis 2 versus axis 1, sample colors correspond with sampling locations (Malibu Ridge (MR) = red, Air Field (AF) = green, Treatment Plant (TP) = blue); **B.** STRUCTURE-related results: plot of deltaK versus values of K 2-4, barplots for K = 3 and K = 4; **C.** Unrooted maximum likelihood phylogenetic tree.

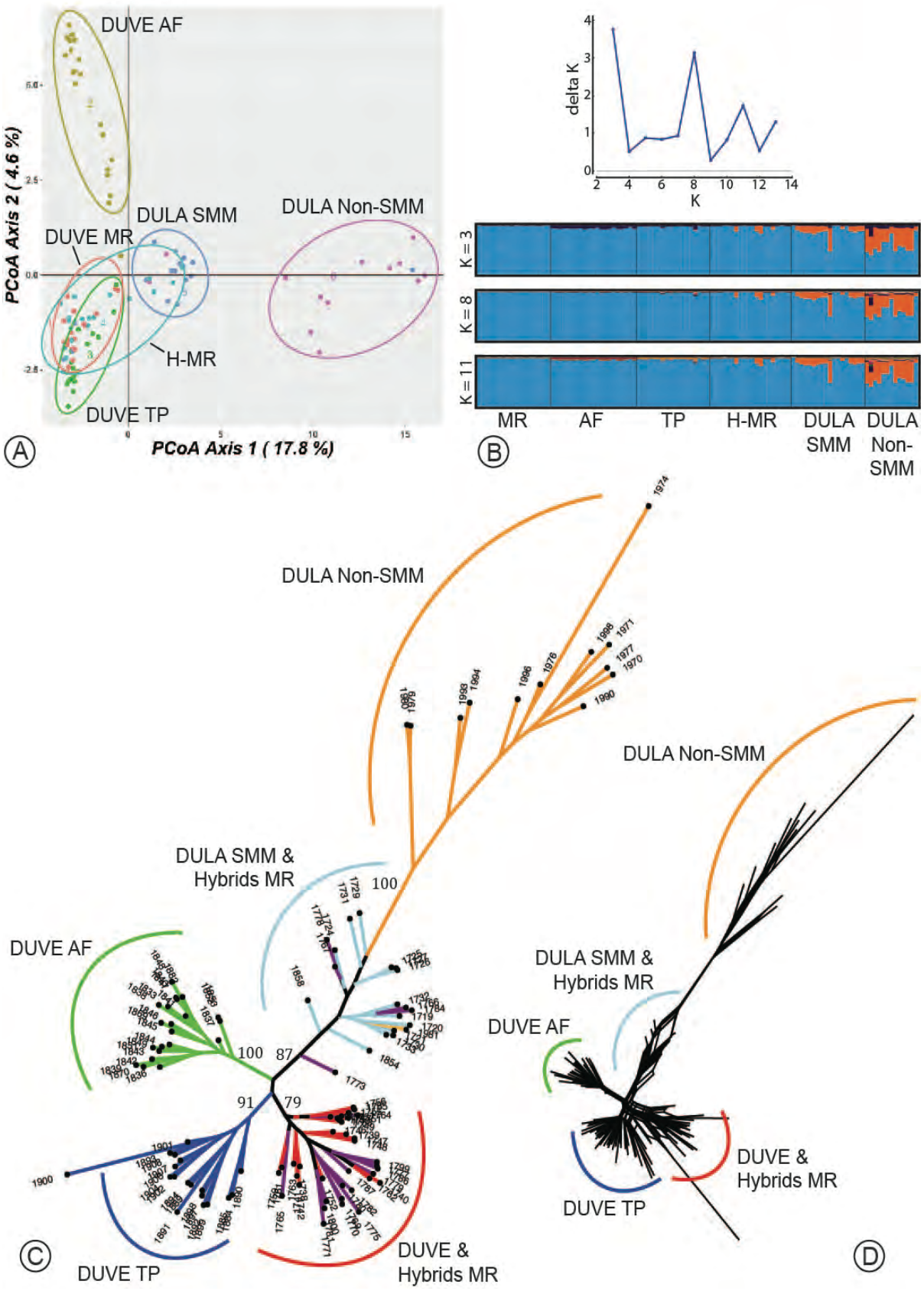
DUVE AF
DULA SMM
DULA Non-SMM
DUVE MR
H-MR
DUVE TP
PCoA Axis 2 (4.6 %)
PCoA Axis 1 (17.8 %)
A
delta K
K
K = 3
K = 8
K = 11
MR
AF
TP
H-MR
DULA SMM
DULA Non-SMM
B
DULA Non-SMM
DULA SMM & Hybrids MR
DUVE AF
DUVE TP
DUVE & Hybrids MR
100
87
91
79
C
DULA Non-SMM
DULA SMM & Hybrids MR
DUVE AF
DUVE & Hybrids MR
DUVE TP
D

Figure 6A-D (preceding page). Graphical results from analyses of Dataset 2. **A.** Scatterplot of principal coordinate axis 2 versus axis 1, sample colors correspond to taxa and sampling locations within a taxon (*D. verityi* Malibu Ridge (MR) = orange, *D. verityi* Air Field (AF) = gold, *D. verityi* Treatment Plant (TP) = green, *D. verityi* and *D. lanceolata* putative hybrids Malibu Ridge (H-MR) = blue, *D. lanceolata* Santa Monica Mountains = blue-gray, *D. lanceolata* Non-Santa Monica Mountains = lavender); **B.** STRUCTURE-related results: plot of deltaK versus values of K 2-4, barplots for K = 3 and K = 4; **C.** Unrooted maximum likelihood phylogenetic tree from RAxML; **D.** Tree network diagram from SplitsTree.

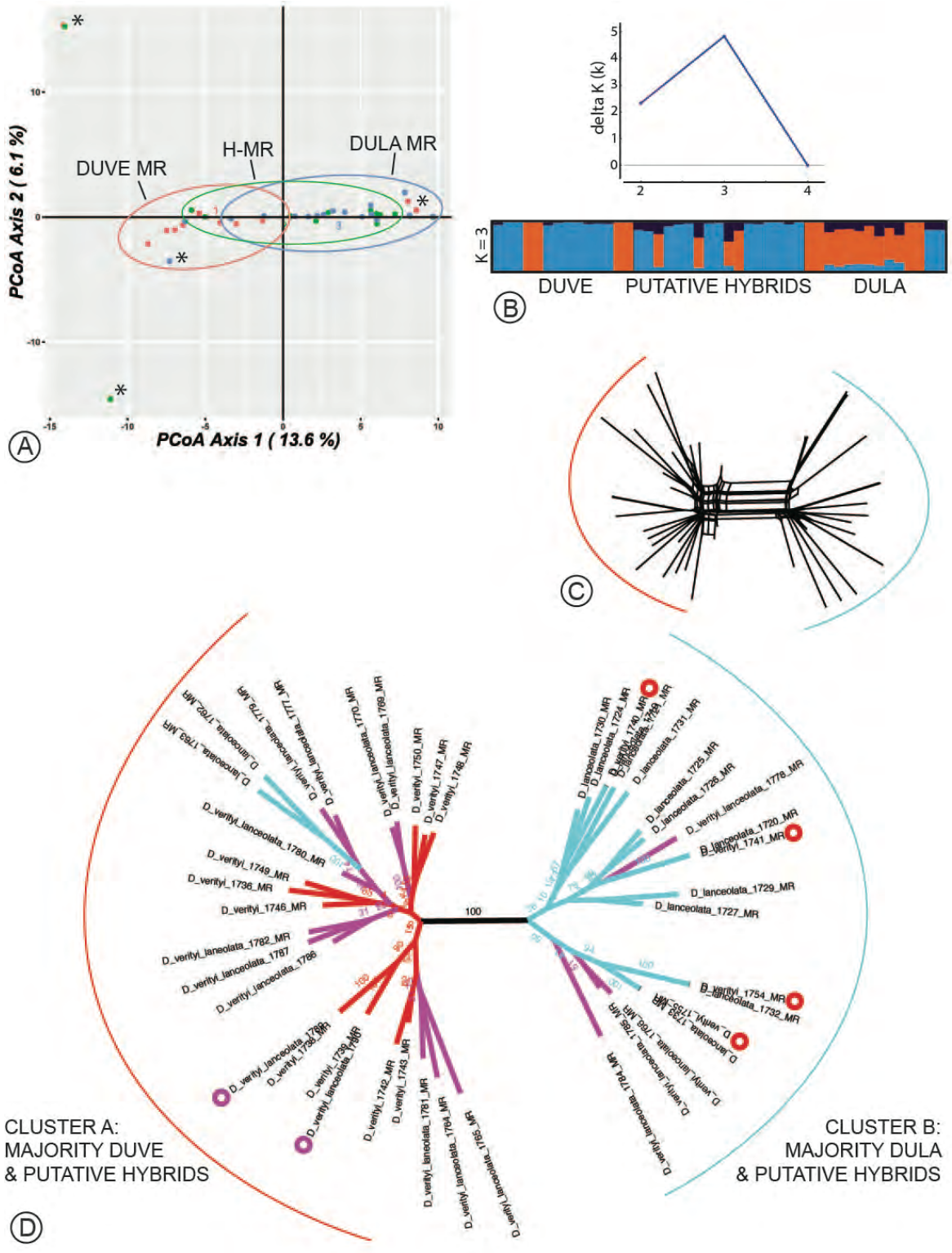

Figure 7A-D. Graphical results from analyses of Dataset 3 from Malibu Ridge. **A.** Scatterplot of principal coordinate axis 2 versus axis 1, sample colors correspond to sampling categories (*D. verityi* = orange, *D. verityi* and *D. lanceolata* putative hybrids (H-MR) = green, *D. lanceolata* = blue-gray), asterisks indicate outlier samples; **B.** STRUCTURE-related results: plot of deltaK versus values of K 2-4, barplot for K = 3; **C.** Tree diagram from

SplitsTree; **D.** Unrooted maximum likelihood phylogenetic tree from RAxML, branch colors and open circles correspond to sampling categories (*D. verityi* = red, *D. verityi* and *D. lanceolata* putative hybrids = purple, *D. lanceolata* = light blue), open circles indicate the presence and sampling category of an obscured branch.

Figures 7C and 7D show the tree network diagram from the SplitsTree analysis and the unrooted maximum likelihood phylogenetic tree from the RAxML analysis, respectively. The overall tree network shape and phylogenetic tree topology are similar in resolving two main groups or clusters. The tree network diagram (Figure 7C) shows considerable parallel branches between these clusters. Figure 7D has branches color-coded to sampling category, with *D. verityi* in red, putative hybrids in purple, and *D. lanceolata* in light blue. Cluster A comprises most but not all samples of *D. verityi*, most but not all of the putative hybrids, and two samples of *D. lanceolata*. Cluster B comprises nearly all samples of *D. lanceolata*, some samples of putative hybrids, and four samples of *D. verityi*.

Results of Analyses of Dataset 4 - Figure 8 shows the rooted phylogenetic tree from the RAxML analysis of Dataset 4. Support for deeper nodes in the tree among phylogenetic outgroups as assessed by ML BS values is strong in general (e.g., ML BS > 95). In this analysis, *D. lanceolata* was non-monophyletic. Samples within the current circumscription of *D. lanceolata* from San Luis Obispo County were recovered with strong MS BS support among phylogenetic outgroups (ML BS > 95). All other samples of *D. lanceolata* form a well-supported clade (ML BS = 98) with samples of *D. verityi*.

Within the *D. lanceolata* + *D. verityi* clade, the earliest phylogenetic split yields one moderately supported (ML BS = 62) clade of *D. lanceolata* samples from locations outside of the Santa Monica Mountains, and another moderately supported clade (ML BS = 76) of *D. lanceolata* samples from the Santa Monica Mountains and all samples of *D. verityi*. In this latter clade, samples of *D. lanceolata* are paraphyletic with respect to a strongly supported (ML BS = 100) clade of *D. verityi* samples with two samples of *D. lanceolata* from the Malibu Ridge location. Within the *D. verityi* clade, samples form strongly supported (ML BS = 100) subclades by sampling location. The samples of *D. lanceolata* from Malibu Ridge are nested in the *D. verityi* subclade from Malibu Ridge.

Discussion

Population differentiation of D. verityi - The results of analyses of Dataset 1, limited to *D. verityi*, show clear differentiation among the sampled populations. Fst values are moderately high, suggesting strong differentiation and minimal gene flow. This conclusion is supported by each subsequent analysis (Figure 5A-C). The PCoA scatterplot shows clear, non-overlapping genetic groupings of samples by location, the STRUCTURE analysis summary barplot shows genetic subdivisions that correspond exactly to sampling locations, and the unrooted maximum likelihood phylogenetic tree resolves three groups corresponding to sampling locations. Collectively, analysis of Dataset 1 shows strong differentiation and from this, likely limited to no gene flow.

The strong differentiation detected here is likely attributable to the patchiness of the substrates on which this species occurs. *D. verityi* is limited to low elevation rock outcrops and cliffs of Conejo Volcanic rock south of the City of Camarillo. While exposures of Conejo Volcanic rock are not uncommon within the restricted range of *D. verityi*, they are patchily distributed and separated from each other by valley bottom and non-rocky, earthen slope settings. Plants are likely further limited to Conejo rock outcrops with sufficient cover of lichen and moss substrate (Riefner et al. 2003; Guilliams and Hasenstab-Lehman personal observations). Thus, geographic isolation due to substrate specificity likely serves as an effective barrier to gene flow for *D. verityi*. Sampling additional EOs of *D. verityi* would be useful to confirm this pattern and should be considered a high priority future activity.

Interactions between D. verityi *and* D. lanceolata - Based on field observations and the results presented here, it is clear that *D. verityi* and *D. lanceolata* plants are interacting in nature. This project was motivated by observations in the field that plants morphologically intermediate between the two taxa were growing in ecotonal settings between

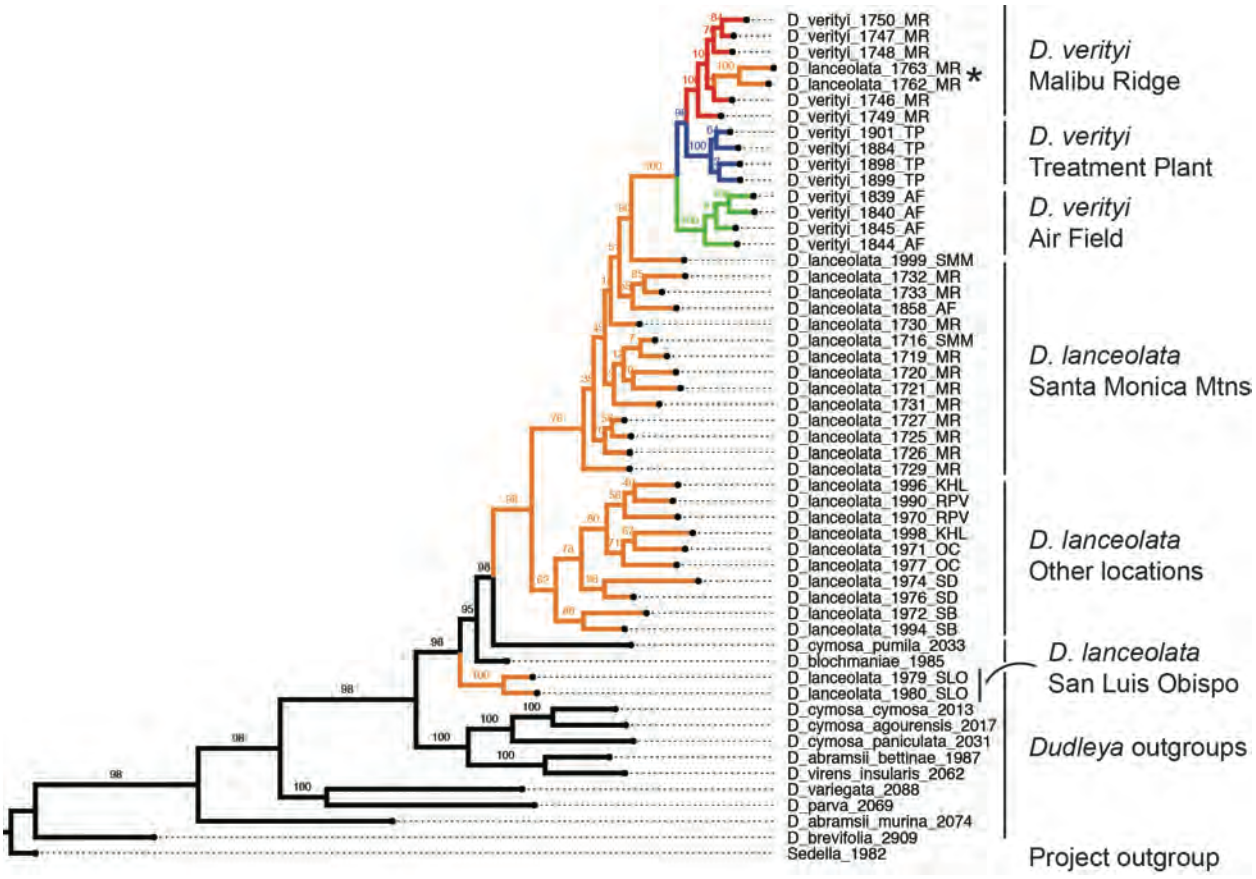

Figure 8. Rooted phylogenetic tree from RAxML from analysis of Dataset 4. Numbers on branches are maximum likelihood bootstrap values. Branches are colored by taxon or sampling locality: *D. lanceolata* = orange, *D. verityi* from the Malibu Ridge locality = red, *D. verityi* from Treatment Plant locality = blue, *D. verityi* from the Air Field locality = green, outgroup taxa = black; the asterisk marks two *D. lanceolata* samples nested within the *D. verityi* subclade from Malibu Ridge.

the Conejo Volcanic outcrops where *D. verityi* occurs, and the adjacent earthen slopes where *D. lanceolata* occurs. Based on morphological and ecological intermediacy, these plants were suspected to be hybrids. Given the broad interfertility of *Dudleya* taxa and close relatives in Crassulaceae in general (Verity in Nakai 1983 McCabe, unpublished data, Zika et al. 2018), this was a reasonable hypothesis with important conservation implications.

Dataset 2 was designed to examine genetic patterns among the total sampling of *D. verityi* and broad, geographically representative sampling of *D. lanceolata* from throughout its range. An outcome in support of the hybrid origin of morphological intermediates would have been the demonstration of two clear groups corresponding to each taxon, with putative hybrid samples occupying an intermediate position between the groups. For the STRUCTURE analysis, an outcome in support of the hybrid origin of morphological intermediates would have been the inference of two primary genetic subdivisions corresponding to each taxon, with putative hybrids assigned with some probability to both subdivisions.

Analyses of Dataset 2 did show some of these patterns, but interpretation of the results is complicated by the finding of significant genetic variation in the wide-spread *D. lanceolata*. The PCoA scatterplot (Figure 6A) shows that samples of *D. lanceolata* from the Santa Monica Mountains appear to be more genetically similar to *D. verityi* than to *D. lanceolata* from the rest of its range. The unrooted ML phylogeny (Figure 6C) and the SplitsTree network

diagram (Figure 6D) similarly shows greater genetic variation (i.e., longer internal and terminal branches) in the *D. lanceolata* group from outside the Santa Monica Mountains than is present in the rest of those tree figures. In both these cases, samples of *D. lanceolata* from the Santa Monica Mountains are resolved closer to *D. verityi* than to other samples of *D. lanceolata*, but a phylogenetic outgroup (as in Dataset 4) is required to understand if this closeness can be interpreted as recency of common ancestry. In both tree-based analyses, morphological intermediates are intercalated among samples of *D. verityi* from Malibu Ridge and *D. lanceolata* from the Santa Monica Mountains (Figures 6C-D), as hypothesized. Furthermore, the SplitsTree network diagram shows numerous parallel branches between putative hybrids and *D. verityi* from Malibu Ridge (Figure 6D). Both of these findings support the hypothesis that morphological intermediates represent hybrid plants. The results of the STRUCTURE analysis of Dataset 2 are only moderately informative, but do show some gradation in inferred population subdivision membership between *D. verityi*, on one extreme, and *D. lanceolata* from outside the Santa Monica Mountains, on the other (Figure 6B).

Dataset 3 was assembled to examine local genetic patterns at one sampling location, Malibu Ridge, where *D. verityi*, *D. lanceolata*, and putative hybrids are all abundant. This location may be atypical within the range of *D. verityi*, as it is an earthen slope with numerous, small outcrops of Conejo Volcanic rock throughout. Typical *D. verityi* occurs on some outcrops and typical yellow-flowered *D. lanceolata* occurs on the earthen slope. Given the numerous small outcrops of Conejo Volcanic rock embedded in an earthen slope matrix at this location, ecotonal environments are abundant. Intercalated rock and earthen slopes would permit the ready exchange of genetic material between *D. verityi* and *D. lanceolata*. Is there evidence for this genetic exchange in the sequence dataset?

Analyses of Dataset 3 suggest significant interactions between *D. verityi* and *D. lanceolata* at the Malibu Ridge location. The PCoA scatterplot (Figure 7A) shows moderately distinct clusters of *D. verityi* and *D. lanceolata* samples when outliers are excluded, with samples of putative hybrids broadly overlapping with samples of *D. verityi*. Given that the putative hybrids are morphologically intermediate between these two distinctive taxa and occur in ecotonal environmental settings, it is illuminating that they are nevertheless genetically similar to *D. verityi* in this analysis.

The STRUCTURE analysis of Dataset 3 (Figure 7B) reveals similar patterns, with the two main genetic subdivisions being assigned more often to one or the other of the taxa. This pattern is not consistent across all individuals, however, with some *D. verityi* being assigned to the genetic subdivision that is more common among *D. lanceolata* samples, and vice versa. Putative hybrid samples are in aggregate assigned to both genetic subdivisions, but each individual sample is usually assigned primarily to one of the genetic subdivisions.

The two main clusters or groupings of samples in the ML phylogenetic tree (Figure 7C) and the SplitsTree network diagram (Figure 7D) correspond reasonably well to *D. verityi* + putative hybrids, and *D. lanceolata* + putative hybrids. Keeping in mind that each putative hybrid was identified and sampled on the basis of morphological and ecological intermediacy, the finding that sometimes these sampled intermediates were inferred to be more closely related to one or the other of the taxa suggests a range of genetic admixture among the sampled intermediates. Indeed, occasionally samples identified on the basis of morphology and ecological setting to be either pure *D. verityi* or pure *D. lanceolata* were placed in the opposing group of samples.

Genetic patterns within D. lanceolata - The finding from analyses of Dataset 2 that the Santa Monica Mountains samples *D. lanceolata* were potentially more genetically similar to *D. verityi* than other samples *D. lanceolata* from outside of the Santa Monica Mountains compelled a deeper inquiry into the evolutionary history and circumscription of *D. lanceolata*. The rooted phylogenetic analysis presented in Figure 8 provides important context for these earlier analyses. Even excluding the *D. lanceolata* samples from Malibu Ridge marked with an asterisk and samples from San Luis Obispo County, *D. lanceolata* is non-monophyletic. The early phylogenetic split yielding two main clades containing *D. lanceolata* samples-- one of core *D. lanceolata* and another of Santa Monica Mountains *D. lanceolata* + *D. verityi* -- confirms the close relationship between plants in the latter clade. It may also help to explain

apparently fixed petal color differences between plants in the core *D. lanceolata* clade (petals orange to red) and some *D. lanceolata* from the western Santa Monica Mountains (petals yellow).

The non-monophyly of *D. lanceolata* in the broad sense requires further study and possibly one or more nomenclatural changes so that only monophyletic groups are recognized taxonomically. Should the genetic patterns inferred here persist with increased sampling, then it may be necessary to provide new names for some clades of non-monophyletic *D. lanceolata*. Further documentation of the geographic distribution of the flower color forms is an important next step. The type for *D. lanceolata* (Nuttall s.n., from "St. Diego") would fall within the core *D. lanceolata* clade on the basis of morphology (flowers reported in the description as red and yellow) and collection locality. New species names would be needed for the clades of *D. lanceolata* from the Santa Monica Mountains and from San Luis Obispo County.

Possible peripheral isolate speciation of D. verityi - The rooted phylogenetic tree presented here (Figure 8) may shed light on the evolutionary origin of *D. verityi*. The clade of *D. lanceolata* + *D. verityi* inferred in the rooted phylogenetic tree shows a paraphyletic *D. lanceolata* from the Santa Monica Mountains with respect to a nested clade of *D. verityi*. Node support outside of the *D. verityi* subclade is mixed in this tree, however. Should further study confirm the general topology recovered here, namely a paraphyletic *D. lanceolata* from the Santa Monica Mountains with respect to a nested clade of *D. verityi*, it may suggest that *D. verityi* arose through peripheral isolate speciation from a common ancestor with some part of *D. lanceolata* from the Santa Monica Mountains.

Peripheral isolate speciation, also called peripatric speciation, is a mode of speciation in which a new species is formed from an isolated population or group populations at a range edge. Such populations may be isolated from other populations by distance, with or without a geographic barrier, or by adaptation to a new ecological setting. The most common way that peripheral isolate speciation may occur is when range-edge populations of a widespread taxon adapt to novel ecological settings, further limiting or even precluding the possibility of gene flow between the incipient species and other populations of the widespread species. Interestingly, because it is likely that the peripheral isolate is closely related to adjacent populations of the widespread species, one possible phylogenetic pattern that could result in the case of peripatric speciation is paraphyly of the widespread species with respect to a nested peripheral isolate. In this special case, non-monophyly of the widespread species would typically not motivate nomenclatural changes (within the paraphyletic, widespread species) to align taxonomy with strictly monophyletic groups unless other compelling lines of evidence exist (e.g., morphological or ecological). Given enough time, it is understood that gene flow among populations of the widespread species and gradual loss of internal phylogenetic lineages may result in eventual reciprocal monophyly between the peripheral isolate and the widespread species. Despite the theoretical plausibility of this mode of speciation, it has rarely been documented using phylogenetic methods (Gottlieb 2004; Baldwin 2005; Valtueña et al. 2017)

Perhaps the best-known example of peripheral isolate speciation in the California Flora is the evolution of *Layia discoidea* D.D. Keck (Asteraceae) from a common ancestor shared with populations of the widespread *L. glandulosa* (Hook.) Hook. & Arn. (Baldwin 2005). *Layia glandulosa* is a common species, widely distributed on sandy substrates from Washington, USA to Baja California, MX, east to New Mexico, USA (Baldwin and Bainbridge 2012). It has relatively showy heads of flowers, each with between 3 and 14 white or yellow rays. *Layia discoidea* is a rare plant that occurs on barren, serpentine soils in Fresno and San Benito counties, California, USA. It has non-showy heads, having completely lost the rays of its close relative. In examining the origin of *L. discoidea*, Baldwin (2005) found a monophyletic *L. discoidea* nested within a paraphyletic *L. glandulosa*, with a divergence time of < 1 MA. Thus, this represents an excellent case of rapid peripheral isolate speciation accompanied by rapid ecological and morphological adaptation to a novel environment.

The evolutionary history of *D. verityi* may be similar to the well-known story of *L. discoidea*. The potential evolution of *D. verityi* from a common ancestor with Santa Monica Mountains *D. lanceolata* may have been accompanied by a shift from a generalist ecological niche (i.e., earthen slopes) to a more specialized one (i.e.,

Conejo Volcanic rock outcrops and cliff faces). Future work could confirm this hypothesis by sampling additional populations of both *D. verityi* and *D. lanceolata* from the Santa Monica Mountains along with numerous other outgroups. Importantly, as the focus of this study was on *D. verityi* and *D. lanceolata*, outgroup sampling was not sufficient to rule out other possible evolutionary scenarios involving other *Dudleya* taxa from the Santa Monica Mountains, e.g., *D. cymosa*.

Broader significance in genus - *Dudleya* has long been viewed as a difficult genus -- and rightly so -- by both taxonomists and taxonomic end-users. In the introduction we highlight a number of factors that contribute toward this perceived difficulty, which include: high-levels of taxonomic diversity in California (especially in Southern California); taxonomically meaningful variation nearly entirely limited to continuous characters with overlapping character state ranges (or conversely, lack of discrete morphological characters); plant succulence, resulting in imperfect herbarium specimen; lack of barriers to gene flow; and resulting inferred hybridization. Because there are several widespread *Dudleya* taxa in Southern California (e.g., *D. cymosa* sspp*., D. lanceolata, D. pulverulenta*) along with dozens of more local taxa, it is not uncommon that many of these factors will be present wherever one chooses to study the genus in the southern half of the state.

A number of these difficulties played a role in the origin of the present study. First, this study was motivated by a desire to understand if the common, widespread *D. lanceolata* was interacting through hybridization with a rare, edaphic endemic *D. verityi*. Hybridization between these taxa could have important consequences for *D. verityi*. For this reason, a finding in support of the hybridization hypothesis would likely motivate further action on the part of resource agencies charged with protecting this plant under the Federal Endangered Species Act. Because these two taxa lack discrete, diagnostic morphological characters to distinguish them, putative hybrid individuals identified on the basis of intermediate morphology and habitat preferences could not be confidently diagnosed in the field.

The high throughput sequencing approach used here was successful in achieving the goals of the project, and also resulted in potentially generalizable results for the genus as a whole. First, when sampling was limited to only individuals fitting clearly within the circumscription of *D. verityi*, the sequence data and resulting population genetic and phylogenetic inferences were clean and highly informative. From this it might be inferred that this data type would work well in the genus at relatively shallow phylogenetic depths of divergence near to the present time, during which the potential confounding effects of gene flow or reticulation may be expected to be relatively minor. Second, this study suggests that putative hybrids of intermediate morphology are likely the result of local interaction between *D. lanceolata* and *D. verityi*. Therefore, we conclude that morphology and ecology were reliable indicators of hybridization in the field in this case, and may be useful in other instances as well. Third, this study revealed non-monophyly in the widespread *D. lanceolata*, which in part may explain the presence of some unusual features in some of the Santa Monica Mountains populations of this taxon (e.g., yellow petals). Should these findings persist with further study, it may serve to highlight a case of a taxon concept in *D. lanceolata* s.l. in which the broad, continuous morphological features used to circumscribe it resulted in an unnatural (=non-monophyletic) grouping. Similar patterns may exist in other widespread dudleyas (e.g., *D. abramsii* sspp*., D. brittonii, D. cymosa* sspp*., D. pulverulenta*). Finally, the relationship inferred between *D. verityi* and populations of *D. lanceolata* from the Santa Monica Mountains suggests that *D. verityi* may have formed via adaptation of a peripheral population to lichen-covered, Conejo Volcanic rock. We hypothesize that several of the narrow endemic taxa may have evolved in a similar fashion from other widespread species (e.g., *D. abramsii, D. cymosa*). We expect further phylogenomic work to be fruitful in revealing similar patterns.

Summary of findings

This study contributes substantially to our understanding of *D. verityi* and its interaction with widespread congener, *D. lanceolata*. This study demonstrates considerable genetic differentiation among the sampled populations of *D. verityi*, which we attribute to limited gene flow between the isolated outcrops of Conejo Volcanic rock on which it occurs. Broader analyses of *D. verityi* and *D. lanceolata* support the genetic distinctiveness of *D. verityi*, but reveal a

complicated evolutionary history in *D. lanceolata*. A set of analyses focusing on one sampling location, Malibu Ridge, showed that morphological and ecological intermediates between *D. verityi* and *D. lanceolata* were likely the result of hybridization. Finally, a rooted phylogenetic analysis with additional outgroup taxa shed light on earlier analyses that suggested a close relationship between *D. verityi* and Santa Monica Mountains *D. lanceolata*. Collectively these analyses drive our understanding of *D. verityi* forward, answering some questions and revealing others.

Recommendations for future conservation actions – The following are recommendations for future conservation actions based on the results of this study:

1. Based on inferred strong differentiation in *D. verityi*, do not unintentionally mix plant material between EOs;
2. Gather sequence data from additional EOs of *D. verityi* to confirm the generalizability of the strong genetic differentiation inferred here;
3. Perform morphometric analysis of the herbarium vouchers of each tissue sample included in this study to quantify the morphological features associated with *D. verityi*, *D. lanceolata*, and (especially) hybrids;
4. Perform a census of *D. verityi* EOs to establish baseline population numbers for each EO;
5. Develop and implement a field study to evaluate the potential impacts to *D. verityi* resulting from direct and indirect competition with *D. lanceolata* and hybrids, including but not limited to evaluating the effects of pollen pollution (on *D. verityi* stigmas) and *D. verityi* pollen waste (on *D. lanceolata* stigmas);
6. Perform direct surveys in the Santa Monica Mountains to document the distribution of *D. lanceolata* with yellow petals, augmented by study of herbarium specimens and images from observation-based records (e.g., iNaturalist);
7. Based on findings of surveys for yellow-petaled *D. lanceolata*, evaluate evidence for recognizing as a new taxon.

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Appendix 1. Sequencing statistics by sample. Reads_raw = the starting or total number of reads; Trim_adapt = number of positions trimmed due to presence of adaptors; Trim_qual = number of positions trimmed due to quality; Filt_Ns = reads filtered due to high numbers of Ns; Filt_leng = reads filtered due to length; Passed_filt = reads that passed trim and filter quality control steps.

Sample	reads_raw	Trim_adapt	Trim_qual	Filt_Ns	Filt_leng	Passed_filt
D_lanceolata_1716	4375634	614896	20376961	145	529853	3845636
D_lanceolata_1717	4893565	758435	21038953	163	664343	4229059
D_lanceolata_1718	5376108	715986	25475705	173	654995	4720940
D_lanceolata_1719	5935712	438959	7396355	281	144547	5790884
D_lanceolata_1720_MR	6751844	331074	5262725	351	19636	6731857
D_lanceolata_1721_MR	5084730	355079	4655928	262	28695	5055773
D_lanceolata_1722_MR	6266980	817418	25625617	221	720986	5545773
D_lanceolata_1723_MR	755100	123056	4003818	17	102875	652208
D_lanceolata_1724_MR	4653576	494448	12177308	201	330688	4322687
D_lanceolata_1725_MR	4618453	545683	13867487	197	392972	4225284
D_lanceolata_1726_MR	6352133	792120	18458914	259	624769	5727105
D_lanceolata_1727_MR	5857115	835760	21936091	216	684397	5172502
D_lanceolata_1728_MR	1686083	216058	4766525	71	164143	1521869
D_lanceolata_1729_MR	10135587	768796	8359641	543	108793	10026251
D_lanceolata_1730_MR	2738462	282434	6581836	115	194411	2543936
D_lanceolata_1731_MR	9054365	443251	7978061	481	68790	8985094
D_lanceolata_1732_MR	3077922	347048	6881382	143	174759	2903020
D_lanceolata_1733_MR	4258418	489381	12521542	168	373285	3884965
D_lanceolata_1762_MR	5361737	406787	4321364	261	104609	5256867
D_lanceolata_1763_MR	7648939	723441	15140401	330	418649	7229960
D_lanceolata_1855_AF	8219	162	9615	1	77	8141
D_lanceolata_1856_AF	8468	159	22064	0	182	8286
D_lanceolata_1857_AF	12344	1718	47224	0	1452	10892
D_lanceolata_1858_AF	9990405	792626	11568760	514	231313	9758578
D_lanceolata_1859_AF	2573	127	6885	0	82	2491
D_lanceolata_1860_AF	7388	462	14356	0	153	7235
D_lanceolata_1970_PalosVerdes	9827033	518111	9649582	495	98771	9727767
D_lanceolata_1971_OC	10141932	549600	9710859	538	87335	10054059
D_lanceolata_1972_SB	6339101	759620	21495419	243	651880	5686978
D_lanceolata_1973_VC	10637	367	18348	0	127	10510
D_lanceolata_1974_SD	2330520	125949	2457357	153	51799	2278568
D_lanceolata_1975_LA	1497129	156130	6389974	60	134682	1362387
D_lanceolata_1976_SD	2878651	245855	7422995	115	168060	2710476
D_lanceolata_1977_OC	9393012	566827	7918789	500	85368	9307144
D_lanceolata_1978_SLO	656923	100585	2380503	24	80339	576560
D_lanceolata_1979_SLO	8252986	697072	13707851	441	360538	7892007
D_lanceolata_1980_SLO	6976479	384414	6211734	362	72717	6903400
D_lanceolata_1981_VC	2447604	363602	6789457	105	274927	2172572

D_lanceolata_1990	7349345	486058	8432468	348	133400	7215597
D_lanceolata_1991	2458	13	2697	0	11	2447
D_lanceolata_1993	184	10	560	0	4	180
D_lanceolata_1994	5882432	605139	18161866	232	455352	5426848
D_lanceolata_1996	4650545	391212	10393244	191	253239	4397115
D_lanceolata_1997	5539561	964671	21391052	209	785695	4753657
D_lanceolata_1998	7534712	419793	8629982	320	156289	7378103
D_lanceolata_1999	3878907	191765	4498613	185	66863	3811859
D_verityi_1736_MR	4654092	199602	4672213	213	40383	4613496
D_verityi_1738_MR	6592746	581559	12743369	306	309992	6282448
D_verityi_1739_MR	4980257	376821	8151527	225	204227	4775805
D_verityi_1740_MR	5935712	438959	7396355	281	144547	5790884
D_verityi_1741_MR	6751844	331074	5262725	351	19636	6731857
D_verityi_1742_MR	3157380	223414	1891575	668	155494	3001218
D_verityi_1743_MR	2055246	131974	1861601	420	101577	1953249
D_verityi_1744_MR	630600	30837	516750	145	26422	604033
D_verityi_1746_MR	3708114	111013	881609	937	50283	3656894
D_verityi_1747_MR	2826373	207912	2158060	575	153262	2672536
D_verityi_1748_MR	3943624	207912	1812337	890	134417	3808317
D_verityi_1749_MR	4066860	139764	1665804	927	80184	3985749
D_verityi_1750_MR	5324706	88682	852369	1356	22779	5300571
D_verityi_1751_MR	2773893	291205	2345830	578	194921	2578394
D_verityi_1752_MR	2127085	57701	596697	529	29568	2096988
D_verityi_1753_MR	3977712	284309	2256132	895	171848	3804969
D_verityi_1754_MR	3077922	347048	6881382	143	174759	2903020
D_verityi_1755_MR	4258418	489381	12521542	168	373285	3884965
D_verityi_1756_MR	4375634	614896	20376961	145	529853	3845636
D_verityi_1757_MR	4893565	758435	21038953	163	664343	4229059
D_verityi_1758_MR	5376108	715986	25475705	173	654995	4720940
D_verityi_1759_MR	7388	462	14356	0	153	7235
D_verityi_1761_MR	2573	127	6885	0	82	2491
D_verityi_1833_AF	9990405	792626	11568760	514	231313	9758578
D_verityi_1834_AF	12344	1718	47224	0	1452	10892
D_verityi_1835_AF	3710846	347906	4849182	653	279653	3430540
D_verityi_1836_AF	6808416	91512	1130866	1579	15906	6790931
D_verityi_1837_AF	1473562	86238	1398488	302	68164	1405096
D_verityi_1838_AF	3236444	48362	527900	795	9671	3225978
D_verityi_1839_AF	6230989	154604	769803	1618	18276	6211095
D_verityi_1840_AF	3298626	98770	1048061	761	44114	3253751
D_verityi_1841_AF	1075744	30562	689730	226	21882	1053636
D_verityi_1842_AF	2597913	148115	3734392	484	116738	2480691
D_verityi_1843_AF	3390843	287922	2353479	720	219723	3170400
D_verityi_1844_AF	5593478	91653	732199	1512	14669	5577297

D_verityi_1845_AF	4903264	269632	2578052	1162	167282	4734820
D_verityi_1846_AF	5525369	625655	18626000	208	486449	5038712
D_verityi_1846_AF	5541928	221662	1976679	1328	96821	5443779
D_verityi_1847_AF	1935477	93451	787593	447	58536	1876494
D_verityi_1848_AF	2152089	73775	685195	538	36507	2115044
D_verityi_1849_AF	884254	34880	449976	219	24463	859572
D_verityi_1850_AF	184	10	560	0	4	180
D_verityi_1851_AF	4057409	310527	6582994	161	156284	3900964
D_verityi_1852_AF	5882432	605139	18161866	232	455352	5426848
D_verityi_1869_AF	4650545	391212	10393244	191	253239	4397115
D_verityi_1870_AF	5539561	964671	21391052	209	785695	4753657
D_verityi_1871_AF	7534712	419793	8629982	320	156289	7378103
D_verityi_1872_AF	3878907	191765	4498613	185	66863	3811859
D_verityi_1883_AF	825953	16652	363471	205	12174	813574
D_verityi_1884_TP	4802423	127093	1749130	1146	67138	4734139
D_verityi_1885_TP	2595688	18976	285736	715	2120	2592853
D_verityi_1886_TP	2346348	362965	2432756	513	275699	2070136
D_verityi_1887_TP	642	12	382	0	7	635
D_verityi_1888_TP	144993	1503	78493	29	1238	143726
D_verityi_1889_TP	25718	819	57872	0	506	25212
D_verityi_1890_TP	2968318	86879	2558280	636	44749	2922933
D_verityi_1891_TP	1312919	75797	128338	342	10269	1302308
D_verityi_1892_TP	2009416	24284	554914	473	13934	1995009
D_verityi_1893_TP	5443393	166403	1262833	1298	64199	5377896
D_verityi_1894_TP	5257893	296907	2018125	1332	165270	5091291
D_verityi_1895_TP	2407451	160195	2192699	500	126576	2280375
D_verityi_1896	4349	255	16682	0	242	4107
D_verityi_1897_TP	4243353	398466	3330704	909	304770	3937674
D_verityi_1898_TP	4090512	105251	1339544	1034	53816	4035662
D_verityi_1899_TP	3339305	61232	743994	855	25302	3313148
D_verityi_1900_TP	651442	1889	72691	158	132	651152
D_verityi_1901_TP	2070984	135802	843689	453	93986	1976545
D_verityi_1902_TP	1170900	65991	584436	257	45105	1125538
D_verityi_1903_TP	846499	56120	867332	168	44929	801402
D_verityi_1904_TP	3026837	53458	417280	789	10207	3015841
D_verityi_1905_TP	49	5	167	0	3	46
D_verityi_1907_TP	3659082	121905	2906866	752	85156	3573174
D_verityi_1908_TP	4006508	242084	4216720	879	181906	3823723
D_verityi_1909_TP	3399733	276856	3912118	667	209714	3189352
D_verityi_lanceolata_1764_MR	4008650	126651	2369163	937	72787	3934926
D_verityi_lanceolata_1765_MR	6794210	118939	1065915	1686	13721	6778803
D_verityi_lanceolata_1766_MR	3815159	246928	5805712	756	193670	3620733
D_verityi_lanceolata_1769_MR	2944422	180770	1339568	680	113588	2830154

D_verityi_lanceolata_1770_MR	2529979	202272	1449053	577	136443	2392959
D_verityi_lanceolata_1771	3261805	108339	1071159	829	49805	3211171
D_verityi_lanceolata_1772	2659747	325679	2223352	534	236771	2422442
D_verityi_lanceolata_1773	3304097	255421	5380374	621	210238	3093238
D_verityi_lanceolata_1774	4036621	389633	4186796	800	299467	3736354
D_verityi_lanceolata_1775	4332786	325961	4920045	871	210827	4121088
D_verityi_lanceolata_1776_MR	4143024	556243	7362081	718	456636	3685670
D_verityi_lanceolata_1777_MR	2629979	195190	1191980	593	112016	2517370
D_verityi_lanceolata_1778_MR	4634823	406589	2427332	1061	263482	4370280
D_verityi_lanceolata_1779_MR	2911593	140829	3845497	573	117535	2793485
D_verityi_lanceolata_1780_MR	3783580	509599	2882958	817	391062	3391701
D_verityi_lanceolata_1784_MR	6086044	80169	931432	1557	13052	6071435
D_verityi_lanceolata_1785	4654092	199602	4672213	213	40383	4613496
D_verityi_lanceolata_1785_MR	4119866	305720	4467486	872	232433	3886561
D_verityi_lanceolata_1786	2514233	209700	6478866	112	73557	2440564
D_verityi_lanceolata_1787	5525369	625655	18626000	208	486449	5038712
D_verityi_lanceolata_1789	6592746	581559	12743369	306	309992	6282448
D_verityi_lanceolata_1790	4980257	376821	8151527	225	204227	4775805
D_verityi_laneolata_1781_MR	2086266	134812	1166144	470	95134	1990662
D_verityi_laneolata_1782_MR	3699698	231261	4289550	703	195251	3503744