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Phylogenetic Relationships and Evolution in *Dudleya* (Crassulaceae)

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Abstract*—Dudleya* (Crassulaceae) is a genus of succulent perennials endemic to western North America. Interspecific relationships within *Dudleya* have been difficult to discern due to a lack of synapomorphic characters for the genus or subgenera, highly variable morphologies within species, and extensive polyploidy. Here we present the first molecular phylogeny of diploid members of the genus using sequences from nrDNA and cpDNA. We cloned ITS alleles from six known polyploid individuals to determine their evolutionary origin. We have been able to resolve four clades within *Dudleya*, but many relationships are still uncertain. Our analyses support the monophyly of the genus and show that *Dudleya* is more closely related to North American *Sedum* species of the Sedoideae subfamily than to members of its currently circumscribed subfamily, the Echeverioideae. The current subgeneric distinctions within *Dudleya* are polyphyletic and should be revised in future taxonomic treatments. We tested the monophyly of several highly variable species and found that *D. virens*, *D. cymosa*, and *D. abramsii* are polyphyletic. The ITS alleles from polyploid taxa were not variable enough to resolve polyploid origins.

***Keywords*—**California flora, cpDNA, ETS, ITS, nrDNA, polyploidy, recent radiation, *trnL–trnF*.

The genus *Dudleya* Britton & Rose (Crassulaceae) consists of approximately 45 species of succulent perennials (Thiede 2003; Mabberley 2008). The genus displays a range of highly diverse forms, from small, delicate geophytes measuring less than 10 cm across, to large rosettes up to half a meter in diameter. *Dudleya* species are endemic to western North America, from central Oregon to the tip of the Baja California peninsula, Mexico, and inland to Nevada, Arizona, and Sonora, Mexico. The genus is largely restricted to the winter-rainfall region of western North America (Thiede 2004), with the center of diversity in southern California and northern Baja California, mainly in coastal habitats (Moran 1960; Thiede 2003; McCabe 2012).

Using morphological characters to discern relationships within the Crassulaceae has proven challenging, and *Dudleya* has undergone several revisions. *Dudleya* species were originally placed into three other recognized genera, *Echeveria* DC., *Cotyledon* L., and *Sedum* L. (DeCandolle 1828; Bentham and Hooker 1865). Subsequent revisions of North American Crassulaceae classified these species within the newly recognized genera *Dudleya* Britton & Rose, *Hasseanthus* Rose, and *Stylophyllum* Britton & Rose (Britton and Rose 1903; Britton and Rose 1905). Moran (1942a, b, c) later proposed *Stylophyllum* as a subgenus of *Dudleya*, but kept *Hasseanthus* as a distinct genus. Later Uhl and Moran (1953) showed that *Dudleya*, *Hasseanthus*, and *Stylophyllum* have the same base chromosome number (n = 17), which was thought to be unique within the Crassulaceae at the time. As a result of this synapomorphy, Moran (1953) transferred all *Hasseanthus* species to *Dudleya.* In his only published treatment of the genus *Dudleya*, Moran (1960) concluded the genus consisted of three subgenera, *Dudleya*, *Hasseanthus*, and *Stylophyllum*. Previous molecular phylogenies of the Crassulaceae (35 genera and 1500 species) have included at most three *Dudleya* species (Van Ham and Hart 1998; Mort et al. 2001; Carrillo-Reyes et al. 2009). Limited sampling in these studies suggest that *Dudleya* is monophyletic, but *Sedum*, *Echeveria*, and *Graptopetalum*, close relatives of *Dudleya*, were found to be highly polyphyletic.

The subgeneric distinctions within *Dudleya* are based on petal orientation and stem morphology (Moran 1960; McCabe 2012). *Dudleya* subgenus *Dudleya* lacks an underground stem and has erect petals that form a tube-like corolla. Subgenus *Hasseanthus* has an underground stem and widely spreading petals. Subgenus *Stylophyllum* also has spreading petals but lacks an underground stem. Unfortunately, none of the morphological characters used to define these subgenera are synapomorphic, and they are convergent with other members of the Crassulaceae (Moran 1951a, 1960). Additionally, allozyme and limited sequencing data have cast doubt on the monophyly of the subgenera (Dodero 1995; Burton 2002; Thiede 2004).

Individual species within *Dudleya* exhibit diverse morphologies that often intergrade with other recognized taxa. This intraspecific variation has resulted in the recognition of species complexes. For example, *D. cymosa* (Lem.) Britton & Rose is so variable that eight subspecies are recognized by McCabe (2012). Members of *D. abramsii* Rose have been treated as subspecies of *D. cymosa* and currently six subspecies of *D. abramsii* are recognized by McCabe (2012). The *D. virens* (Rose) Moran complex, associated with the Santa Barbara Channel Islands, is currently divided into three subspecies. The monophyly of these species is uncertain, and it is unclear how much of the morphological variation within each species complex is the result of changing environmental variables across the range, hybridization, or divergent selection.

Understanding relationships within *Dudleya* is further complicated by hybridization and polyploidy. Approximately 35% of all *Dudleya* species are polyploid, with n = 34, 51, 68, 85, and ca. 119 recorded (Uhl and Moran 1953; McCabe 2012). Most species are known to be interfertile in the greenhouse regardless of morphology and ploidy level, and natural

hybrids have been reported in the genus (Moran 1951b; Uhl and Moran 1953). The extent of hybridization in nature is unclear, however. Uhl and Moran (1953) concluded that natural hybridization between diploid *Dudleya* and *Hasseanthus* was uncommon. Hybridization and allopolyploidy obscure the typical divergence patterns that molecular phylogenetics are able to reconstruct (Cronquist 1987; McDade 1990). Therefore, phylogenetic trees that include diploid hybrids or allopolyploids are unlikely to accurately depict true evolutionary relationships without additional sources of evidence and analyses.

Here we construct a molecular phylogeny of all diploid members of the genus using ITS, ETS, and *trnL–trnF* sequences. ITS and ETS are spacers associated with 18S-26S nuclear ribosomal DNA (nrDNA). The nrDNA gene can occur thousands of times in a plant genome, which makes isolation and amplification easy, and it undergoes rapid concerted evolution to homogenize conflicting gene copies (Baldwin et al. 1995; Baldwin and Markos 1998). While these regions have been very valuable for phylogenetic reconstruction in plants, high copy number and incomplete concerted evolution can be problematic especially when recent hybridization or allopolyploidy are suspected (Álvarez and Wendel 2003). We chose, therefore, to reconstruct the diploid relationships first using both nrDNA and cpDNA (Brown et al. 2002; Beck et al. 2010). Then using cloned ITS alleles from six known polyploid taxa, we generate hypotheses for the evolutionary origin of these polyploids.

Materials and Methods

***Taxon Sampling*—**In total, 41 *Dudleya* taxa, representing 27 diploid species from California and Mexico, were included in this study along with six polyploid species (Appendix 1). To assure monophyly of variable species, multiple specimens from different populations of some species were included. In total 84 individual *Dudleya* species were sampled. DNA was acquired from field-collected plants in the native range of individual taxa. California species were identified according to McCabe (2012). Baja California species were identified according to Moran (1960) and Thiede (2003). Taxa included in the analysis, collection locations, and voucher numbers are listed in Appendix 1. Seven of the nine outgroup taxa studied were chosen to represent the traditionally circumscribed Echeverioideae (Berger 1930), a subfamily of the Crassulaceae, as well as certain *Sedum* species that are reportedly closely related to some echeverioid genera, including *Dudleya* (Van Ham and Hart 1998; Mort et al. 2001). The goal of this sampling design was not necessarily to find the sister taxon to *Dudleya*, but to determine if our sequence data supported the results from Mort et al. (2001), which suggested that *Dudleya* is more closely related to several taxa in the Sedoideae than to Echeverioideae. *Kalanchoe fedtschenkoi* Raym.-Hamet & H. Perrier (subfamily Kalanchoideae) and *Aeonium lindleyi* Webb & Berthel. (subfamily Sempervivoideae) were selected as distant outgroups to root the entire tree (Van Ham and Hart 1998; Mort et al. 2001).

***DNA Extraction and PCR Amplification*—**Total genomic DNA was extracted from frozen leaf tissue stored at –80°C. Leaf tissue was ground in liquid nitrogen or disrupted using stainless steel beads and a MiniBeadbeater-16 (BioSpec Products, Bartlesville, Oklahoma) prior to extraction. Extractions were made using the DNeasy® plant mini kit (QIAGEN®, Valencia, California) according to the manufacturer's instructions. Target DNA regions were amplified via PCR using the primer combinations ETS-IGSf/18S-ETS (Baldwin and Markos 1998; Acevedo-Rosas et al. 2004), ITS1/ITS4 (White et al. 1990), and the universal trn primers C and F (Taberlet et al. 1991). The PCR was performed using GoTaq® green master mix (Promega, Fitchburg, Wisconsin). The PCR mix consisted of 12 μL GoTaq green, 1.2 μL of each primer at 10 μmol/L, 7.6 μL of water, and 2 μL of extracted DNA for a total volume of 24 μL. The PCR program ran at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and ended at 72°C for 5 min. For all diploid taxa, amplification and sequencing were performed using all three pairs of primers. Polyploid taxa were amplified with ITS primers only. All amplicons were purified using ExoSAP-IT® (Affymetrix, Santa Clara, California) according to the manufacturer's instructions.

***Cloning*—**Single alleles of the ITS region from six polyploid taxa were selected using the TOPO® TA Cloning® kit for sequencing with OneShot® TOP10 chemically competent *E. coli* (Invitrogen™, Carlsbad, California), according to the manufacturer's instructions. Kanamycin was used to select for recombinants. Ten single colonies were selected from each polyploid individual using toothpicks. Each toothpick was gently swirled in a microcentrifuge tube containing 6 μL GoTaq® green master mix (Promega), 4 μL water, and 1 μL of each M13 primer. Amplification conditions were as previously described, but with an additional 10 min 94°C incubation step at the beginning of the thermocycling program. The PCR products from the cloning reactions were purified as described above.

***Sequencing and Alignment*—**Amplified fragments were sequenced in both directions at UC Berkeley's DNA Sequencing Facility on an ABI 3100 Sequencer (Applied Biosystems, Foster City, California) using ABI dye terminator chemistry. We inspected all chromatograms for ambiguous bases and assembled contigs from the forward and reverse sequences using Geneious Pro 5.4.5 (BioMatters Limited, Auckland, New Zealand). We aligned contigs of each region first using the default parameters in MUSCLE (Edgar 2004) followed by hand editing in Mesquite (ver. 2.75; Maddison and Maddison 2011).

***Phylogenetic Analyses*—**Trees were constructed using Bayesian inference (BI) and maximum likelihood (ML). We used jModelTest (ver. 0.1.1; Posada 2008) to compare models of nucleotide substitution with unequal base frequencies, a proportion of invariable sites, and rate variation among sites. These options resulted in the testing of 88 models on a fixed BIONJ-JC tree. The combined ITS/ETS data matrix was evaluated separately from the *trnL–trnF* data matrix. The selection of the best-fit model of substitution was based on the ΔAIC and the associated Akaike weights (Posada and Buckley 2004). We coded gaps using SeqState (ver.1.4.1; Müller 2005) in two ways: Simple indel coding (SIC; Simmons and Ochoterena 2000) and multiple complex indel coding (MCIC; Müller 2006). We constructed trees using a combined ITS/ETS/*trnL–trnF* matrix, an ITS/ETS matrix, and a *trnL–trnF* matrix. Each matrix contained an indel partition with either binary SIC coded gap characters or multistate MCIC coded gap characters. The BI analyses were performed in MrBayes (ver. 3.1.2; Huelsenbeck and Ronquist 2001). A general time reversible (GTR) + gamma prior was specified for the combined ITS/ETS matrix and a GTR prior was specified for the *trnL–trnF* matrix. The –lnL and ΔAIC values for the GTR+ gamma model were 5,939.05 and 0.00, respectively. The –lnL and ΔAIC values for the GTR model were 1,593.16 and 0.00, respectively. All indel partitions were coded as variable with gamma distributed rates. Each BI analysis was conducted with two independent runs with four incrementally heated Markov Chain Monte Carlo chains. Trees were sampled every 1,000 generations for 20,000,000 generations, producing 20,000 trees. A majority rule consensus tree and posterior probabilities (PP) for each node were calculated from the trees after the first 25% of trees were discarded as burn-in. Convergence was checked in all cases to ensure the proper burn in value was used. Maximum likelihood inference of relationships with bootstrap support was made using RAxML (ver. 7.0; Stamatakis 2006) RAxML bootstrap analyses of 50,000 replicates were conducted with the GTR-gamma approximation. We used RAxML to place polyploid ITS alleles on the constrained topology of the ITS/ETS MCIC Bayesian tree of all diploid *Dudleya*.

Results

GenBank accession numbers for the ITS, ETS, and *trnL–trnF* regions are provided in Appendix 1, and data matrices are available on TreeBASE (study number S13362). The number of DNA characters and indel characters under SIC and MCIC are presented in Table 1. The combined Bayesian ITS/ETS/*trn*

Table 1. Summary of taxa and characters included in each analysis. Gaps were coded in two ways; simple indel coding (SIC) according to Simmons and Ochoterena (2000) and multiple complex indel coding (MCIC) according to Müller (2006).

	Taxa	Base pairs	MCIC indels	SIC indels
ITS/ETS	41	1,255	87	133
*trn*L–*trn*F	40	806	16	21

majority rule consensus tree is presented in Fig. 1. The nrDNA majority rule consensus tree is presented in Fig. 2. The cpDNA majority rule consensus tree is presented in Fig. 3. Bayesian posterior probabilities are presented above each branch. There is no difference in topology between trees constructed with MCIC or SIC and we therefore only present the results from MCIC trees.

Monophyly of Dudleya—All three data matrixes show *Dudleya* species form a well-supported monophyletic clade that corresponds to Moran's treatment of the genus (1.00 BI PP for the combined analysis). *Sedum spathulifolium* Hook. is the sister taxon to *Dudleya* in our analyses. The outgroup taxa fail to support the monophyly of *Echeveria* or *Sedum*.

Monophyly of Subgenera—Within *Dudleya*, there is generally weak support for deeper nodes within the tree. In the combined analysis, however, four clades are well resolved (Fig. 1). These clades have been labeled to facilitate discussion. All four major clades, the *Virens*, *Ingens*, *Formosa,* and *Blochmaniae* clades, are recovered with strong support in the nrDNA tree (Fig. 2). The cpDNA tree (Fig. 3) shows little resolution and the four major clades are not recovered in this analysis. Shading on all trees (Figs. 1–3) indicates the subgeneric distinctions. In all analyses, the subgenera within *Dudleya* are not monophyletic. Members of all three subgenera occur in the *Virens* clade. The *Ingens* clade is a mix of subgenera *Dudleya* and *Stylophyllum*. The *Blochmaniae* clade is predominately made up of members of subgenus *Hasseanthus* but also includes *D. verityi* K. M. Nakai, a member of subgenus *Dudleya*. The small *Formosa* clade consists entirely of some members of *Stylophyllum*.

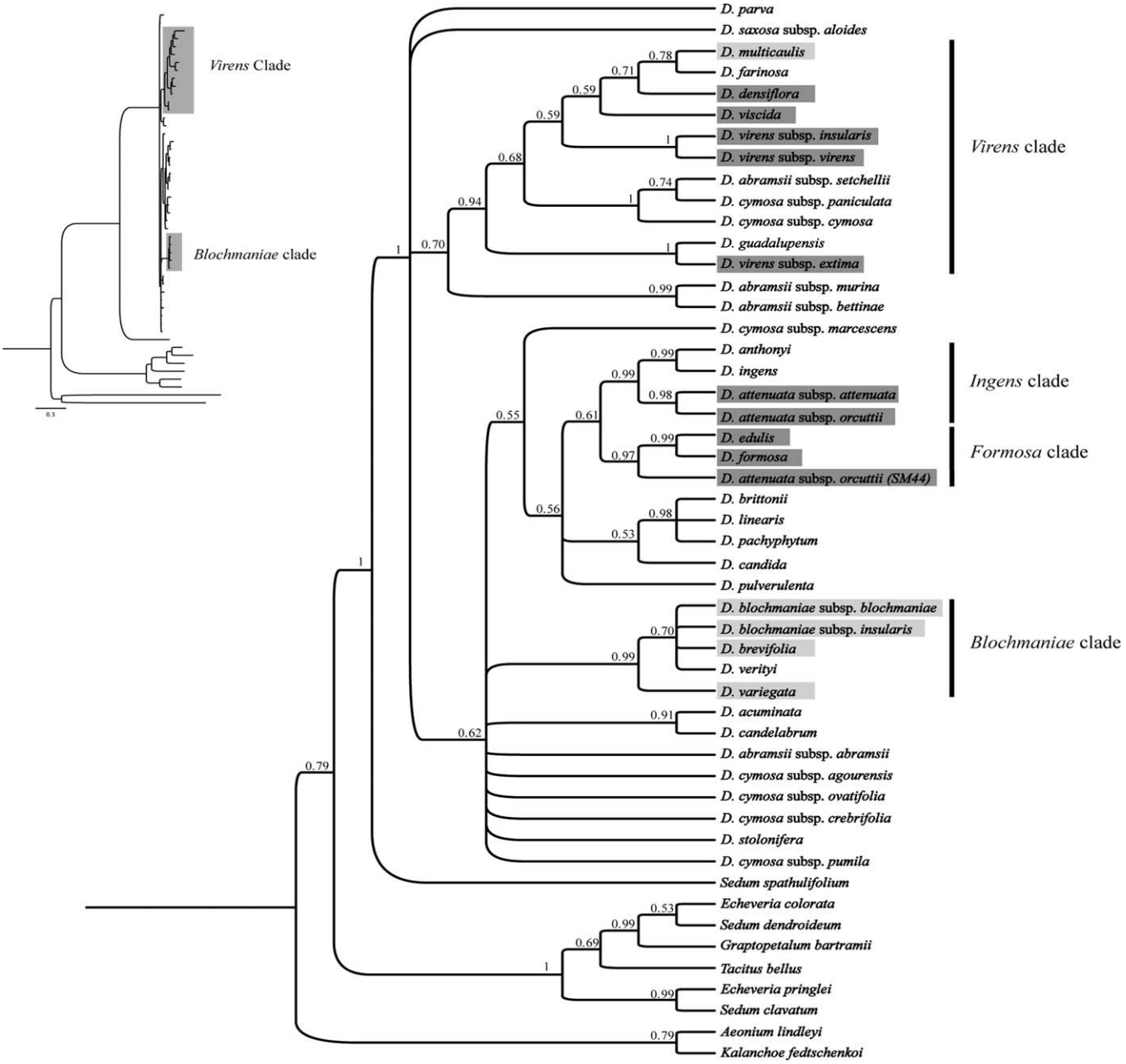

Fig 1. Bayesian consensus tree of combined ITS/ETS/*trnL–trnF* sequences. Bayesian posterior probabilities are reported for nodes over 0.50. Major clades are labeled to facilitate discussion. Inset tree shows branch lengths. Subgenus *Hasseanthus* is shaded in light grey, subgenus *Stylophyllum* in dark grey, and subgenus *Dudleya* is not shaded.

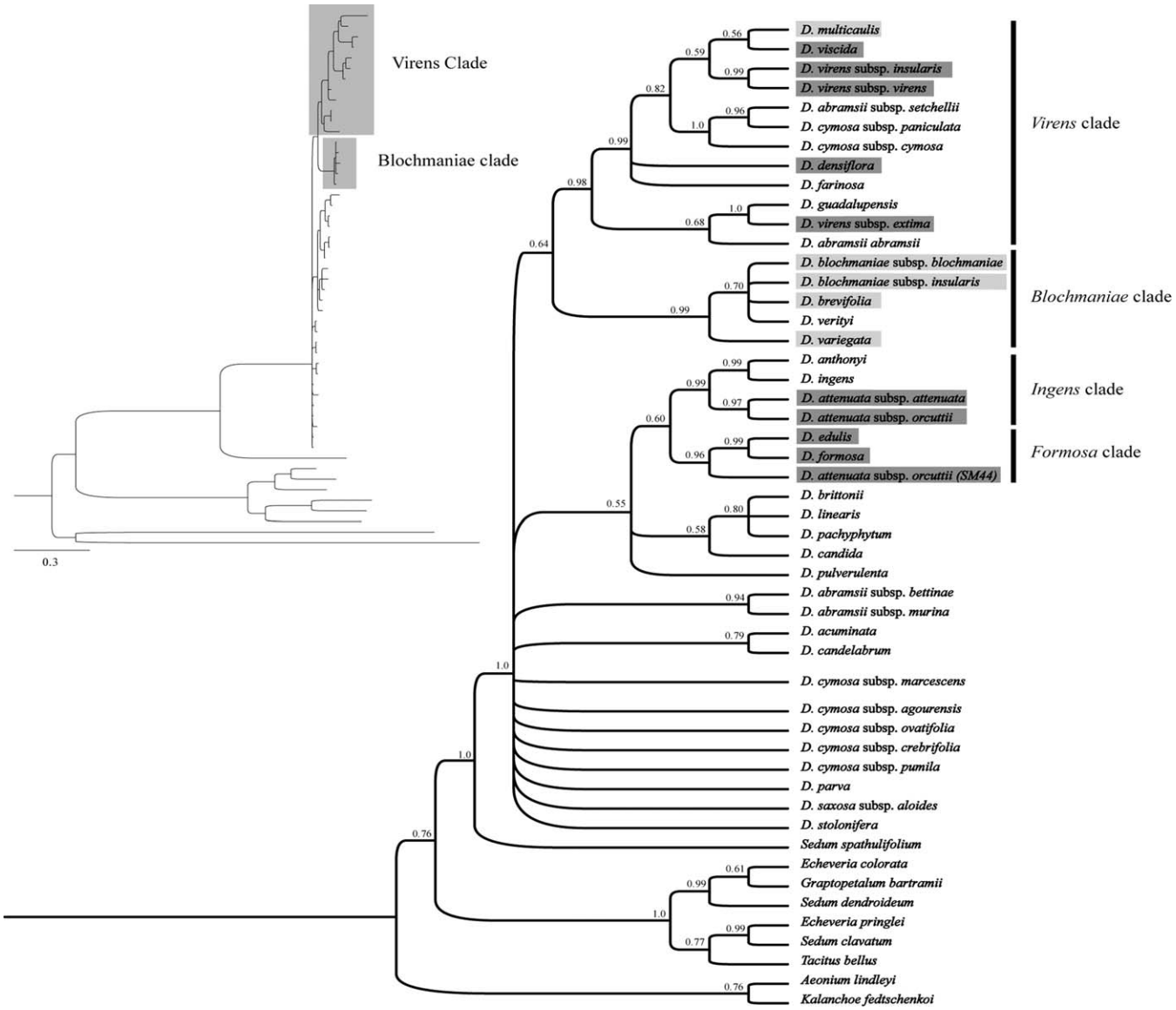

FIG 2. Bayesian consensus tree of ITS/ETS sequences. Bayesian posterior probabilities are reported for nodes over 0.50. Major clades are labeled to facilitate discussion. Inset tree shows branch lengths. Subgenus *Hasseanthus* is shaded in light grey, subgenus *Stylophyllum* in dark grey, and subgenus *Dudleya* is not shaded.

Species Complexes—While all three subspecies of *D. virens* occur in the *Virens* clade, the species is polyphyletic. *Dudleya virens* subsp. *insularis* (Rose) Moran and *D. v.* subsp. *virens* (Rose) Moran are strongly supported as sister taxa (1.0 BI PP; Fig. 1) but are not allied with *D. v.* subsp. *extima* Moran. Instead, *D. guadalupensis* Moran and *D. v.* subsp. *extima* are strongly supported as sister taxa (1.00 BI PP; Fig. 1). The same relationship is recovered in the nrDNA tree (Fig. 2). The cpDNA tree shows moderate support (0.88 BI PP) for a paraphyletic *D. virens,* with *D. guadalupensis* embedded within *D. virens* (Fig. 3). Two subspecies of *D. cymosa*, *D. cymosa* subsp. *paniculata* (Jeps.) K. M. Nakai and *D. c.* subsp. *cymosa* (Lem.) Britton & Rose, are most closely related to *D. abramsii* subsp. *setchellii* (Jeps.) Moran (1.00 BI PP) in the *Virens* clade. The nrDNA tree places *D. cymosa* subsp. *paniculata* sister to *D. abramsii* subsp. *setchellii* (0.96 BI PP), whereas the cpDNA tree places *D. cymosa* subsp. *cymosa* sister to *D. abramsii* subsp. *setchellii* (0.95 BI PP). The close alliance of *D. c.* subsp. *cymosa* with *D. c.* subsp. *paniculata* and *D. a.* subsp. *setchellii* may be an artifact of gene flow between taxa since collections for these three taxa came from Santa Clara County. The four other subspecies of *D. cymosa* are unresolved. *Dudleya abramsii* subsp. *bettinae* (Hoover) Bartel and *D. a.* subsp. *murina* (Eastw.) Moran, are strongly supported sister taxa in the combined tree (0.99 BI PP) and in the nrDNA tree (0.94 BI PP), but their relationship to other *Dudleya* remain uncertain. *Dudleya a.* subsp. *abramsii* Rose is unresolved in the combined analysis and is loosely associated with the *Virens* clade in the nrDNA tree (Fig. 2).

The *Ingens* clade (0.99 BI PP) consists of four taxa from Baja California and the nearby islands: *D. anthonyi* Rose, *D. ingens* Rose, and the two subspecies of *D. attenuata*, *D. a.* subsp. *attenuata* (S. Watson) Moran and *D. a.* subsp. *orcuttii* (Rose) Moran. The two *D. a.* subsp. *orcuttii* accessions that we used were not each other's closest relatives, with one being basal to *D. formosa* Moran and *D. edulis* (Nutt.) Moran in the *Formosa* clade and the other sister to *D. a.* subsp. *attenuata*.

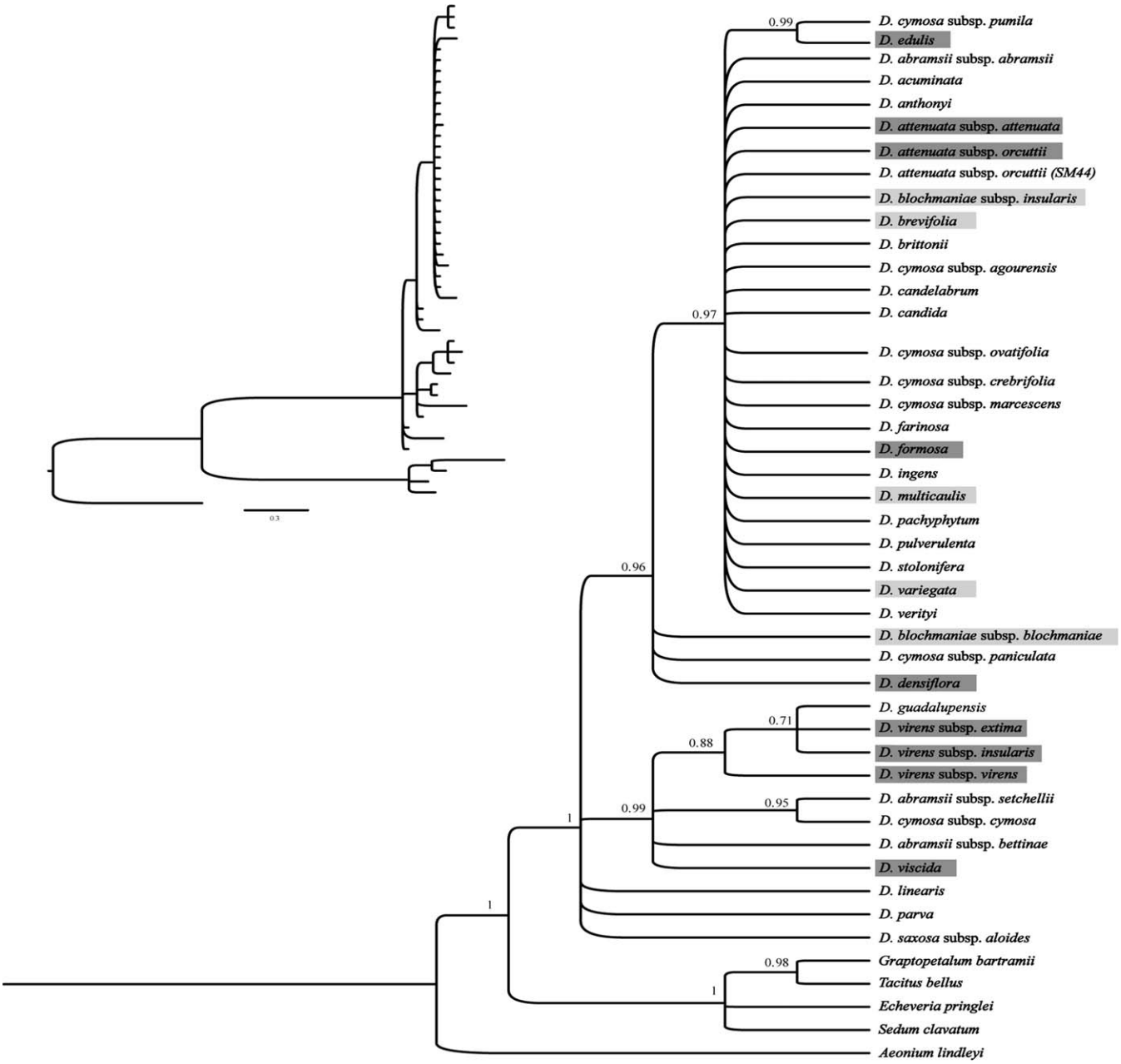

FIG 3. Bayesian consensus tree of chloroplast *trnL–trnF* sequences. Bayesian posterior probabilities are reported for nodes over 0.50. Inset tree shows branch lengths. Subgenus *Hasseanthus* is shaded in light grey, subgenus *Stylophyllum* in dark grey, and subgenus *Dudleya* is not shaded.

Polyploid Taxa—We amplified cloned ITS alleles from six polyploid taxa. We attempted to amplify at least ten alleles per individual, but the number of sequences obtained ranged from three to nine per species (Appendix 1). Polyploid ITS alleles were incorporated into the constrained topology of the diploid ITS/ETS Bayesian tree using RAxML. Maximum likelihood bootstrap values above 50% are shown in Fig. 4. One allele of *D. anomala* (Davidson) Moran occurs in the *Ingens* clade, sister to *D. anthonyi* Rose (64% ML BS). Seven *D. anomala* alleles are unresolved and basal to the *Ingens* clade, and one allele is unresolved within *Dudleya*. All seven *D. virens* subsp. *hassei* (Rose) Moran alleles occur together in a monophyletic group and are closely associated with three alleles of *D. traskiae* (Rose) Moran. These polyploids are weakly supported as sister to the *Blochmaniae* clade (74% ML BS). *Dudleya gnoma* S.W. McCabe, *D. nesiotica* (Moran) Moran, *D. greenei* Rose, and two alleles of *D. traskiae* are unresolved within *Dudleya*.

DISCUSSION

Our results indicate that *Dudleya* is a well supported monophyletic group within a polyphyletic subfamily of the Crassulaceae. *Sedum spathulifolium*, a wide ranging member of the Sedoideae subfamily, is sister to *Dudleya* in all phylogenetic reconstructions in which it was included. While it is unlikely that *S. spathulifolium* is the true sister taxon to *Dudleya*, due to incomplete sampling, our analyses indicate that *Dudleya* is more closely related to North American members of the Sedoideae than it is to members of the traditionally circumscribed Echeverioideae. Thiede and Eggli (2007) and

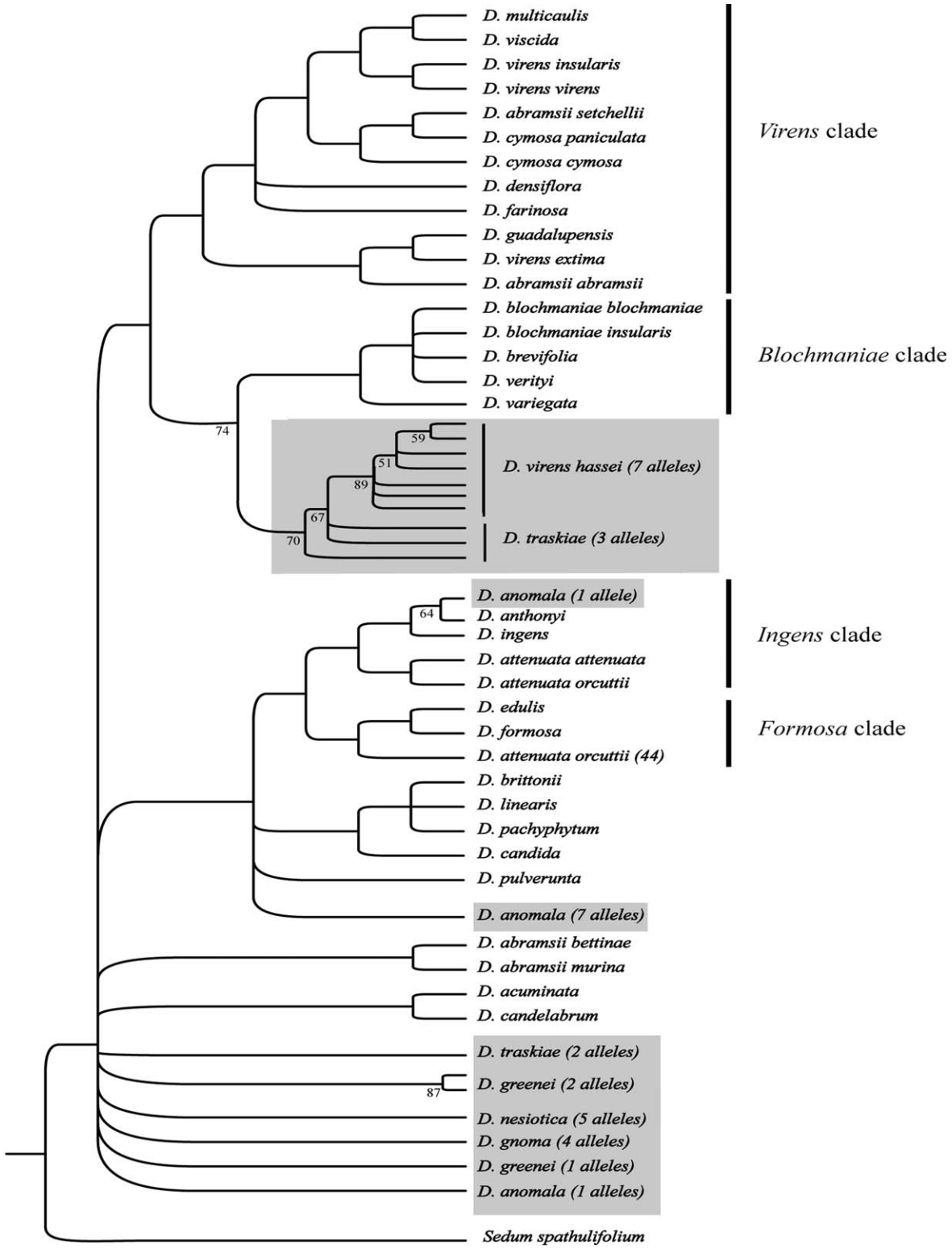

FIG 4. Bayesian consensus tree of chloroplast ITS/ETS sequences with ITS polyploid alleles. The topology of the tree was constrained to the ITS/ETS diploid tree and polyploid alleles were added using maximum likelihood. Bootstrap values are reported for each polyploid allele. ML BS values below 50% are not reported. Polyploid taxa are shaded in grey.

Gontcharova and Gontcharova (2009) concluded that *Dudleya* may be sister to *Sedum* subg. *Gormania* sect. *Gormania*. The relationships that we have found among the outgroup taxa are consistent with previous phylogenetic reconstructions of the family (Van Ham and Hart 1998; Burton 2002; Mort et al. 2002), and support the conclusion that subfamilies Echeverioideae and Sedoideae are not monophyletic (Carrillo-Reyes et al. 2009).

While our analyses strongly support the monophyly of the genus *Dudleya*, they do not support the monophyly of the

subgenera. Our results show that the subgenera of *Dudleya* are polyphyletic, and they should therefore be reconsidered in future taxonomic treatments. Traditionally used morphological characters such as petal orientation and stem morphology are not good indicators of relatedness in the genus. Petal orientation is known to be variable even within a species (Aigner 2004; Aigner 2005). A drought deciduous habit, often associated with subgenus *Hasseanthus*, can be observed in many *Dudleya* species if drought conditions are prolonged (D. Burton pers. obs.).

Our results suggest that some of the most variable species complexes within *Dudleya* are polyphyletic. The complexity surrounding *D. virens,* which has been treated with up to four subspecies (McCabe 2012), could result from misclassification or recent gene flow. *Dudleya virens* subsp. *virens* and *D. v.* subsp. *insularis* are sister taxa closely related to *D. multicaulis* and *D. viscida*. *Dudleya virens* subsp. *extima*, a rare species from Guadalupe Island, is sister to *D. guadalupensis*, also from the island. The genetic similarity between these two taxa may have resulted from hybridization on Guadalupe Island, or they may share a recent common ancestor. The fact that *D. guadalupensis* is embedded within a *D. virens* clade in the cpDNA reconstruction supports the possibility of recent gene flow occurring between *D. virens* subsp. *extima* and *D. guadalupensis*. The fourth subspecies of *D. virens, D. v.* subsp. *hassei* (Rose) Moran is a polyploid from Catalina Island, off the California coast. All of the ITS alleles cloned from *D. v.* subsp. *hassei* suggest autopolyploid origins for the species and a close relationship not with the other *D. virens* subspecies but with members of the *Blochmaniae* clade.

Within the *Virens* clade are a number of subspecies of *D. cymosa* and *D. abramsii*. *Dudleya cymosa* is a highly variable taxon that occurs throughout the foothills of California, and has been treated as nine different subspecies (McCabe 2012). Many of the relationships among subspecies remain unresolved with *D. c.* subsp. *agourensis* K. M. Nakai, *D. c.* subsp. *ovatifolia* (Britton) Moran, *D. c.* subsp. *crebrifolia* K. M. Nakai & Verity, *D. c.* subsp. *pumila* (Rose) K. M. Nakai, and *D. c.* subsp. *marcescens* Moran stemming from a large polytomy at the base of the tree. However, two of the *D. cymosa* subspecies, *D. c.* subsp. *cymosa* and *D. c.* subsp. *paniculata*, occur in the *Virens* clade as sister to *D. abramsii* subsp. *setchellii*, a federally listed endangered species from Santa Clara County, California. Interestingly, these three taxa have overlapping distributions in the coastal ranges of the eastern San Francisco Bay Area and gene flow could be occurring. *Dudleya abramsii* subsp. *setchellii* has in the past been treated as a subspecies of *D. cymosa* (Moran 1951a) or as a species (Britton and Rose 1903; Bartel 1993) but is currently treated as a subspecies of *D. abramsii* (McCabe 2012). The taxonomy of *D. abramsii* subsp. *setchellii* should be reevaluated. Within other *D. abramsii* taxa, the relationship of *D. a.* subsp. *abramsii* is unresolved, but we are confident in the close relationship between *D. a.* subsp. *bettinae* and *D. a.* subsp. *murina*, two well supported sister taxa. These two taxa are both endemic to San Luis Obispo County and occur on serpentine soils. It appears then that all three species complexes under investigation, *D. virens*, *D. cymosa*, and *D. abramsii*, show some degree of polyphyly.

The *Blochmaniae* clade is a well-supported group with some morphological unity. Most of the taxa in this clade have small, round or club shaped leaves, which are unique within *Dudleya,* and resemble *Sedum*. For this reason, Burton (2002) hypothesized that the traditionally circumscribed subgenus *Hasseanthus* is basal within the genus. Our results are inconclusive as to the placement of the *Blochmaniae* clade within the genus; it could stem from the base of the phylogeny. *Dudleya verityi*, a narrow endemic of the northern Santa Monica Mountains of Ventura County, California, is an exception to the morphological unity of this clade, in that it has medium sized linear leaves and semi-erect to spreading petals. *Dudleya verityi* is sympatric with *D. blochmaniae* in the Santa Monica Mountains and this close relationship could result from hybridization. Known hybrids between the two species have been reported (Nakai 1983). *Dudleya multicaulis* has been treated in subgenus *Hasseanthus* but our results place it within the *Virens* clade. The leaves of *D. multicaulis*, like those of the *Blochmaniae* clade species, are drought deciduous, but unlike the *Blochmaniae* clade species, the leaves of *D. multicaulis* are much longer, widest at the base, and have an acute apex. Our result is corroborated by allozyme data that placed *D. multicaulis* outside of Dodero's *Hasseanthus* clade (Dodero 1995).

The *Ingens* clade contains four species from the same geographic region. These include *D. anthonyi*, *D. ingens*, and the two subspecies of *D. attenuata*, *D. a.* subsp. *attenuata* and *D. a.* subsp. *orcuttii*, all of which occur in Baja California and the surrounding islands. Both of our collections of *D. a.* subsp. *orcuttii* came from Punta Banda, Baja California. One falls in the *Ingens* clade, sister to *D. a.* subsp. *attenuata,* as would be expected, but the other *D. a.* subsp. *orcuttii* collection falls in the *Formosa* clade along with *D. edulis*, and *D. formosa*. These three species are sympatric in southern coastal California and northern Baja California, and it is possible that some gene flow is occurring between them.

Considering that 35% of the species in the genus *Dudleya* are polyploids, polyploidy has likely contributed to the diversification of the genus. Polyploidy often confers immediate reproductive isolation between parental taxa and the polyploid offspring, and is therefore an important mechanism of speciation (Grant 1981; Coyne and Orr 2004; Rieseberg and Willis 2007; Wood et al. 2009; Mayrose et al. 2011). It is unclear how many polyploid species within *Dudleya* have resulted from hybridization (allopolyploidy) or genome doubling (autopolyploidy). Our results suggest that *D. virens* subsp. *hassei* is likely of autopolyploid origin, but more work is necessary to determine the evolutionary origins of other polyploid *Dudleya*.

This study represents a comprehensive phylogeny of diploid *Dudleya*, but many questions remain. Using nrDNA and cpDNA, we were able to resolve four well supported clades within *Dudleya*. The ITS, ETS, and *trnL–trnF* regions do not provide sufficient variation to fully resolve *Dudleya* and other markers should be employed to understand how the genus is diversifying. The lack of variation at these loci could indicate gene flow followed by concerted evolution at nuclear loci or very short divergence times between taxa. Future studies employing single-copy or low-copy DNA regions will be useful in further elucidating relationships within the genus and polyploid origins (Sang 2002). Based on results from the markers employed in this study, no significant divergence exists among the major clades of *Dudleya*, and it remains unclear which clades within *Dudleya* are basal or derived. Additionally, the true sister group to *Dudleya* will remain unknown until more members of the Crassulaceae, especially North American *Sedum*, are sampled for phylogenetic analysis.

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Appendix 1. Dudleya species included in our analyses. Taxa are grouped according to traditionally treated subgenera. SEM = subfamily Sempervivoideae, SED = subfamily Sedoideae, ECH = Echeverioideae, KAL = Kalanchoideae. UCSC = U. C. Santa Cruz Herbarium. SDSU = San Diego State University Herbarium. - = no sequence obtained. Polyploid taxa are bolded. Data are presented in the order of taxon, ploidy, location, GenBank numbers for ITS, ETS, trnL–trnF, and voucher number. Unless otherwise noted, specimens are from California, U. S. A.

Subgenus* Dudleya—D. abramsii* subsp. *abramsii, n* = 17, Descanso, San Diego County, JX960490, JX960453, JX960531, UCSC SM64-1220. *D. abramsii* subsp. *bettinae, n* = 17, Cayucos, San Luis Obispo County, -, JX960454, JX960532, UCSC SM101-A4124-216. *D. abramsii* subsp. *murina, n* = 17, San Luis Obispo County, JX960491, JX960455, -, UCSC SM65-A1235-35. *D. abramsii* subsp. *setchellii, n* = 17, Tulare Hill, Santa Clara County, -, JX960456, JX960533, UCSC SM66. *D. acuminata, n* = 17, MEXICO. Cedros Island, Baja California, JX960492, JX960457, JX960534, UCSC SM67-16340. *D. anthonyi, n* = 17, MEXICO. Isla San Martin, Baja California, JX960493, JX960458, JX960535, UCSC SM68. *D. brittonii, n* = 17, MEXICO. Rio San Miguel, Baja California, JX960500, JX960464, JX960542, UCSC SM55-450. *D. candelabrum, n* = 17, Santa Rosa Island, Santa Barbara County, JX960501, JX960465, JX960543, UCSC SM49-02-242. *D. candida, n* = 17, MEXICO. Los Coronados Island, Baja California, JX960502, JX960466, JX960544, UCSC SM57. *D. cymosa* subsp. *agourensis, n* = 17, Santa Monica Mountains, Los Angeles County, -, JX960467, JX960545, UCSC SM100. *D. cymosa* subsp. *crebrifolia, n* = 17, Fish Canyon, Los Angeles County, JX960503, JX960468, JX960546, UCSC SM74. *D. cymosa* subsp. *cymosa, n* = 17, Santa Clara County, JX960504, JX960469, JX960547, UCSC SM75-1223. *D. cymosa* subsp. *marcescens, n* = 17, Ventura County, -, JX960470, JX960548, UCSC SM85-A912-3. *D. cymosa* subsp. *ovatifolia, n* = 17, Topanga Canyon, Los Angeles County, JX960505, JX960471, JX960549, UCSC SM94-A926-19. *D. cymosa* subsp. *paniculata, n* = 17, Pacheco Pass, Santa Clara County, JX960506, JX960472, JX960550, UCSC SM87.

D. cymosa subsp. *pumila, n* = 17, San Bernardino Mountains, San Bernardino County, JX960507, -, JX960551, UCSC SM88-934. *D. farinosa, n* = 17, Carmel, Monterey County, JX960510, JX960474, JX960554, UCSC SM13-A1119-26. ***D. gnoma* (4 alleles),** *n* = 34, Santa Rosa Island, Santa Barbara County, KC426965-KC426968, -, -, UCSC SM50-792. ***D. greenei* (3 alleles),** *n* = 34, Santa Cruz Island, Santa Barbara County, KC426969-KC426971, -, -, UCSC SM17-A977-4. *D. guadalupensis, n* = 17, MEXICO. Zapato Island, Baja California, JX960512, JX960476, JX960556, UCSC SM41-87.333. *D. ingens, n* = 17, 34, MEXICO. Northern Baja California, JX960513, JX960477, JX960557, UCSC SM90-676. *D. linearis, n* = 17, W. MEXICO. San Benito Island, Baja California, JX960514, JX960478, JX960558, UCSC SM47-86.568. *D. pachyphytum, n* = 17, MEXICO. Cedros Island, Baja California, JX960516, JX960480, JX960560, UCSC SM45. *D. parva, n* = 17, Mont Clef Ridge, Ventura County, JX960517, JX960481, JX960561, UCSC SM92-723. *D. pulverulenta, n* = 17, Coast, Santa Barbara County, JX960518, JX960482, JX960562, UCSC SM24-A1122-2. *D. saxosa* subsp. *aloides, n* = 17, San Felipe, San Diego County, -, JX960483, JX960563, UCSC SM102-1222. *D. stolonifera, n* = 17, Laguna Canyon, Orange County, JX960519, -, JX960564, UCSC SM97-603. *D. verityi, n* = 17, Santa Monica Mountains, Los Angeles County, JX960521, JX960485, JX960566, UCSC SM98-A558-8.

Subgenus* Hasseanthus—D. blochmaniae* subsp. *blochmaniae, n* = 17, 34, 54, Santa Cruz Island, Santa Barbara County, JX960497, JX960461, JX960539, UCSC SM48-1006. *D. blochmaniae* subsp. *insularis, n* = 17, Santa Rosa Island, Santa Barbara County, JX960498, JX960462, JX960540, UCSC SM72. *D. brevifolia, n* = 17, Torrey Pines, San Diego County, JX960499, JX960463, JX960541, UCSC SM73-5. *D. multicaulis, n* = 17, Laguna Canyon, Orange County, JX960515, JX960479, JX960559, UCSC SM91.

***D. nesiotica* (5 alleles),** *n* = 34, Santa Cruz Island, Santa Barbara County, KC426972-KC426976, -, -, UCSC SM61-751.

D. variegata, n = 17, Mission Trails Regional Park, San Diego County, JX960520, JX960484, JX960565, SDSU MS3180.

***Subgenus* Stylophyllum—*D. anomala* (9 alleles),** *n* = 34, MEXICO. Todos Santos Island, Baja California, KC426956-KC426964, -, -, UCSC SM46-89.255. *D. attenuata* subsp. *attenuata, n* = 17, MEXICO. Nueva York, Baja California, JX960494, JX960459, JX960536, UCSC SM109-A123611. *D. attenuata* subsp. *orcuttii, n* = 17, 34, MEXICO. Punta Banda, Baja California, JX960495, JX960460, JX960537, UCSC SM44-441. *D. attenuata* subsp. *orcuttii, n* = 17, 34, MEXICO. Punta Banda, Baja California, JX960496, -, JX960538, UCSC SM108-660D. *D. densiflora, n* = 17, San Gabriel Canyon, Los Angeles County, JX960508, -, JX960552, UCSC SM11-86.189. *D. edulis, n* = 17, San Diego, San Diego County, JX960509, JX960473, JX960553, UCSC SM12-941, SDSU DB100. *D. formosa, n* = 17, MEXICO. Rio San Miguel, Baja California, JX960511, JX960475, JX960555, UCSC SM43-454. ***D. traskiae* (5 alleles),** *n* = 34, Santa Barbara Island, Santa Barbara County, KC426977-KC426981, -, -, UCSC SM26-147. ***D. virens hassei* (7 alleles),** *n* = 34, Emerald Cove, Catalina Island, KC426982-KC426988, -, -, UCSC SM29. *D. virens* subsp. *extima, n* = 17, MEXICO. Guadalupe Island, Baja California, JX960522, JX960486, JX960567, UCSC SM42-A1222-3. *D. virens* subsp. *insularis, n* = 17, San Nicolas Island, Ventura County, -, JX960487, JX960568, UCSC SM30-806, UCSC SM31-842, UCSC SM32-88.48. *D. virens* subsp. *virens, n* = 17, 34, San Clemente, Orange County, -, JX960488, JX960569, UCSC SM33-937, UCSC SM34-936, UCSC SM36-939, UCSC SM37-935. *D. viscida, n* = 17, Oceanside, San Diego County, JX960523, JX960489, JX960570, UCSC SM63.

Outgroups—*Aeonium lindleyi,* SEM,, JX960524, -, AY082248. *Echeveria colorata,* ECH,, JX960525, -, -. *Echeveria pringlei,* ECH,, AY545687, AY540517, AY540555. *Graptopetalum bartramii,* ECH,, JX960526, AY540520, AY540557. *Kalanchoe fedtschenkoi,* KAL,, JX960527, -, -. *Sedum clavatum,* SED,, AY545713, AY540542, AY540576. *Sedum dendroideum,* SED,, JX960528, -, -., *Sedum spathulifolium,* SED,, JX960529, -, -. *Tacitus bellus,* ECH,, JX960530, AY540547, AY540579.,