

# Directionally biased habitat shifts and biogeographically informative cytonuclear discordance in the Hawaiian silversword alliance (Compositae)

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## Abstract

**Premise:** Expanded phylogenetic analyses of the Hawaiian silversword alliance (*Argyroxiphium*, *Dubautia*, *Wilkesia*; Compositae) were undertaken to assess evolutionary and biogeographic informativeness of cytonuclear discordance and any biases in evolutionary directionality of ecological transitions within this prominent example of adaptive radiation.

**Methods:** Samples spanning the geographic and ecological distributions of all recognized taxa were included in phylogenetic and biogeographic analyses of nuclear ribosomal DNA (nrDNA) and cpDNA sequences. Bayesian model testing approaches were used to model ecological evolution and the evolution of nuclear chromosomal arrangements while accounting for phylogenetic uncertainty.

**Results:** Cytonuclear discordance detected previously appears to reflect chloroplast capture, at least in part, with nrDNA trees being largely congruent with nuclear chromosomal structural data and fine-scale taxonomy. Comparison of biogeographic histories estimated from the posterior distributions of nrDNA and cpDNA trees, including inferred chloroplast-capture events, provides additional resolution of dispersal history, including a back-dispersal to Maui Nui from Hawai'i. A newly resolved major nrDNA clade of endemic Kaua'i taxa that mostly were described as new-to-science since the 1980s strengthens the earlier hypothesis that diversification on Kaua'i has not waned since the island began to decline in area through subsidence and erosion. Bias in habitat shifts was estimated, with transitions from dry-to-mesic or -wet and from wet-to-mesic or -bog habitats dominating diversification of the silversword alliance from a dry-adapted tarweed ancestor.

**Conclusions:** The habitat-transition biases estimated here may indicate more limited pathways of ecological evolution than proposed previously for an adaptive radiation involving such major ecological shifts.

## KEYWORDS

adaptive radiation, Asteraceae, dispersal, evolutionary bias, habitat transitions, Hawaii, hybridization, island biogeography, Madiinae, silverswords

Diversification involving evolutionary transitions between highly contrasting environments or ecological niches is a hallmark of adaptive radiation on islands (Schluter, 2000). Examples of repeated evolution of ecologically disparate forms in different island-like settings have been resolved in a diversity of organismal lineages (e.g., Mahler et al., 2013; Gillespie et al., 2018; Pouchon et al., 2018), as has evidence for deep hybridization spurring major radiations (Marques et al., 2019). Even with refined understanding of processes associated with insular evolution, uncertainty often remains about the

directionality of ecological change and any biases in evolutionary transitions that may reflect constraints or extrinsic limitations associated with diversification. Such uncertainty can stem from lack of phylogenetic resolution or conflicting lines of phylogenetic evidence in young clades, wherein hybridization, incomplete lineage sorting (ILS), and short branch-duration times can complicate interpretation of evolutionary and biogeographic histories (see Crawford and Archibald, 2017). A model-testing framework that utilizes posterior distributions of phylogenetic trees can help account for some of these

limitations in phylogenetic uncertainty and a lack of a priori understanding of the mode of ecological evolution (Huelsenbeck et al., 2000). In addition, enhanced taxon sampling across clades has potential to allow interpretations of phylogenetic incongruence that are evolutionarily and biogeographically meaningful (e.g., Hedtke et al., 2006).

A case in point is the endemic Hawaiian clade known as the silversword alliance (*Argyroxiphium*, *Dubautia*, and *Wilkesia*; Compositae–Madiinae), which has been studied from diverse perspectives (see Carlquist et al., 2003) and is a textbook example of insular adaptive radiation or evolutionary radiation more generally (Judd et al., 2016; Urry et al., 2021). Studies of the silversword alliance have yielded well-documented examples of multiple ecological shifts between contrasting habitats (Baldwin and Robichaux, 1995) associated in part with anatomical and ecophysiological innovations (e.g., Carlquist, 1957, 2003a, b; Robichaux et al., 1990), extensive divergence across lineages (disparification) in life forms (Ackerly, 2009; Nürk et al., 2019) and leaf functional traits (Blonder et al., 2016), rapid diversification (Baldwin and Sanderson, 1998; Landis et al., 2018), and chromosomal structural evolution including dysploidization (Carr and Kyhos, 1981, 1986; Carr, 2003a). Understanding of evolutionary and biogeographic patterns and processes in the group has been hampered to some extent, however, by uncertainty about the causes of phylogenetic incongruence between plastid and nuclear DNA datasets (Baldwin, 1997, 2003), incomplete phylogenetic resolution within some diverse clades (Baldwin and Robichaux, 1995), and lack of sampling in previous phylogenetic investigations of some ecologically distinctive rare, inaccessible, or recently described taxa, especially from montane bog habitats.

Higher congruence has been found between nuclear genomic arrangements (Carr and Kyhos 1981, 1986; Carr, 2003a) and molecular phylogenetic trees of the silversword alliance when structural genomes, differing by one or more whole-arm chromosomal interchanges, were mapped onto nuclear ribosomal DNA (nrDNA) clades than when these genomic arrangements were mapped onto cpDNA clades (Baldwin et al., 1990; Baldwin, 1997, 2003). Those findings, and the strong incongruence between nrDNA and cpDNA trees in general, have been interpreted as evidence for cpDNA capture via hybridization, rather than cpDNA ILS, based on deep separation of some taxa bearing the same genomic arrangements in cpDNA trees (Baldwin, 1997, 2003). The sole example from earlier studies of congruence between cpDNA and nrDNA trees but incongruence of both of those lines of phylogenetic evidence with nuclear genomic arrangements involved a taxon implicated in having a recombinant or homoploid hybrid origin, *Dubautia scabra* [authors of names of plant taxa are given in Appendix 1] (Friar et al., 2008). Sampling of additional populations and taxa has been needed to explore further the basis for phylogenetic incongruence across data sets, especially given evidence for natural hybridization (Carr and Kyhos, 1981; Carr, 1995, 2003b; Friar et al., 2007; Garcia-Verdugo et al., 2013) and gene flow (Caraway et al., 2001; Lawton-Rauh et al., 2007; Remington and Robichaux, 2007) between species of the silversword

alliance, wherein natural and artificial hybrids of all 70 different intergeneric and interspecific combinations examined have been found to be partially to fully fertile (Carr, 2003b).

Previous studies based on nrDNA internal transcribed spacer (ITS) sequences have indicated that silversword alliance taxa endemic to the same island, island group, or volcano often belong to a locally endemic clade or grade that spans a diversity of habitats and often growth forms (Baldwin and Robichaux, 1995; Baldwin, 2003; Landis et al., 2018), consistent with ecological diversification. In contrast, some insular plant and animal groups evidently have extensively diversified primarily allopatrically, with dispersal between similar habitats on different islands or volcanoes (see Baldwin et al., 1998; Gillespie and Baldwin, 2010). Landis et al. (2018) obtained additional biogeographic and ecological resolution, with strong support for the hypothesis that the silversword alliance follows the progression rule of Hawaiian biogeography—i.e., with dispersal primarily from older to younger islands or island groups (Funk and Wagner, 1995). Landis et al. (2018) also found strong evidence against the related hypothesis that diversification has been primarily limited to the growth phase of islands, with evidence for much diversification on Kauaʻi after loss of island area to subsidence and erosion. Landis et al. (2018) noted that erosional dissection of the terrain may have spurred continued diversification by creating local habitat diversity and opportunities for isolation, in keeping with the often-narrow distributions of Kauaʻi taxa and Whittaker et al.'s (2008) general dynamic theory of oceanic island biogeography. Directionality of habitat shifts and any biases thereof in the silversword alliance that might indicate evolutionary constraints, or lack of reciprocal evolutionary lability more generally, have remained unresolved.

Here, we present phylogenetic results from expanded analyses of nrDNA, including ITS and external transcribed spacer (ETS) sequences, and cpDNA sequences of four rapidly-evolving gene regions from representatives of populations spanning much of the geographic and ecological distributions of all currently recognized taxa in the silversword alliance. These results are evaluated in comparison with published chromosomal structural data, current taxonomic concepts, and vouchered insular and ecological occurrence records to ask: (1) Is cytonuclear discordance in the silversword alliance evolutionarily or biogeographically informative? (2) Do biogeographic analyses with expanded taxon sampling provide additional resolution of dispersal events between islands or volcanoes that bear on the progression rule of Hawaiian biogeography? And (3) What does the history of ecological shifts reveal about adaptive radiation of the silversword alliance?

## MATERIALS AND METHODS

### Population sampling

All 45 minimum-rank taxa (in 33 species) of *Argyroxiphium*, *Dubautia*, and *Wilkesia* recognized in the *Flora of the Hawaiian Islands* (Wagner et al., 2005) were sampled from all islands and

most volcanoes of known occurrence for sequences of nrDNA (ITS and ETS) and cpDNA (3'-*ndhF* gene, 5'-*trnK* intron, *rpl16* intron, and *psbA-trnH* intergenic spacer). The likely extinct *A. virescens* was represented by a putative natural hybrid (*A. "Pu'u 'Alaea"*) with *A. sandwicense* subsp. *macrocephalum* (Carr and Medeiros, 1998). *Dubautia kenwoodii*, also probably extinct, was sampled from the type specimen. Individuals representing extensive morphological diversity in *Dubautia* from the summit bog of Wai'ale'ale, Kaua'i were sampled for ITS and ETS sequences to verify their identities, including parentage of putative hybrids (see "Assessment of natural hybridization" below). All DNAs examined earlier for cpDNA restriction sites and ITS sequences by Baldwin et al. (1990), Baldwin and Robichaux (1995), and Baldwin and Sanderson (1998) were included among the samples. North American tarweeds of subtribe Madiinae, including the "Madiia" lineage sensu Baldwin (2003) to which the silversword alliance belongs, and the sister group to Madiinae, *Arnica* (Baldwin and Wessa, 2000), were also sampled. A representative of tribe Madiaceae sensu Baldwin (Baldwin and Wessa, 2000) from outside the *Arnica*-Madiinae clade, *Hulsea algida*, served as the outgroup (see Appendix 1 for voucher information).

## DNA sequencing

Standard DNA extraction and polymerase chain reaction (PCR) methods were used to amplify nrDNA and cpDNA regions, as detailed in Appendix S1. The 3' end of the nrDNA ETS, upstream of the 18S gene, was amplified and sequenced using primers ETS-Hel-1 and 18S-ETS (Baldwin and Markos, 1998). The four cpDNA regions were amplified and sequenced using the following primer pairs: (1) *trnK*-3914F (Johnson and Soltis, 1994) and 884R (Panero and Crozier, 2003) for the 5'*trnK* intron and 5' end of *matK*; (2) 1587MADIA and 607R (Panero and Crozier, 2003) for the 3' end of *ndhF* and *ndhF-ycf1* intergenic spacer; (3) *psbAF* and *trnHR* (McGlaughlin and Friar, 2011) for the 3' end of *psbA* and *psbA-trnH* intergenic spacer; and (4) F71 (Jordan et al., 1996) and R1516 (Kelchner and Clark, 1997) for the *rpl16* intron.

ITS amplification products from 10 samples across *Dubautia* (chosen based on nucleotide polymorphism at phylogenetically informative sites or prior phylogenetic evidence for a hybrid history of the taxon) were cloned using the zero blunt TOPO cloning kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA), following the manufacturer's protocol. For these samples, i.e., BGB 667 (*D. imbricata* subsp. *imbricata*), BGB 1386 (*D. kalalauensis*), Carr 1047 (*D. knudsenii* subsp. *knudsenii*), BGB 675 (*D. latifolia*), Carr 1044 (*D. microcephala*), BGB 668 (*D. pauciflorula*), and BGB 530, 1184, 1213, and 1267 (both subspecies of *D. scabra*), products were pooled prior to cloning from three replicate PCRs per sample, with only 20 rounds of cycling per reaction to limit possible PCR drift or selection (see Wagner et al., 1994), in an attempt to detect any variation across nrDNA repeats that might reflect a history of hybridization.

Sanger sequencing of both DNA strands for all PCR products was performed at the University of California, Berkeley DNA Sequencing Facility (Barker Hall). For sequencing of the ITS region, ITS5 (White et al., 1990) was used instead of ITS-I (Urbatsch et al., 2000). Opposing DNA strands for each sample were assembled and reconciled using Geneious 6.1.8 (see Data Availability for GenBank accession numbers).

## Phylogenetic and chromosomal analyses

DNA sequences of each gene region were aligned separately using MAFFT version 7.017, as implemented in Geneious 6.1.8 with default parameters, and refined manually using the similarity criterion of Simmons (2004). Resulting sequence matrices were concatenated into an nrDNA (ETS + ITS) matrix and a four-gene cpDNA matrix after preliminary phylogenetic analysis of separate gene regions (not shown) indicated lack of conflict between ETS and ITS trees or among trees of each of the four cpDNA regions based on comparisons of clade support from bootstrap values and posterior probabilities. Prior to phylogenetic analysis of each concatenated matrix, samples assignable to the same minimum-rank taxon of Carr and colleagues (see Wagner et al., 2005) were merged into a common operational taxonomic unit (OTU) if they were identical at all sites or potentially so with resolution of sequence polymorphisms (hereafter called identical). The cpDNA matrix represented 111 OTUs, with a combined length of 3410 bp plus 28 insertions/deletions (indels) treated as additional characters using simple indel coding (Simmons and Ochoterena, 2000); the nrDNA matrix included 98 OTUs, with a combined length of 1307 bp.

For phylogenetic analyses, the combined spacers (ETS, ITS-1, and ITS-2) and the 5.8S gene were treated as two distinct partitions in the nrDNA matrix and the four plastome regions were treated as a single partition in the cpDNA matrix based on results using PartitionFinder version 1.1.1 (Lanfear et al., 2012). The best-fitting nucleotide substitution model was chosen by ModelFinder (Kalyaanamoorthy et al., 2017), using the Bayesian information criterion. The GTR substitution model (Tavaré, 1986; Rodriguez et al., 1990) with rate variation across sites modeled as a discretized gamma distribution (Yang, 1994) was applied to the ETS, ITS-1, and ITS-2. For the 5.8S gene we used a TrN substitution model (Tamura and Nei, 1993) with a proportion of invariable sites. For the cpDNA matrix we applied the GTR substitution model uniformly to all nucleotide sites with rate variation across sites modeled as a discretized gamma distribution. For the cpDNA indels we used the F81 substitution model (Felsenstein, 1981). Maximum likelihood phylogenetic analyses were conducted using IQ-Tree version 1.6.12 for MacOSX (Nguyen et al., 2015). Branch support was obtained with ultrafast (UF) nonparametric bootstrapping (1000 replicates), and a Shimodaira-Hasegawa (SH)-like approximate likelihood ratio test (aLRT; 1000 replicates) (Guindon et al., 2010). For Bayesian inference of phylogeny, Markov chain Monte Carlo (MCMC; Metropolis

et al., 1953; Hastings, 1970) analyses were run in RevBayes (Höhna et al., 2016) for 40,000 iterations, with each iteration consisting of 944 independent Metropolis-Hastings steps. Trees were sampled every 10 iterations, and the first 25% of all samples were discarded as burnin. The maximum a posteriori (MAP) tree was then calculated from the 3000 sampled trees. Secondary calibrations for divergence time estimation were obtained from Landis et al.'s (2018) re-analysis of a fossil-calibrated cpDNA dataset for Compositae (Barreda et al., 2015), with inclusion of sequences from additional taxa of subtribe Madiinae and close relatives (see Data Availability for information on accessing sequence matrices and trees).

The history of nuclear chromosomal arrangements (Carr and Kyhos, 1986; Carr, 2003a) was estimated on both the cpDNA and nrDNA trees, as done earlier for a subset of the samples and sequences studied here (Baldwin, 1997, 2003), to evaluate clade incongruence between cpDNA and nrDNA trees based on the relatively low likelihood of homoplasy in chromosomal rearrangements compared to nucleotide substitutions in general (e.g., O'Grady et al., 2001). The evolution of nuclear chromosomal arrangements was modeled using a seven-state model, where each state represented a different chromosomal arrangement. The seven chromosomal arrangements were characterized by Carr and Kyhos (1986) and Carr (2003a) as *Dubautia* genomes 1–5 and *Argyroxiphium* genomes 1 and 2. *Dubautia* genome 2 was treated as structurally identical to the *Wilkesia* genome of Carr and Kyhos (1986) and Carr (2003a), consistent with available cytogenetic data. The rate of chromosomal translocations,  $t$ , was estimated using an exponential prior with the mean rate of one translocation over the length of the phylogeny. The rate of transition between each of the chromosomal arrangements was set to  $t/n$ , where  $n$  is the number of rearrangements differentiating the two genomes according to Carr and Kyhos (1986) and Carr (2003a). The number of chromosomal rearrangements differentiating *Argyroxiphium* genomes 1 and 2 from some of the other genomes is uncertain (Carr and Kyhos, 1986). For those transitions, we estimated each rate using a mixture distribution in which the rate could be  $t$ ,  $t/2$ ,  $t/3$ , or  $t/4$ , with equal prior probability. Ancestral chromosomal arrangements and parameters were estimated using MCMC analyses in RevBayes (Höhna et al., 2016). Phylogenetic uncertainty was accounted for by sampling across 3000 trees of the posterior distributions estimated as described above. The MCMC was run for 5000 iterations, with each iteration consisting of eight separate Metropolis-Hastings steps. The first 10% of samples were discarded as burnin.

## Assessment of natural hybridization

Previous documentation of a wide diversity of natural hybrids in the silversword alliance (Carr and Kyhos, 1981; Carr, 2003b) warranted instances of sampling to detect unconfirmed hybridization, especially in the summit bog of Wai'ale'ale, Kaua'i, where four taxa of *Dubautia* (*D. imbricata* subsp. *acronaea*, *D. laxa* subsp. *hirsuta*, *D. paleata*, and *D. waialealae*) are

sympatric and evidently hybridize (Carr, 1985, 1999). Direct ETS and ITS sequences of putative hybrids were examined for additivity of mutations diagnostic for each of the four sympatric taxa, i.e., for double-peaks in electropherograms corresponding to nucleotide states that differentiate taxa. Pollen stainability of flowering hybrids was also assessed by light microscopy from a sample of 200+ grains per individual as an estimate of fertility to consider intrinsic potential for gene flow and possible chromosomal differentiation between parental taxa of unknown genomic arrangement. Pollen from late-stage floral buds of dried specimens was stained overnight with cotton blue in lactophenol prior to microscopic examination.

## Estimation of biogeographic history

Biogeographic history was estimated for the nrDNA and cpDNA trees at the scale of island or, for the prehistoric island Maui Nui (including modern-day islands of Lana'i, Maui, and Moloka'i), island group using the Dispersal-Extinction-Cladogenesis (DEC) model (Ree and Smith, 2008), as implemented in RevBayes (Höhna et al., 2016). Probabilities for biogeographic states were obtained from sampling across 3000 trees of the posterior distribution for each of the two sets of Bayesian-inference trees, from nrDNA and cpDNA sequences. All non-Hawaiian taxa were coded as North American. OTUs representing samples from multiple geographic areas were coded so that the tip state probabilities were equal among that OTU's observed geographic areas. Ancestral ranges were estimated during an MCMC analysis that was run for 5000 iterations, with each iteration consisting of 15 separate Metropolis-Hastings steps. The first 10% of samples were discarded as burnin.

In addition, isolation-by-distance (Wright, 1943) analysis were performed for the nrDNA and cpDNA datasets using Mantel (1967) tests to compare log-transformed phylogenetic distance in molecular branch lengths and log-transformed geographic distance among all samples. For each of the two datasets, 1000 Mantel tests were performed, with sampling across the posterior distribution of trees to account for phylogenetic uncertainty. Mantel tests were performed using the *ade4* package (Dray and Dufour, 2007) in the R programming language (R Core Team, 2017).

## Estimation of growth-form and habitat transitions

Each Hawaiian population sampled for the phylogenetic analysis was assigned to one of five growth-form states—cushion-plant, liana, rosette-plant, shrub, or tree—following the habit designations of Blonder et al. (2016), which align closely with those of Robichaux et al. (1990). Each Hawaiian population also was assigned to one of three habitat states—dry, wet, or bog—based on the ecological designations in table 1 of Robichaux et al. (1990) and Blonder et al. (2016), with “cinder and lava” treated as “dry” except for *Dubautia scabra*, which

likely accesses abundant water at depth (Robichaux, 1984) and was scored only as “wet.” Other taxa or populations not scored previously for habitat under the above classification were assigned designations consistent with that system, based on plant associations, local climate, and published descriptions. As an alternative approach to ecological categorization, each Hawaiian population was assigned to one of four habitat states—dry, mesic, wet, or bog. The bog designation remained unchanged and the other three designations were based on annual rainfall (dry, <1200 mm; mesic, 1200–2500 mm; wet, >2500 mm), following Gagné and Cuddihy's (1990) moisture regimes for Hawaiian vegetation and mean annual rainfall estimates for collection localities (Giambelluca et al., 2013; <http://rainfall.geography.hawaii.edu/>). For taxa from Lana'i high montane sites, where fog precipitation is extensive relative to rainfall, habitats were scored as “wet,” as reflected by field records and moisture zonation estimates for the Hawaiian Islands (Price et al., 2012; <https://hub.arcgis.com/datasets/HiStateGIS::moisture-zones>). For North American taxa, habitat designations for both sets of habitat categorizations were based on average annual precipitation estimates from PRISM data (period 1981–2010; <http://www.prism.oregonstate.edu/normals/>).

Growth-form and habitat evolution under both systems of habitat classification were each estimated for the nrDNA trees in RevBayes (Höhna et al., 2016). Reversible-jump MCMC (Green, 1995) was used to explore a set of continuous-time Markov models of character evolution and to infer ancestral states while marginalizing over the posterior distribution of trees. The growth-form analyses were restricted to the silversword alliance because of lack of overlap in habit between North American and Hawaiian members of the “*Madia*” lineage (Baldwin, 2003). The set of models of character evolution considered included all models in which each transition rate was either estimated using an exponential prior with the mean rate of one transition over the length of the phylogeny or fixed to zero. The reversible-jump MCMC sampled from the models in proportion to their posterior probability. This approach enabled model-fit comparisons through Bayes factors (Kass and Raftery, 1995) and provided the opportunity to account for both phylogenetic and model uncertainty by making model-averaged ancestral state and parameter estimates (Madigan and Raftery, 1994; Kass and Raftery, 1995; Huelsenbeck et al., 2004; Freyman and Höhna, 2018). The MCMC was run for 5000 iterations, with each iteration consisting of 360 separate Metropolis-Hastings steps. The first 10% of samples were discarded as burnin.

## RESULTS

### nrDNA and cytogenetic congruence

The high level of congruence between nrDNA trees and nuclear chromosomal data (Carr and Kyhos, 1986; Carr, 2003a) for the silversword alliance shown earlier

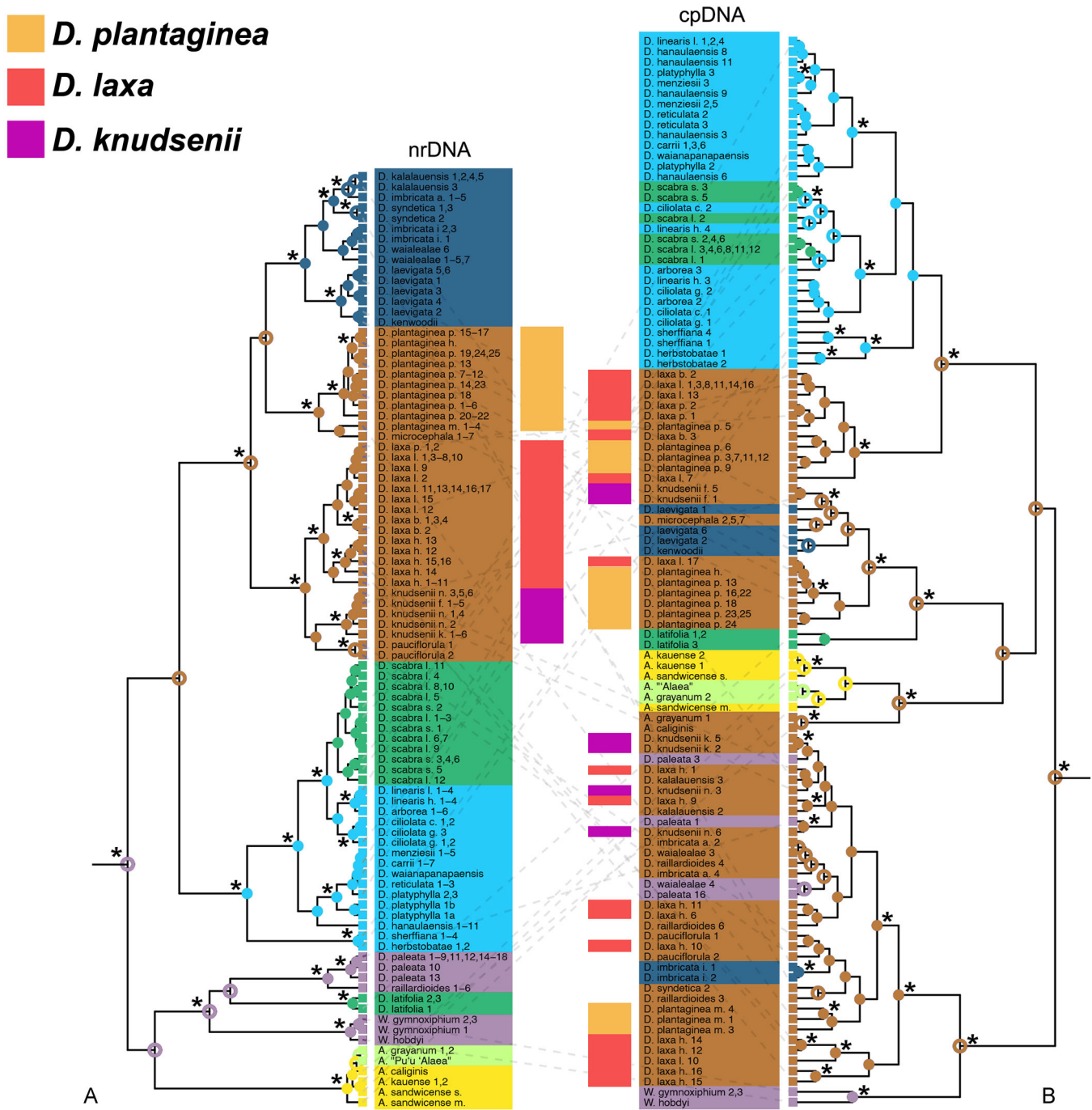
(Baldwin, 1997, 2003) remains evident with extended sequencing of nrDNA to include ETS and sampling of additional taxa and populations (Figure 1A; see Appendix S1 for summary of nrDNA clades). Single origins of four of the five nuclear chromosomal arrangements documented for *Dubautia* by Carr and Kyhos (1986) can be inferred from the nrDNA trees, with the caveat that chromosomal structural data are lacking for some taxa (see below). Only *Dubautia* genome 4 (Carr and Kyhos, 1986), shared by *D. latifolia* and *D. scabra* (Carr, 2003a), is mapped with two origins on the nrDNA tree. As shown by Friar et al. (2008), the nested position of *D. scabra*, with 14 pairs of chromosomes, among the other taxa of *Dubautia* sect. *Railliardia*—all of which have 13 pairs of chromosomes and share a common genomic arrangement (*Dubautia* genome 5; Carr and Kyhos, 1986)—in cpDNA and nrDNA trees is not corroborated by MADS-box gene trees, which often place *D. scabra* outside a clade containing the  $x = 13$  taxa. Resolution of a robust *Dubautia* genome 4 clade containing *D. latifolia* and *D. scabra* based on phylogenetic analysis of *APETALA1-A* data of Barrier et al. (1999) for a subset of taxa in the silversword alliance also indicates that the deep separation of *D. latifolia* and *D. scabra* in both the nrDNA and cpDNA trees probably does not reflect homoplasy in nuclear chromosomal arrangements but instead a history of hybridization in the *D. scabra* lineage.

### cpDNA and cytogenetic incongruence

The incongruence between structural nuclear chromosomal data (Carr and Kyhos, 1986; Carr, 2003a) and cpDNA trees based on restriction site mutations (Baldwin et al., 1990) discussed earlier for a subset of the samples studied here (Baldwin, 1997, 2003) was reinforced based on inclusion of representatives of more populations and species than in previous studies (Figure 1B). As shown by Baldwin et al. (1990), each of the three deepest, well-supported cpDNA clades containing members of *Dubautia* include taxa with *Dubautia* genome 1 (Carr and Kyhos, 1986; Carr, 2003a), namely *D. knudsenii*, *D. laxa*, *D. microcephala*, or *D. plantaginea*, together with taxa with other genomic arrangements. Although ancestral state estimates based on the cpDNA trees indicated *Dubautia* genome 1 as homologous across these three clades, multiple origins of *Dubautia* genome 3 (in *D. imbricata* subsp. *imbricata* and *D. laevigata*), as well as *Dubautia* genome 4 (in *D. latifolia* and *D. scabra*), were resolved (also see Appendix S1).

### nrDNA and taxonomic congruence

Sampling to include representatives of multiple populations within minimum-rank taxa demonstrated high congruence between fine-scale nrDNA clades or



**FIGURE 1** Maximum a posteriori (MAP) trees, displayed as a tanglegram, for the Hawaiian silversword alliance based on Bayesian phylogenetic analyses of (A) 18S-26S nuclear ribosomal (nrDNA) internal and external transcribed spacer (ITS and ETS) region sequences, and (B) cpDNA sequences (3' end of *ndhF* and *ndhF-ycf1* intergenic spacer, 5' *trnK* intron and 5' end of *matK*, *rpl16* intron, and *psbA-trnH* intergenic spacer) of the Hawaiian silversword alliance (and relatives, not shown). Nuclear chromosomal arrangements (Carr and Kyhos, 1986; Carr, 2003a) are shown at nodes (and, for terminal taxa, as colored background) as estimated by MCMC analysis using a model informed by the number of reciprocal translocations differentiating pairs of genomes (filled circles,  $\geq 95\%$  posterior probability (PP); open circles,  $< 95\%$  PP). Asterisks indicate clades with  $\geq 95\%$  PP. For generic abbreviations in taxon names, A. = *Argyroxiphium*; D. = *Dubautia*; W. = *Wilkesia*. Numbers following names of terminal taxa correspond to numbering of different vouchered collections in Appendix 1. Colored blocks corresponding to three species (*D. knudsenii*, *D. laxa*, and *D. plantaginea*) are shown to highlight congruence of nrDNA clades and incongruence of cpDNA clades with current taxonomic hypotheses. See Appendix S1 for more details on clades resolved here

sequence variants and circumscriptions of species or subspecies by Carr and colleagues (Figure 1A), who used multiple lines of evidence to delimit taxa (e.g., Carr, 1985). Possible instances of incongruence between

nrDNA clades and taxonomic concepts involved only unresolved or questionable nrDNA relationships, with  $< 95\%$  posterior probability or bootstrap support, or  $< 80\%$  SH-aLRT support (see Appendix S1).

## cpDNA and taxonomic incongruence

Sampling within species and subspecies demonstrates that incongruence between cpDNA trees and nuclear chromosomal arrangements extends to incongruence between cpDNA trees and taxon delimitations of Carr and colleagues (see Wagner et al., 2005) (Figure 1B). For example, samples of the most widespread species of the silversword alliance—*Dubautia plantaginea*—from three different sets of islands, namely, (1) Kauaʻi, (2) Lanaʻi, Molokaʻi, and Oʻahu, and (3) Maui and Hawaiʻi (Big Island), are separated across the three major, well-supported cpDNA clades that contain taxa of *Dubautia*. Sequences of *D. laxa* were also resolved in each of the three major cpDNA clades that include members of *Dubautia*, with representatives of *D. laxa* subsp. *hirsuta* deeply separated from representatives of *D. laxa* subsp. *bryanii*, subsp. *laxa*, and subsp. *pseudoplantaginea* (except for one sample of subsp. *laxa*—BGB 1391, from Mt. Olympus, southern Koʻolau Range, Oʻahu—in the same cpDNA clade as members of subsp. *hirsuta*). The only sample of *D. laxa* subsp. *laxa* from East Maui (Oppenheimer H61224), where it is rare and at the geologically youngest edge of its geographic distribution, was resolved in a cpDNA clade with all sequences of (co-occurring and putatively fully interfertile) *D. plantaginea* from Maui and deeply separated from all other representatives of *D. laxa*. Similarly, sequences of *D. knudsenii* were separated across two of the three major cpDNA clades, with representatives of subsp. *knudsenii* and subsp. *nagatae* in a different major cpDNA clade than representatives of subsp. *filiformis*.

The deep separation of conspecific cpDNA lineages of *D. knudsenii*, *D. laxa*, and *D. plantaginea* evidently does not reflect major taxonomic problems in circumscriptions of each of the three species, i.e., conspecific sequences of *D. knudsenii* and *D. laxa* were resolved as exclusive nrDNA clades, and conspecific sequences of *D. plantaginea* constituted two nrDNA clades in an unresolved polytomy with *D. microcephala*, consistent with potential monophyly of *D. plantaginea*. Within *D. laxa*, all representatives of subsp. *laxa*—including BGB 1391 (placed with subsp. *hirsuta* in cpDNA trees) and Oppenheimer H61224 (placed with *D. plantaginea* from Maui in cpDNA trees), mentioned above—were resolved in an nrDNA clade with representatives of subsp. *bryanii* and subsp. *pseudoplantaginea*, which in turn constituted a deeper *D. laxa* clade also including all representatives of subsp. *hirsuta*. [Note: McGlaughlin and Friar's (2011) representative of subsp. *laxa* from the southern Koʻolau Range (Puʻu Konahuanui) also was placed with subsp. *hirsuta* in their cpDNA trees.]

Incongruence between clade composition and taxonomic delimitations of Carr and colleagues was also apparent within major cpDNA clades (Figure 1B), although lack of resolution and support for cpDNA relationships at that level was an obstacle to evaluating the extent of such conflict. For example, *Dubautia kalalauensis* in part (BGB 1441) and *D. laxa* subsp. *hirsuta* in part (KRW 12190)

constituted a clade that is not reflected by nrDNA trees, wherein representatives of each of the two species were united strongly as separate subclades within different major clades of the silversword alliance. Similarly, *D. knudsenii* subsp. *nagatae* in part (Carr 1322) and *D. paleata* in part (Carr 1375), from nearby habitats on Kauaʻi, constituted a robust cpDNA clade but belong to well-supported nrDNA clades of only conspecific samples. Maui Nui endemic *D. linearis* subsp. *linearis* was resolved as part of a cpDNA clade containing all Maui Nui endemic *Dubautia* taxa whereas Big Island (Hawaiʻi) endemic *D. linearis* subsp. *hillebrandii* was resolved to be part of a (modestly supported) cpDNA clade with all Big Island endemic *Dubautia* taxa. In contrast, nrDNA sequences of all representatives of both subspecies of *D. linearis* and of *D. arborea* were identical, consistent with potential monophyly of *D. linearis*, and were part of a well-supported clade containing all species of sect. *Railliardia* that occur at least in part on the Big Island.

Within *Argyroxiphium*, incongruence between cpDNA relationships and taxonomic delimitations of Carr and colleagues (Figure 1B) is difficult to evaluate with lack of nrDNA phylogenetic resolution and uncertainty of nuclear chromosomal arrangements in the true silverswords and greenswords. As resolved earlier from cpDNA data (Baldwin et al., 1990; McGlaughlin and Friar, 2011), the West Maui endemic ʻEke silversword, *A. caliginis*, and the greensword, *A. grayanum*, from West Maui were united as a lineage to the exclusion of *A. grayanum* from East Maui in the cpDNA trees. All representatives of the Mauna Loa silversword, *A. kauense*, and Mauna Kea silversword, *A. sandwicense* subsp. *sandwicense*, constituted a robust Big Island cpDNA clade to the exclusion of the Maui endemic Haleakala silversword, *A. sandwicense* subsp. *macrocephalum*, as also resolved in cpDNA trees of McGlaughlin and Friar (2011).

## Cloning results and sequence polymorphisms

Examination of cloned ITS sequences from select taxa, including *D. latifolia* and *D. scabra*, did not provide convincing evidence for conflicting nrDNA phylogenetic signals that reflect a history of hybridization, which may have been obscured by concerted evolution. Cloned ITS sequences for the taxa examined (see Data Availability for GenBank accession numbers) were mostly either identical to direct sequences of the same sample or taxon, or had a distinct state that was not found at that site in other Hawaiian taxa. For individual cloned sequences, nucleotide states not shared with the direct sequence for that sample but shared with at least one sample (including cloned sequences) of other Hawaiian taxa occurred at only one or, less commonly, two position(s) at most. When treated as OTUs in phylogenetic analyses, cloned sequences were resolved together with members of the same, finest-scale, well-supported clade (results not shown).

## Characterization of spontaneous natural hybrids

Additivity for diagnostic parental-taxon nucleotide states in direct sequences of ETS and ITS allowed for documentation of multiple hybrids of each pairwise combination between the four taxa of *Dubautia* in the Wai'ale'ale summit bog, Kaua'i (Figure 2), including the previously undocumented hybrid combinations *D. imbricata* subsp. *acronaea* × *D. laxa* subsp. *hirsuta*, and *D. laxa* subsp. *hirsuta* × *D. waialealae*, as well as two other previously undocumented hybrid combinations from elsewhere on Kaua'i: *D. imbricata* subsp. *imbricata* × *D. laxa* subsp. *hirsuta*, and *D. paleata* × *D. raillardoides* (Appendix 2). Pollen stainabilities in molecularly verified hybrids from Wai'ale'ale were as follows: *D. imbricata* subsp. *acronaea* × *D. laxa* subsp. *hirsuta* (51.9%, KRW 13339), *D. imbricata* subsp. *acronaea* × *D. waialealae* (99.5%, KRW 12530), and *D. laxa* subsp. *hirsuta* × *D. waialealae* (61%, KRW 12505; 98.8%, KRW 13343—a putative backcross or later generation hybrid). Implications of these data for chromosomal arrangements

are presented in Appendix S1 and are consistent with estimates of uncertain chromosomal states in the nrDNA tree (Figure 1A).

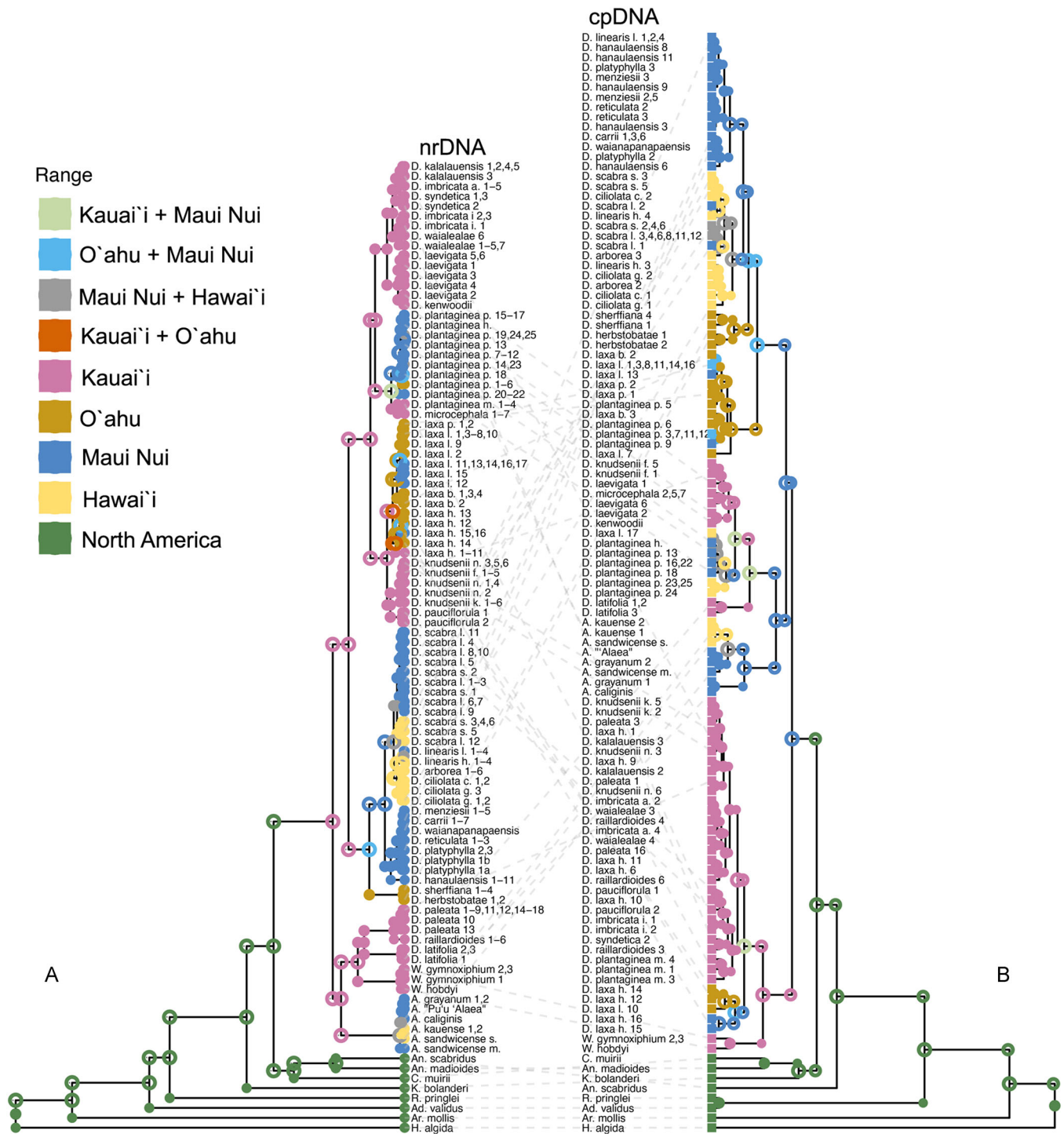
## Biogeographic history

Timing of estimated biogeographic transitions (Appendices S2, S3) was consistent with estimated origination dates for each island or island group (Clague and Sherrod, 2014; Lim and Marshall, 2017). Biogeographic analysis based on the nrDNA trees yielded highest posterior probabilities for Kaua'i ancestry at basal and backbone nodes of the silversword alliance (Figure 3A), with multiple dispersal events to younger islands. Only one dispersal event was estimated from nrDNA trees to explain all species of *Dubautia* endemic to a single major island or island group. Phylogenetic resolution within *Argyroxiphium* was too limited in nrDNA trees for detailed biogeographic inference. Maui Nui ancestry at basal and backbone nodes of the cpDNA trees was favored weakly (Figure 3B), in line with low support for corresponding



**FIGURE 2** Taxa of *Dubautia* that occur in sympatry and hybridize in all pairwise combinations in the summit bog of Wai'ale'ale, Kaua'i. (A) *Dubautia imbricata* subsp. *acronaea*; (B) *Dubautia waialealae*; (C) *Dubautia paleata*; and (D) *Dubautia laxa* subsp. *hirsuta* (photos: K. R. Wood). Estimations of habitat transitions in the silversword alliance indicate that each taxon represents an independent shift to bog habitat





**FIGURE 3** Estimates of island or island-group occurrence based on biogeographic analysis using the Dispersal-Extinction-Cladogenesis (DEC) model for (A) 18S-26S nuclear ribosomal (nrDNA) internal and external transcribed spacer (ITS and ETS) region sequences, and (B) cpDNA sequences (3' end of *ndhF* and *ndhF-ycf1* intergenic spacer, 5' *trnK* intron and 5' end of *matK*, *rpl16* intron, and *psbA-trnH* intergenic spacer) of the Hawaiian silversword alliance and relatives, shown as a tanglegram. Probabilities for biogeographic occurrences were obtained from sampling 3000 trees of the posterior distribution (filled circles,  $\geq 95\%$  PP; open circles,  $< 95\%$  PP). For generic abbreviations in taxon names, A. = *Argyroxiphium*; An. = *Anisocarpus*; Ar. = *Arnica*; C. = *Carlquistia*; D. = *Dubautia*; H. = *Hulsea*; K. = *Kyhosia*; R. = *Raillardella*; W. = *Wilkesia*. Numbers following names of terminal taxa correspond to numbering of different vouchered collections in Appendix 1. Maui Nui = prehistoric (Pleistocene) island including modern islands of Lana'i, Moloka'i, and Maui, treated here as one biogeographic unit

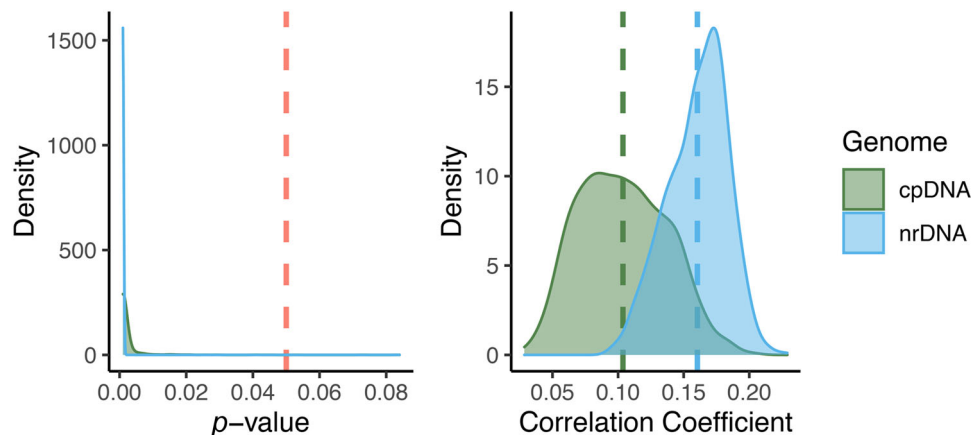
relationships (Figure 1B). Given that nuclear chromosomal arrangements and fine-scale taxonomy were much more congruent with nrDNA trees than with cpDNA trees, the nrDNA trees are taken here to be the primary source of biogeographic evidence, with cpDNA results providing complementary evidence of dispersal and biogeographically informative hybridization. Results of the Mantel tests showed that isolation-by-distance was significant for both cpDNA and nrDNA in the silversword alliance, with correlation coefficients for phylogenetic distance and geographic distance that were significantly non-zero for each set of trees and mean  $p$ -values  $<0.01$  (Figure 4).

Based on nrDNA trees, each of the two most diverse clades of young-island endemics, corresponding to *Argyroxiphium* and *Dubautia* sect. *Railliardia*, was estimated with highest probability to have descended from a stem ancestor on the oldest modern high island, Kaua'i (Figure 3A). A general pattern of dispersal down the chain, from older to younger islands, was estimated for both groups (Figure 5A, B). The Big Island taxa of *Argyroxiphium* were resolved as a clade nested among Maui lineages in the cpDNA trees (Figure 3B), as resolved earlier (McGlaughlin and Friar, 2011). Similarly, the Big Island taxa of *Dubautia* sect. *Railliardia* are nested among lineages of older islands in the mostly congruent nrDNA and cpDNA clades (Figure 3A, B), with uncertainty about whether the main dispersal pattern was strictly from older to younger islands or whether the O'ahu endemic clade represents a back-dispersal from Maui Nui (Figure 5B). Back-dispersal from the Big Island to Maui Nui followed by cpDNA capture (see Discussion) is evidently represented by the Maui Nui endemic *D. linearis* subsp. *linearis*, which was resolved in nrDNA trees in a clade otherwise representing all native taxa of the Big Island in sect. *Railliardia*—including conspecific *D. linearis* subsp. *hillebrandii*—but was resolved in cpDNA trees in a clade representing all Maui Nui endemic taxa of the section (Figure 3A, B).

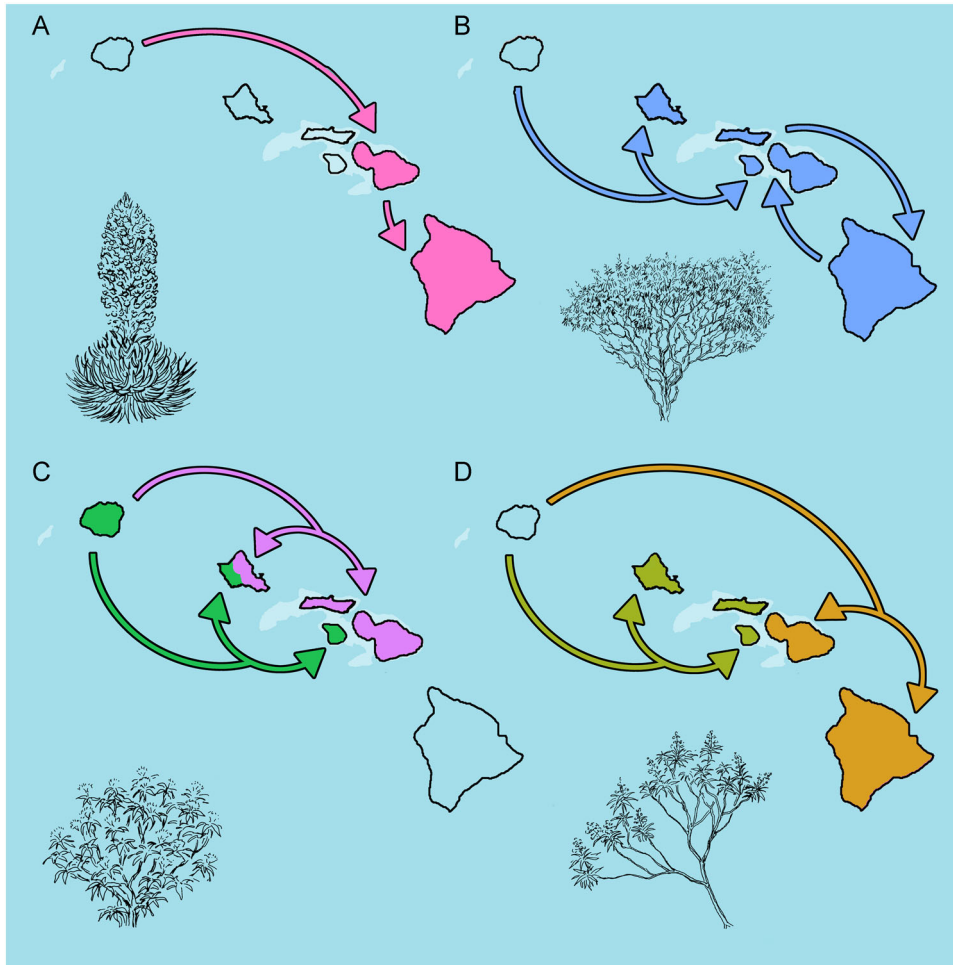
Biogeographic estimates based on nrDNA versus cpDNA trees for *Dubautia laxa* and *D. plantaginea*—the only species of the silversword alliance other than those of *Argyroxiphium*

and *Dubautia* sect. *Railliardia* that occur (in part) on islands younger than Kaua'i—differed greatly, as did clade resolution for representatives of these two taxa in nrDNA versus cpDNA trees, as noted above (Figures 1 and 3). Chloroplast capture evidently has occurred in both *D. laxa* and *D. plantaginea* (see Discussion). Conspecific samples of each species from the same volcano bear cpDNAs representing the same major plastid clade of the silversword alliance, with one exception (see below). In *D. laxa*, samples placed in different major plastid clades of the silversword alliance correspond to (1) subsp. *hirsuta*, of Kaua'i, Lana'i, and the Wai'anae Range of western O'ahu; (2) subsp. *laxa* of West Maui, Moloka'i, and the Ko'olau Range of eastern O'ahu, and subsp. *bryanii* and subsp. *pseudoplantaginea*, both endemic to the Ko'olau Range; and (3) subsp. *laxa* of East Maui. In *D. plantaginea*, samples representing different major plastid clades of the silversword alliance correspond to: (1) subsp. *magnifolia*, endemic to Kaua'i; (2) subsp. *plantaginea* of O'ahu, Moloka'i, and Lana'i; and (3) subsp. *humilis* of West Maui and subsp. *plantaginea* of Maui and the Big Island. The sole exception to the above plastid-clade distribution is the only sample of *D. laxa* from the southern Ko'olau Range, O'ahu (Mt. Olympus); the Mt. Olympus sample (BGB 1391), with morphology of subsp. *laxa*, is in an nrDNA clade with representatives of subsp. *bryanii*, subsp. *pseudoplantaginea*, and subsp. *laxa* but in a robust cpDNA clade with O'ahu representatives of subsp. *hirsuta*, similar to cpDNA findings by McGlaughlin and Friar (2011) for their sample of *D. laxa* from the southern Ko'olau Range (Pu'u Konahuanui).

Extensive incongruence between cpDNA and nrDNA trees and phylogenetic uncertainty within clades representing *D. laxa* and *D. plantaginea* allow for only tentative dispersal scenarios for each species. Two independent dispersals out of Kaua'i for each species cannot be ruled out, as noted by McGlaughlin and Friar (2011) for *D. laxa*, with subsp. *hirsuta* on western O'ahu (Wai'anae Range) and Lana'i and the clade containing subsp. *bryanii*, subsp. *laxa*, and subsp. *pseudoplantaginea* on eastern O'ahu (Ko'olau Range), Moloka'i, and Maui possibly representing two different out-of-Kaua'i colonizations of younger



**FIGURE 4** Results of isolation-by-distance analyses using Mantel tests for cpDNA and nrDNA sequences of the Hawaiian silversword alliance. Density plots of Mantel test  $p$ -values (left) and correlation coefficients (right) indicate significant isolation-by-distance for both cpDNA and nrDNA



**FIGURE 5** Inter-island dispersal hypotheses for the Hawaiian silversword alliance based on comparison of biogeographic estimates from nuclear ribosomal DNA (nrDNA) and cpDNA trees of taxa that occur on multiple islands or island groups: (A) *Argyroxiphium*; (B) *Dubautia* sect. *Railliardia*; (C) *D. laxa*; and (D) *D. plantaginea* (except Kauai endemic subsp. *magnifolia*, not shown). Viable alternative hypotheses involving only one dispersal event out of Kauai for *D. laxa* (involving subsp. *hirsuta*, shown in green) and *D. plantaginea* (involving the Maui/Hawai'i (Big Island) lineage, shown in orange) are presented in the text (see *Biogeographic history* section, under Results)

islands (Figure 5C). Populations of *D. plantaginea* on O'ahu, Lana'i, and Moloka'i and populations on Maui and the Big Island potentially represent two distinct out-of-Kauai dispersals, as well (Figure 5D). Alternatively, a single dispersal event out of Kauai for *D. laxa* is also plausible, with subsp. *hirsuta* dispersed out of Kauai and ancestral (paraphyletic) to the other three, strictly younger-island subspecies of *D. laxa*, as suggested by McGlaughlin and Friar (2011). A more complex scenario is required to explain the findings for *D. plantaginea* by a single dispersal event out of Kauai—directly to Maui/Big Island, followed by back-dispersal to Moloka'i/O'ahu and chloroplast capture from the putatively resident clade of *D. laxa* (represented by subsp. *bryanii*, *pseudoplantaginea*, and *laxa*), with subsequent dispersal to Lana'i, where *D. laxa* subsp. *bryanii*, *pseudoplantaginea*, and *laxa* do not occur.

A different major plastid clade is represented by *D. laxa* (subsp. *laxa*) on East Maui, where the species is rare and only recently rediscovered, on the youngest volcano in its geographic distribution and putatively at the leading edge of

colonization. The sole sample of *D. laxa* from East Maui was identical in cpDNA sequences to some of the relatively common individuals of *D. plantaginea* on Maui, in a well-supported cpDNA clade otherwise representing all sampled Maui populations of *D. plantaginea* (Figure 3B) but in the nrDNA clade corresponding to *D. laxa* subsp. *laxa*, *bryanii*, and *pseudoplantaginea* (Figure 3A).

## Growth-form evolution

Bayes factors indicated strong support for models that include non-zero transition rates out of the shrub growth-form (Appendix S4) with repeated shrub-to-tree transitions (Appendices S5 and S6). Bayes factors for other models of growth-form evolution indicated approximately equal support for models with zero and non-zero transition rates, probably reflecting phylogenetic uncertainty and lack of sufficient numbers of transitions to yield robust results.

## Habitat shifts

For the analyses based on three habitat categories (dry, wet, or bog), Bayes factors indicated “decisive” support (Kass and Raftery, 1995) for unidirectional or irreversible evolutionary shifts (Table 1), with repeated transitions from dry-to-wet and wet-to-bog habitats (Figure 6A). The highest transition rate was from wet-to-bog habitats and second highest was from dry-to-wet habitats (Figure 6B). The transition rate from bog-to-wet habitats was barely non-zero and transition rates for bog-to-dry, dry-to-bog, and wet-to-dry habitats were each zero. For the analyses based on four habitat categories (dry, mesic, wet, or bog), Bayes factors also supported irreversible evolutionary shifts from dry-to-wet and wet-to-bog habitats but not as overwhelmingly as in the three-habitat analysis (Table 1), with “mild” to “strong” support for irreversibility (Kass and Raftery, 1995). Estimated ancestral habitat-states for the more information-rich, four-habitat analyses were similar to those for the three-habitat analysis, but with fewer transitions from dry-to-wet habitats (Figure 7A) and with transitions that more closely approach parsimony-based estimates of habitat shifts by Baldwin and Robichaux (1995). The only significantly non-zero transition rates for the four-habitat analysis were for dry-to-mesic, dry-to-wet, wet-to-bog, and wet-to-mesic habitats (Figure 7B). The violin plots for both

**TABLE 1** Comparisons of models of habitat evolution. Habitat models were tested using both a 3-state (dry, wet, or bog) and 4-state (dry, mesic, wet, or bog) coding. Each model tested fixed the transition rate between two states to be zero

Habitat coding	Transition rate fixed to zero	Bayes factor
3-state	bog-to-dry	>1000
3-state	bog-to-wet	0.0
3-state	dry-to-bog	>1000
3-state	dry-to-wet	0.0
3-state	wet-to-bog	0.0
3-state	wet-to-dry	>1000
4-state	bog-to-dry	3.5
4-state	bog-to-mesic	4.4
4-state	bog-to-wet	0.7
4-state	dry-to-bog	1.1
4-state	dry-to-mesic	0.0
4-state	dry-to-wet	0.0
4-state	mesic-to-bog	2.2
4-state	mesic-to-dry	4.7
4-state	mesic-to-wet	5.2
4-state	wet-to-bog	0.0
4-state	wet-to-dry	5.8
4-state	wet-to-mesic	1.0

sets of analyses show model-averaged posterior densities of transition rates (Figures 6B, 7B). The transition rates shown are reported in number of transition events per unit of branch length (i.e., per million years).

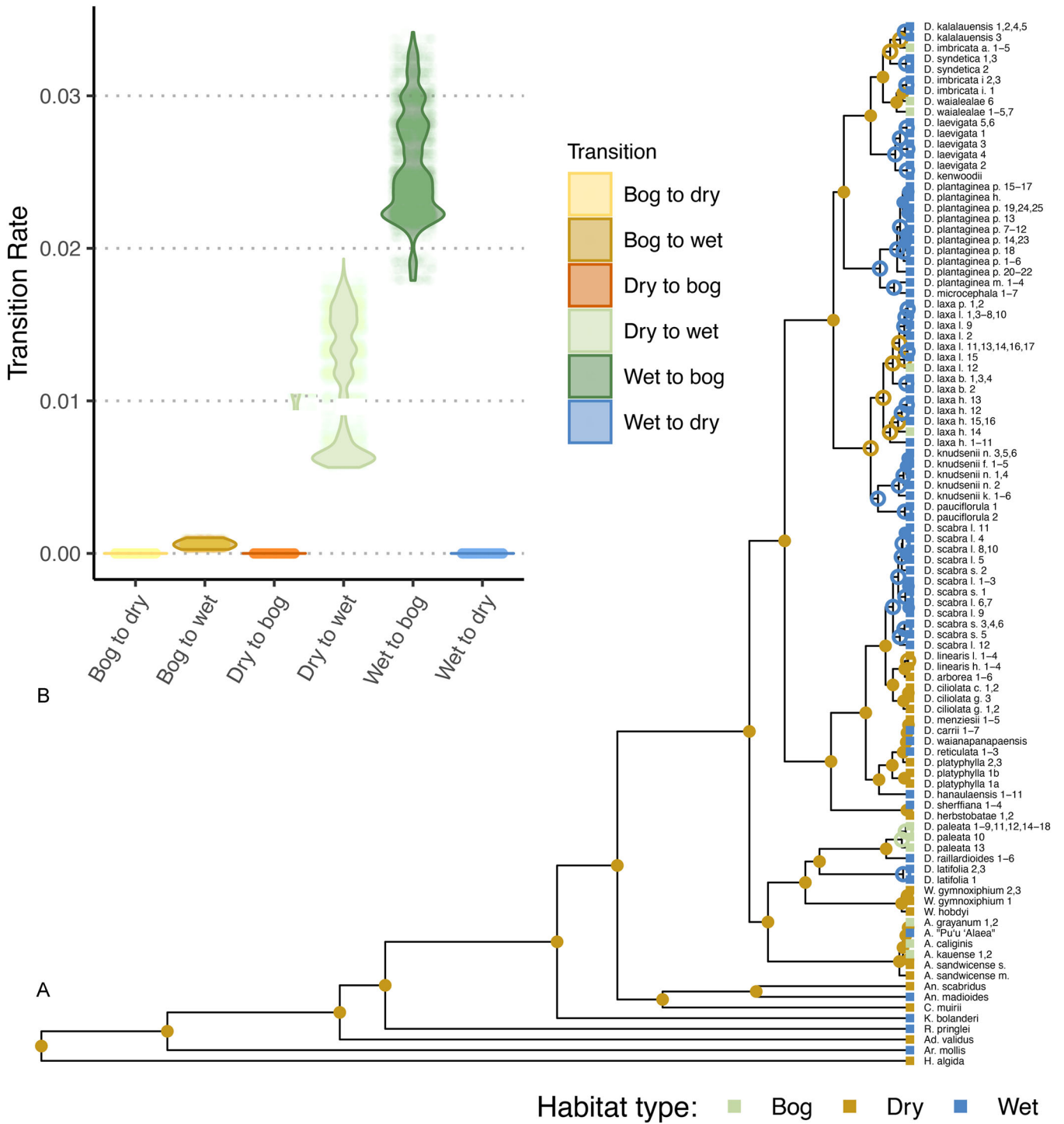
## DISCUSSION

Expanded phylogenetic analyses of the silversword alliance reinforce and extend earlier findings about evolutionary relationships and putative reticulation in the group (Carr and Kyhos, 1986; Baldwin et al., 1990; Baldwin and Robichaux, 1995; Baldwin, 1997; Friar et al., 2008; McGlaughlin and Friar, 2011). In particular, increased representation of populations and species across the archipelago and additional sequence data for all sampled taxa allowed for enhanced phylogenetic, biogeographic, and ecological resolution and reconsideration of the extent of phylogenetic incongruence between cpDNA and nuclear data, i.e., cytonuclear discordance.

### Cytonuclear discordance and island evolution

The extent of cytonuclear discordance in the silversword alliance is difficult to explain unless hybridization affected cpDNA relationships, as reinforced by discordance between cpDNA clades and fine-scale taxonomy. The extent of cpDNA variation occurring within populations and persisting through speciation events that would be necessary to account for such discordance by ILS is difficult to reconcile with levels and patterns of allelic variation estimated across the silversword alliance (Witter and Carr, 1988) and the four-fold elevated rate of coalescence expected for organellar genes as compared to nuclear genes (see Hudson and Coyne, 2002). Congruence of well-supported cpDNA clades with those resolved earlier using cpDNA restriction site variation from across the plastome for a subset of the taxa sampled here (Baldwin et al., 1990) reinforces confidence that the cytonuclear conflicts are not a result of failure to accurately resolve the pertinent, deep cpDNA clades.

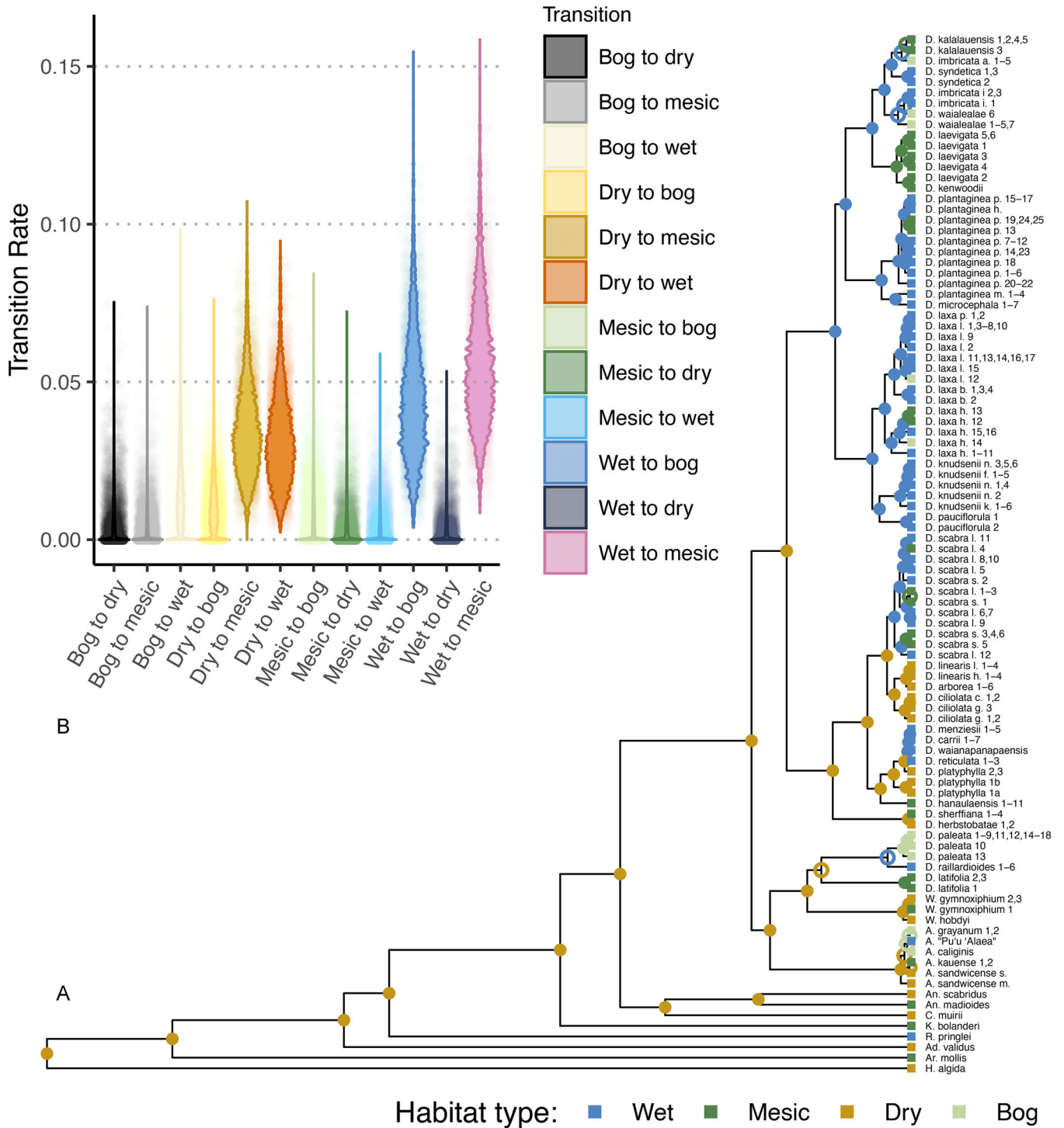
Cytonuclear discordance has been commonly resolved in rapidly radiating clades of young or dynamic habitats, including other Compositae lineages, such as *Espeletia* in Andean paramo (Pouchon et al., 2018) and *Bidens* in the Hawaiian Islands (Knope et al., 2020), and has been particularly well-studied in annual sunflowers (Lee-Yaw et al., 2019). Such discordance is increasingly widely documented in angiosperms (e.g., Winkler et al., 2013; Folk et al., 2017; Garcia et al., 2017) and can arise through hybridization in lineages that have successfully colonized areas already occupied by close, interfertile relatives (Currat et al., 2008; Folk et al., 2018), as in island clades that are sufficiently distinct ecologically to co-exist in adjacent habitats within range of common pollinators (Levin, 2006). Similar effects of hybridization may occur in declining species in close proximity to more common relatives, especially if



**FIGURE 6** Habitat transitions for three-state categorization (dry, wet, and bog) estimated for the nrDNA trees using reversible-jump MCMC to explore all models of habitat shifts and to estimate ancestral states while marginalizing across the posterior distribution of trees. (A) Plot of reconstructed ancestral habitat states (filled circles,  $\geq 95\%$  PP; open circles,  $< 95\%$  PP). For generic abbreviations in taxon names, A. = *Argyroxiphium*; An. = *Anisocarpus*; Ar. = *Arnica*; C. = *Carlquistia*; D. = *Dubautia*; H. = *Hulsea*; K. = *Kyhosia*; R. = *Raillardella*; W. = *Wilkesia*. Numbers following names of terminal taxa correspond to numbering of different vouchered collections in Appendix 1. (B) Model-averaged posterior densities of transition rates (number of transition events per million years) among dry, wet, and bog habitats

mutational load develops in the organismal genome of the rare taxon (Sloan et al., 2017). Selection for cpDNA haplotypes associated with elevated fitness in different habitat conditions (wet vs. dry) could come into play during

hybridization, as well (Sambatti et al., 2008). An alternative possibility of being hybridized out of existence following colonization (Levin, 2006) may have erased an unknown number of interisland colonizations, which seems especially



**FIGURE 7** Habitat transitions for four-state categorization (dry, mesic, wet, and bog) estimated for the nrDNA trees using reversible-jump MCMC to explore all models of habitat shifts and to estimate ancestral states while marginalizing across the posterior distribution of trees.

(A) Plot of reconstructed ancestral habitat states (filled circles,  $\geq 95\%$  PP; open circles,  $< 95\%$  PP). For generic abbreviations in taxon names, A. = *Argyroxiphium*; An. = *Anisocarpus*; Ar. = *Arnica*; C. = *Carlquistia*; D. = *Dubautia*; H. = *Hulsea*; K. = *Kyhosia*; R. = *Raillardella*; W. = *Wilkesia*. Numbers following names of terminal taxa correspond to numbering of different vouchered collections in Appendix 1. (B) Model-averaged posterior densities of transition rates (number of transition events per million years) among dry, mesic, wet, and bog habitats.

likely for self-incompatible taxa with dispersal units containing a single seed, as in the silversword alliance (Carr et al., 1986). Conversely, successful colonization of areas already occupied by close relatives, as appears likely for

*Dubautia laxa* on East Maui and *D. linearis* on Maui Nui based on biogeographic and cytonuclear patterns, provides indirect evidence for ecological segregation of these colonists from resident taxa and lack of pre-emption of the

colonist niche. Niche pre-emption and priority effects or resistance to invasion in general by earlier arrivals (i.e., “lock up” dynamics; see Shaw and Gillespie, 2016) have been widely discussed for islands, where phylogenetic patterns often indicate that successful dispersal events to archipelagos or between islands have been rare.

Widespread parapatry or sympatry of taxa and nrDNA evidence for within-island diversification of the silversword alliance likely reflect conditions favorable for plastid introgression. The much more extensive discordance between clade membership and species-level taxonomy in cpDNA trees than in nrDNA trees contrasts with more highly concordant clades of cpDNA and nuclear trees in another example of extensive radiation in the Hawaiian flora, *Schiedea* (Caryophyllaceae; Willyard et al., 2011). As noted by Willyard et al. (2011), such concordance in *Schiedea* was expected based on the largely allopatric distributions of closely related taxa, especially on younger islands where the concordance was most marked. Natural hybridization is also much less evident in *Schiedea* than in the silversword alliance, although intrinsic potential for introgression is evident from successful production of artificial hybrids across the genus that show levels of fertility inversely correlated with genetic distance between parents (Weller et al., 2001). Cytonuclear discordance in *Schiedea* is primarily evident at deep phylogeny nodes, and has been argued to reflect plastid introgression associated with ancient colonization events (Willyard et al., 2011). Lack of monophyly of morphologically distinctive taxa in cpDNA trees of Hawaiian *Euphorbia* subg. *Chamaesyce* (Euphorbiaceae) also has been attributed to hybridization (Yang et al., 2018), as has cytonuclear discordance in Hawaiian *Plantago* (Plantaginaceae; Dunbar-Co et al., 2008).

### Biogeographic implications of cytonuclear discordance and natural hybridization

By comparison with the nrDNA trees, the more complex evolutionary history indicated by cpDNA trees provides evidence for a less simple biogeographic history than would be concluded from the nuclear data alone. As previously found from studying cytonuclear discordance in Hawaiian crickets (*Laupala*; Shaw, 2002; see Shaw and Gillespie, 2016), the nuclear trees alone do not provide convincing evidence for back-migration or hybridization associated with interisland (or between-volcano) dispersal events. As shown by Landis et al. (2018), the nrDNA trees for the silversword alliance provide strong support for the progression rule of island biogeography (dispersal from older to younger islands), and expanded sampling of taxa and addition of ETS sequence data to the nrDNA dataset did not resolve any robust counter-examples, i.e., of dispersal from younger to older islands (Figure 3A). In contrast, expanded taxon sampling of the silversword alliance in cpDNA trees resolved clades that were incompatible with species delimitations and indicated back-migration (of *Dubautia*

*linearis*) from Hawaii to Maui Nui, as also resolved in mitochondrial DNA trees for Hawaiian crickets (see Shaw and Gillespie, 2016).

The back-dispersal from the Big Island to Maui Nui resolved from evident cpDNA capture in *Dubautia linearis* is compelling because of the likelihood of gene flow with other taxa following dispersal to an older island colonized earlier by closely related, completely interfertile taxa (see Currat et al., 2008). In this instance, morphological differences between Maui Nui endemic subsp. *linearis* and Big Island endemic subsp. *hillebrandii* may in part represent the influence of hybridization with resident taxa after colonization of Maui Nui. At a putative leading edge of colonization, we also found strong evidence of hybridization and chloroplast capture in *D. laxa* on East Maui (Haleakala), where it shares the plastome of Maui *D. plantaginea*. Natural hybrids between *D. laxa* and *D. plantaginea* have not been documented but the two species share the same nuclear genomic arrangement (*Dubautia* genome 1), have overlapping flowering phenologies, and are putatively fully interfertile (Carr, 1985; Carr and Kyhos, 1986).

Significant isolation-by-distance for both cpDNA and nrDNA lineages of the silversword alliance (Figure 4) may have different explanations, with ecological diversification on the same island or volcano and perhaps dispersal primarily between nearby islands (but see Abellán and Ribera, 2017) reflected by the nrDNA results, and hybridization on a local scale contributing to the cpDNA findings. The much wider range of correlation coefficients between phylogenetic and geographic distance for cpDNA compared to nrDNA probably reflects the extent of poorly resolved cpDNA clade structure.

Relationships resolved by nrDNA trees in comparison with documented natural hybridization in the silversword alliance indicate only modest evidence for sister-species hybridization in nature, consistent with ecological and geographic barriers to gene flow associated with evolutionary divergence. Documentation here of a natural hybrid between the bog endemic *Dubautia paleata* and the moist-mesic to wet-forest endemic *D. raillardioides* at the bog-forest ecotone is an exception to this pattern, although only minor overlap in flowering phenology and strong habitat differences between the two taxa (Carr, 1985, 1999) may limit potential for gene flow between them. A putative hybrid between the now-rare *D. microcephala* and closely related (also rare) *D. plantaginea* subsp. *magnifolia* is also known but from only one (historical) collection (see Carr, 1985). Molecular evidence for genetic admixture between *D. arborea* and *D. ciliolata* on Mauna Kea (Lawton-Rauh et al., 2007; Remington and Robichaux, 2007), insofar as it represents hybridization between these fully interfertile taxa, appears on the basis of the nrDNA trees to represent gene flow following evolutionary divergence of *D. arborea* and *D. linearis* from a common ancestor rather than genetic exchange associated with initial divergence of sister species. The extent to which historical natural hybrids between *D. arborea* and *D. linearis* subsp. *hillebrandii* on Hualalai

(Carr, 1985) may indicate past gene flow between these taxa, however, is unknown. On the basis of nuclear microsatellite data, Friar et al. (2006) concluded a lack of evidence for gene flow between species of the Maui Nui clade of *D. sect. Railliardia* that have been documented to hybridize there (Carr, 1985) and that remain of unresolved relationship to one another based on the nrDNA and cpDNA trees.

### Continued diversification on Kauaʻi

Resolution here of a previously unknown nrDNA clade on Kauaʻi that contains most of the taxa not included in earlier phylogenetic studies of the silversword alliance (*Dubautia imbricata* subsp. *acronaea*, *D. kalalauensis*, *D. kenwoodii*, *D. syndetica*, and *D. waialealae*) gives additional strength to the conclusion of Landis et al. (2018) that diversification on Kauaʻi has not abated since the island began to decline in area. Based on estimated lineage ages in the nrDNA trees (Appendix S2), the additional taxa represent more examples of diversification late in the history of Kauaʻi (<1 million yrs ago) and all are geographically narrow endemics (as is the previously sampled *D. imbricata* subsp. *imbricata*) of moist-mesic to wet or bog habitats that may have diverged as Kauaʻi became more topographically and ecologically complex with continuing erosion. Five of these taxa—all but *D. waialealae* and an earlier studied member of the clade, *D. laevigata*—were described as new-to-science since the 1980s and demonstrate the importance to evolutionary biology of continued exploratory fieldwork by Kauaʻi botanists, e.g., in the recently described and poorly known *Dubautia*–*Sadleria* plant community (Wood et al., 2016) in the vicinity of Kauaʻi's highest peak, Kawaikini.

### Biased habitat transitions during radiation

Evidence presented here for diversification dominated by unidirectional habitat shifts (Figures 6 and 7) indicates that adaptive radiation of the silversword alliance may have been more constrained evolutionarily than was previously understood, or that survival has been strongly biased against lineages representing some ecological transitions. A history of repeated transitions from dry-to-wetter (mesic or wet) habitats and wet-to-bog-habitats without transitions back to dry habitats is consistent with the scenario of a high, dry ancestry of the silversword alliance, as suggested by Carlquist (1995, 1997), and in keeping with the temperate North American tarweed relationships of the clade (see Baldwin and Wagner, 2010). If most or all interisland dispersal events that resulted in the evolution of new species involved colonists of dry habitats, as estimated here, then lack of evidence for evolutionary transitions to dry habitats may reflect niche pre-emption by ancestrally dry-adapted relatives. Difficulty of re-evolving characteristics necessary to survive in dry environments once lost is also conceivable, as might be expected if survival under dry conditions

depends on complex suites of traits (Robichaux and Canfield, 1985; Robichaux et al., 1990; Carlquist, 1997, 1998, 2003a, b; also see Aroca, 2012) that are not advantageous in wet habitats. Evidence for constraint or survival bias in habitat transitions does not appear to be explained by general growth form, with shifts from dry-to-wetter habitats estimated for both rosette-plant and shrub lineages, and transitions to the tree habit associated with both wet and dry environments (Appendices S4–S6; Figures 6A and 7A).

A bias toward ecological shifts from dry-to-wet habitats is comparable to the suggestion for Hawaiian *Euphorbia* subg. *Chamaesyce* of multiple transitions from open-to-closed habitats, on Kauaʻi and Oʻahu (Yang et al., 2018). In contrast, in *Schiedea*, multiple shifts to dry or wet habitats from an ancestral occurrence in mesic habitats have been inferred (Sakai et al., 2006). Changes in taxon sampling in the largely xeric-adapted mainland “Madia” lineage to which the silversword alliance belongs (Baldwin, 2003) would be unlikely to affect the findings, with inclusion in the analysis here of all relevant North American taxa that occur outside of dry habitats.

The biases in habitat shifts indicated from the analyses here (Figures 6 and 7) may help to explain some previous findings and new data and observations for the silversword alliance. For example, studies of leaf osmotic properties in taxa of *Dubautia* in dry and wetter habitats yielded ecophysiological results for the mesic or wet forest tree *D. reticulata*, of East Maui, that were intermediate between those for shrubby *D. menziesii*, of drier habitats on East Maui, and those for the tree *D. knudsenii*, of wet habitats on Kauaʻi (Robichaux, 1985). Robichaux (1985) interpreted these findings as possibly representing a recent origin of *D. reticulata* from an ancestor of dry habitats, consistent with the results presented here—*D. reticulata* and *D. menziesii* are members of the same, young endemic Maui Nui clade (also see Friar et al., 2006). On Kauaʻi, phylogenetic data on habitat transitions for taxa that occur sympatrically in the summit bog of Waiʻaleʻale (Figure 2) and were shown here to hybridize in every pairwise combination indicate that no pair of those taxa is sister and that each Waiʻaleʻale taxon appears to represent a separate origin of bog adaptation. Independent transitions to bog life leave open the possibility that the four moderately to highly interfertile taxa of *Dubautia* at Waiʻaleʻale each evolved sufficiently distinct bog adaptations to allow for occurrence in sympatry without merging into a common gene pool through more extensive hybridization.

## CONCLUSIONS

Despite lack of evidence for evolutionary transitions to dry habitats from mesic, wet, or bog situations, more nuanced evolutionary perspectives have potential to modify understanding of ecological radiation of the silversword alliance considerably. At a fine-scale level, for example, studies of gene flow between sympatric members of the silversword alliance have concluded introgression from mesic/wet to dry adapted



taxa, i.e., from *Dubautia scabra* to *D. ciliolata*, although not for alleles known to be associated with survival in different habitats (Caraway et al., 2001). Between dry-adapted taxa, some evidence for gene flow toward the more xeric-adapted taxon also has been found, from *D. arborea* to *D. ciliolata* (Lawton-Rauh et al., 2007). Such findings warrant questioning whether the extreme cytonuclear discordance described here represents a history of hybridization that otherwise had minor evolutionary consequences or if it had a more fundamental impact on adaptive radiation of the silversword alliance. Evidence for an allopolyploid origin of the silversword alliance (Barrier et al., 1999) and growing appreciation for the extent to which wide hybridization can spur adaptive radiations (Marques et al., 2019) warrant a genomic-scale approach to examining ecological change across this fascinating clade, as we are currently pursuing by targeted sequencing of hundreds of nuclear loci from most taxa of tribe Madieae, including those of the silversword alliance.

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### AUTHOR CONTRIBUTIONS

B.G.B., K.R.W., and W.A.F. contributed to the study design and research; B.G.B. drafted the manuscript with contributions from K.R.W. and W.A.F.; all authors approved the final version.

### DATA AVAILABILITY STATEMENT

GenBank accession numbers for DNA sequences are M93787–M93793, M93797–M93800, AF061882–AF061914, MZ313563–MZ313845, and MZ324000–MZ324809. Sequence matrices and phylogenetic trees are available at the

Dryad Digital Repository: <https://doi.org/10.6078/D1FT5G> (Baldwin et al., 2021).

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**Appendix S1.** Methodological notes, well-supported nrDNA and cpDNA clades, and notes on chromosomal structural arrangements and hybrid fertilities.

**Appendix S2.** Chronogram of the Hawaiian silversword alliance and relatives based on nuclear nrDNA sequences.

**Appendix S3.** Chronogram of the Hawaiian silversword alliance and relatives based on cpDNA sequences.

**Appendix S4.** Comparisons of models of growth-form evolution in the Hawaiian silversword alliance.

**Appendix S5.** Ancestral growth-form states in the Hawaiian silversword alliance estimated for the nrDNA trees.

**Appendix S6.** Model-averaged posterior densities of transition rates among growth forms in the Hawaiian silversword alliance estimated for the nrDNA trees.

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## APPENDIX 1

List of Madieae samples included in phylogenetic analyses of the silversword alliance. Numbers in parentheses preceding collections of taxa represented by more than one sample correspond to numbers following taxon names in tree figures. *Index Herbariorum* (Thiers, 2020) abbreviations (in parentheses) follow collector name and number. BGB = Bruce G. Baldwin. KRW = Kenneth R. Wood. See Data Availability for GenBank accession numbers of nuclear ribosomal DNA and cpDNA sequences.

*Adenothamnus validus* (Brandege) D.D. Keck: MEXICO, Baja California, Punta Banda, Witter W86-99 (DAV). *Anisocarpus madioides* Nutt.: USA, California, Inner North Coast Ranges, Mayacamas Mountains, BGB 488 (DAV). *Anisocarpus scabridus* (Eastw.) B.G. Baldwin: USA, California, high North Coast Ranges, Hull Mountain, BGB 676 (DAV). *Argyroxiphium caliginis* C.N. Forbes: USA, Hawaiian Islands, West Maui, Pu'u Kukui, BGB 660 (DAV). *Argyroxiphium grayanum* (Hillebr.) O. Deg.: USA, Hawaiian Islands, (1) West Maui, Pu'u Kukui, BGB 661

- (DAV), (2) **East Maui**, Haleakala, *Medeiros s.n.*, 1987 (DAV). *Argyroxiphium kauense* (Rock & M. Neal) O. Deg. & I. Deg.: USA, *Hawaiian Islands*, **Hawai'i** (Big Island), Mauna Loa, (1) Kahuku, *BGB, Bainbridge & Robichaux 1516* (UC), (2) Waiakea, *BGB 773* (DUKE). *Argyroxiphium sandwicense* DC. subsp. *macrocephalum* (A. Gray) Meyrat: USA, *Hawaiian Islands*, **East Maui**, Haleakala, *Carr 1239* (HAW). *Argyroxiphium sandwicense* DC. subsp. *sandwicense*: USA, *Hawaiian Islands*, **Hawai'i** (Big Island), Mauna Kea, *BGB 657* (DAV). *Argyroxiphium* "Pu'u 'Alaea" (putative *A. sandwicense* subsp. *macrocephalum* × *A. virescens* Hillebr.): USA, *Hawaiian Islands*, **East Maui**, Haleakala, Pu'u 'Alaea, *Medeiros s.n.* (UC). *Arnica mollis* Hook.: USA, *California*, Sierra Nevada, Carson Range, *BGB 680* (DAV). *Carlquistia muirii* (A. Gray) B.G. Baldwin: USA, *California*, Santa Lucia Range, Ventana Double Cone, *BGB 618* (DAV). *Dubautia arborea* (A. Gray) D.D. Keck: USA, *Hawaiian Islands*, **Hawai'i** (Big Island), (1) Hualalai, pit crater 0.4 km northeast of Malokule, *Carr et al. 1072* (BISH), (2) Mauna Kea, Pu'u La'au, *BGB 527* (DAV), (3) Mauna Kea, upper Wailuku drainage, *BGB 656* (DAV), (4) Mauna Kea, above Pu'u Mali, *BGB, Bainbridge & Robichaux 1511-4* (UC); (5) Mauna Kea, Pu'u Mali, *BGB, Bainbridge & Robichaux 1512-1* (UC), (6) *BGB, Bainbridge & Robichaux 1512-4* (UC). *Dubautia carrii* B.G. Baldwin & Friar: USA, *Hawaiian Islands*, **Moloka'i**, (1) Waikolu Valley Lookout, *BGB & Bainbridge 1215-1* (UC), (2) ne Kamoku Flats, *BGB & Bainbridge 1389* (UC), (3) Pu'u Kolekole, *BGB & Bainbridge 1390* (UC), (4) between Kamoku Flats and 'Onini Gulch, *BGB & Bainbridge 1495-1* (UC), (5) sw Kamoku Flats, *BGB & Bainbridge 1495-6* (UC), (6) Pu'u o Ka'eha, *BGB & Bainbridge 1497-1* (UC), (7) *BGB & Bainbridge 1497-8* (UC). *Dubautia ciliolata* (DC.) D.D. Keck subsp. *ciliolata*: USA, *Hawaiian Islands*, **Hawai'i** (Big Island), (1) Humu'ula Saddle, Pu'u Huluhulu, *BGB 525* (DAV), (2) Kilauea, *BGB 529* (DAV). *Dubautia ciliolata* (DC.) D.D. Keck subsp. *glutinosa* G.D. Carr: USA, *Hawaiian Islands*, **Hawai'i** (Big Island), Mauna Kea, (1) upper Wailuku drainage, *BGB 659* (DAV), (2) above Pu'u Mali, *BGB, Bainbridge & Robichaux 1511-1* (UC), (3) Pu'u Kanakaleonui, *BGB, Bainbridge & Robichaux 1513* (UC). *Dubautia hanaulaensis* B.G. Baldwin: USA, *Hawaiian Islands*, **West Maui**, (1) northwest branch Pohakea Gulch, *BGB & Oppenheimer 1502-1* (UC), (2) *BGB & Oppenheimer 1502-5* (UC), (3) *BGB & Oppenheimer 1502-15* (UC), (4) *BGB & Oppenheimer 1502-20* (UC), (5) *BGB & Oppenheimer 1503* (UC), (6) *BGB & Oppenheimer 1504-1* (UC), (7) *BGB & Oppenheimer 1504-5* (UC), (8) ridge north of O'oawa Kilika Gulch, *Oppenheimer 90910* (UC), (9) upper Ka'onohua Gulch, south branch, *Oppenheimer 90906* (UC), (10) northwest branch Pohakea Gulch, *Oppenheimer, KRW & Brosius 100504* (UC), (11) upper Ka'onohua Gulch, south branch, *Oppenheimer 120513* (UC). *Dubautia herbstobatae* G.D. Carr: USA, *Hawaiian Islands*, **O'ahu**, Wai'anae Range, Ohikilolo Ridge, (1) *Carr 1244* (HAW), (2) *Perlman 13656* (PTBG). *Dubautia imbricata* H. St. John & G.D. Carr subsp. *acronaea* G.D. Carr: USA, *Hawaiian Islands*, **Kaua'i**, Wai'ale'ale, (1) *Medeiros 528* (BISH), (2) *KRW 12506* (PTBG), (3) *KRW 12531* (PTBG), (4) *KRW 12535* (PTBG), (5) *KRW 13337* (UC). *Dubautia imbricata* H. St. John & G.D. Carr subsp. *imbricata*: USA, *Hawaiian Islands*, **Kaua'i**, upper Wahiawa Stream, (1) *BGB 667* (DAV), (2) *BGB & KRW 1523* (UC), (3) *BGB & KRW 1524* (UC). *Dubautia kalalauensis* B.G. Baldwin & G.D. Carr: USA, *Hawaiian Islands*, **Kaua'i**, southwest Kalalau Rim, (1) *BGB & Bainbridge 1386* (UC), (2) *BGB & Bainbridge 1441* (UC), (3) W of type locality, *Bainbridge s.n.*, 26 January 2008 (UC), (4) S of type locality, *Bainbridge s.n.*, 11 June 2011 (UC), (5) S of type locality, large-leaved individual, *Bainbridge s.n.*, 11 June 2011 (UC). *Dubautia kenwoodii* G.D. Carr: USA, *Hawaiian Islands*, **Kaua'i**, southwest Kalalau Rim, *KRW 1004* (PTBG). *Dubautia knudsenii* Hillebr. subsp. *filiformis* G.D. Carr: USA, *Hawaiian Islands*, **Kaua'i**, (1) Makaleha Mountains, *Carr 1234* (HAW), (2) Kamanu, *KRW 12769* (PTBG), (3) *KRW 12781* (PTBG), (4) *KRW 12783* (PTBG); (5) upper Wainiha River, *KRW 13268* (UC). *Dubautia knudsenii* Hillebr. subsp. *knudsenii*: USA, *Hawaiian Islands*, **Kaua'i**, (1) southwest edge of Kahuama'a Flat, *BGB & Bainbridge 1442* (UC), (2) southwest Kalalau Rim, *BGB & Bainbridge 1445* (UC), (3) *BGB & Bainbridge 1494* (UC), (4) *BGB & Bainbridge s.n.* (UC), (5) headwaters of Awa'awapuhi Valley, *Carr 1047* (HAW), (6) Kilohana Lookout, *KRW 11523* (PTBG). *Dubautia knudsenii* Hillebr. subsp. *nagatae* (H. St. John) G.D. Carr: USA, *Hawaiian Islands*, **Kaua'i**, (1) Makaleha Mountains, *BGB, Bainbridge, Flynn & Tangalin 1498a* (UC), (2) *BGB, Bainbridge, Flynn & Tangalin 1498b* (UC), (3) *BGB, Bainbridge, Flynn & Tangalin 1500* (UC), (4) *Tangalin 2478* (PTBG), (5) Namolokama Mountain, north face, *KRW 12183* (PTBG), (6) southeast Kalalau Rim, *Carr 1322* (HAW). *Dubautia laevigata* A. Gray: USA, *Hawaiian Islands*, **Kaua'i**, (1) southwest Kalalau Rim, *BGB 671* (DAV), (2) *BGB 777* (DUKE), (3) *BGB & Bainbridge 1492* (UC), (4) *Bainbridge s.n.*, 26 January 2008 (UC), (5) above Kauaikinana Stream (at Mohihi Road), *BGB, Bainbridge & KRW 1490* (UC), (6) eastern Kohua Ridge, *BGB, Bainbridge & KRW 1489* (UC). *Dubautia latifolia* (A. Gray) D.D. Keck: USA, *Hawaiian Islands*, **Kaua'i**, (1) northwestern watershed of Kauaikinana Stream (at Mohihi Road), *BGB 675* (DAV), (2) northern headwaters of Ku'ia Valley (at Nu'alolo Trail), *BGB & Bainbridge 1383* (UC), (3) southern headwaters of Makaha Valley, *BGB & Bainbridge 1384* (UC). *Dubautia laxa* Hook. & Arn. subsp. *bryanii* (Sherff.) G.D. Carr: USA, *Hawaiian Islands*, **O'ahu**, Ko'olau Range, (1) between uppermost Poamoho and Helemano streams (on Poamoho Trail), *BGB & Bainbridge 1518* (UC), (2) summit of Ko'olau Range (on Poamoho Trail), *BGB & Bainbridge 1520* (UC), (3) lowest elevation along Schofield-Waikane Trail, *Burt s.n.* (UC), (4) near summit of Ko'olau Range (on Schofield-Waikane Trail), ca. 700 m elev., *Burt s.n.* (UC). *Dubautia laxa* Hook. & Arn. subsp. *hirsuta* (Hillebr.) G.D. Carr: USA, *Hawaiian Islands*, **Kaua'i**, (1) Alaka'i Swamp south of Kilohana Lookout, *BGB, Brueggmann & Caraway 1174* (UC), (2) *BGB, Brueggmann & Caraway 1175* (UC), (3) *BGB, Brueggmann & Caraway 1179*

(UC), (4) Wai'ale'ale, *Caraway et al. Waialeale #1*, Feb 2003 (UC); (5) *Caraway et al. Waialeale #3*, Feb 2003 (UC), (6) KRW 12515 (UC), (7) KRW 12516 (UC), (8) KRW 12533 (UC), (9) Namolokama Mountain, north face, KRW 12190 (UC), (10) west base of Kapalaoa, *BGB & KRW 1522* (UC), (11) Kahili, *John Game s.n.*, Apr 2007 (UC), **O'ahu**, (12) Wai'anae Range, north of Palikea, *BGB & Bainbridge 1505* (UC), (13) Wai'anae Range, south of Palikea, *BGB & Bainbridge 1507* (UC), (14) Wai'anae Range, Ka'ala, *Carr 833* (HAW), **Lana'i**, (15) north of Pu'u A'alii, *BGB & Bainbridge 1220* (UC), (16) headwaters of Hauola Gulch northwest of Ha'alelepa'akai, KRW 12057 (UC). *Dubautia laxa* Hook. & Arn. subsp. *laxa*: USA, *Hawaiian Islands, O'ahu*, (1) Ko'olau Range, between uppermost Poamoho and Helemano streams (on Poamoho Trail), *BGB & Bainbridge 1517-1* (UC), (2) *BGB & Bainbridge 1517-2* (UC), (3) *BGB & Bainbridge 1519* (UC), (4) *BGB & Bainbridge 1521* (UC), (5) summit of Ko'olau Range (on Manana Trail), *BGB & Bainbridge 1268* (UC), (6) Ko'olau Range, ridge between Manana and Waimano streams (on Manana Trail), *BGB & Bainbridge 1269* (UC), (7) *BGB & Bainbridge 1270* (UC), (8) Ko'olau Range, Schofield-Waikane Trail, near 2193 ft Pu'u, *Burt s.n.* (UC), (9) Ko'olau Range, Schofield-Waikane Trail, just below summit, *Burt s.n.* (UC), (10) Ko'olau Range, Mt. Olympus, *BGB, Bainbridge & Caraway 1391* (UC), **Moloka'i**, (11) Pepe'opae Bog, *BGB & Bainbridge 1218* (UC), (12) *BGB & Bainbridge 1219a* (UC), (13) bog west of Pu'u Ali'i, KRW 11246 (PTBG), **West Maui**, (14) Pu'u Kukui, *BGB 662* (DAV), (15) eastern Kaho'olewa Ridge, southeast of Pu'u Kane, *Oppenheimer H91002* (UC), (16) summit of West Maui Mountains at upper south edge of Ukumehame Gulch, *Oppenheimer & Perlman 100706* (UC), **East Maui**, (17) eastern Honomanu drainage, *Oppenheimer H61224* (UC). *Dubautia laxa* Hook. & Arn. subsp. *pseudoplantaginea* (Skotts.) G.D. Carr: USA, *Hawaiian Islands, O'ahu*, Ko'olau Range, (1) ridge between Manana and Waimano streams (on Manana Trail), *BGB & Bainbridge 1271* (UC), (2) *BGB & Bainbridge 1272* (UC). *Dubautia linearis* (Gaudich.) D.D. Keck subsp. *hillebrandii* (H. Mann) G.D. Carr: USA, *Hawaiian Islands, Hawai'i* (Big Island), (1) Nohona o Hae, *Carr 1147* (BISH), (2) *Team 3732* (BISH519426), (3) southwest base of Mauna Kea (Pohaku-Loa Military Reservation), *BGB 531* (DAV), (4) Kulani, *Friar et al. s.n.* (RSA). *Dubautia linearis* (Gaudich.) D.D. Keck subsp. *linearis*: USA, *Hawaiian Islands*, (1) **Lana'i**, west of Kapano Gulch, *Nagata & Ganders s.n.*, 27 August 1982 (BISH), (2) **West Maui**, Lihau, west of summit, *Welton & Haus 925* (BISH), (3) lower northwest branch of Pohakea Gulch, *Oppenheimer H100514* (UC), (4) **East Maui**, between Pimoe and Hokukano (east of 'Ulupalakua), *BGB 516* (DAV). *Dubautia menziesii* (A. Gray) D.D. Keck: USA, *Hawaiian Islands, East Maui*, Haleakala, (1) south of Pu'u Keokea, *Oppenheimer H40927* (UC), (2) northwest outer slope, *BGB 521* (DAV), (3) *BGB 522* (DAV), (4) Pu'u 'Alaea, *Oppenheimer H100818* (UC), (5) Kaupo Gap, *Welton s.n.*, 18 September 2008 (HALE). *Dubautia microcephala* Skotts.: USA, *Hawaiian Islands, Kaua'i*, (1) southwest

Kalalau Rim, *Bainbridge s.n.*, 7 October 2008 (UC), (2) *BGB & Bainbridge 1387* (UC), (3) *BGB & Bainbridge 1493* (UC), (4) KRW 3616 (HAW), (5) Kahuama'a Flat, *Carr 1044* (HAW), (6) upper Koaie Canyon, KRW 12135 (PTBG), (7) KRW 12138 A (PTBG). *Dubautia paleata* A. Gray: USA, *Hawaiian Islands, Kaua'i*, (1) Alaka'i Swamp south of Pihea, *Carr 1375* (HAW), (2) Alaka'i Swamp between north branch Kawaikoi Stream and Kilohana, *BGB, Brueggemann & Caraway 1173* (UC), (3) *BGB, Brueggemann & Caraway 1176* (UC), (4) *BGB, Brueggemann & Caraway 1177* (UC), (5) *BGB, Brueggemann & Caraway 1178* (UC), (6) *BGB, Brueggemann & Caraway 1180* (UC), (7) *BGB, Brueggemann & Caraway 1181* (UC), (8) *BGB, Brueggemann & Caraway 1185* (UC), (9) Wai'ale'ale, *Caraway et al. Waialeale #4*, Feb 2003 (UC), (10) KRW 12507 (UC), (11) KRW 12508 (PTBG), (12) KRW 12512 (UC), (13) west of Wai'ale'ale, KRW 12517 (PTBG), (14) near Sincock Bog, KRW 12520 (UC), (15) Wai'ale'ale, KRW 12528 (UC), (16) KRW 12534 (UC), (17) KRW 13331 (PTBG), (18) summit ridge below Kawaikini, KRW 7973 (HAW). *Dubautia pauciflorula* H. St. John & G.D. Carr: USA, *Hawaiian Islands, Kaua'i*, (1) upper Wahia Stream, *BGB 668* (DAV), (2) Makaleha Mountains, *BGB, Bainbridge, Flynn & Tangalin 1499* (UC). *Dubautia plantaginea* Gaudich. subsp. *humilis* G.D. Carr: USA, *Hawaiian Islands, West Maui*, Black Gorge, *Carr 1183* (HAW). *Dubautia plantaginea* Gaudich. subsp. *magnifolia* (Sherff) G.D. Carr: USA, *Hawaiian Islands, Kaua'i*, (1) Blue Hole, *BGB, Givnish, Perlman & KRW 776* (DUKE), (2) Kamanu, KRW 12768 (PTBG), (3) KRW 12780 (PTBG), (4) KRW 12784 (PTBG). *Dubautia plantaginea* Gaudich. subsp. *plantaginea*: USA, *Hawaiian Islands, O'ahu*, (1) Wai'anae Range, Ka'ala, *Carr 838-9* (HAW), (2) *Carr 838-10* (HAW), (3) *Carr 838-11* (HAW), (4) Wai'anae Range, north of Pu'u Kaua, *Palmer 1171* (HAW), (5) Wai'anae Range, south of Palikea Peak, *BGB & Bainbridge 1506* (UC); (6) Ko'olau Range, ridge between Kaluanui and Punaluu streams (on Castle Trail), *Carr 1180* (HAW), **Moloka'i**, (7) upper rim of Waikolu Valley, KRW 11281 (PTBG), (8) West Fork Kawela Gulch, *BGB & Bainbridge 1216-1* (UC), (9) ridge between East and West forks of Kawela Gulch, *BGB & Bainbridge 1496b-1* (UC), (10) *BGB & Bainbridge 1496b-4* (UC), **Lana'i**, (11) upper Kaiholena Gulch, *BGB & Bainbridge 1221-1* (UC), (12) Pu'u Ali'i vicinity, *Oppenheimer & Perlman H110702* (UC), **West Maui**, (13) West Maui Mountains, ridge between north and south branches of Kaua'ula Stream, *Oppenheimer et al. 100710* (UC), (14) West Maui Mountains, southern headwaters of Kaua'ula Stream, *Oppenheimer H120808* (UC), (15) West Maui Mountains, head of Waihe'e River (Wall of Tears), *Oppenheimer H110801* (UC), (16) West Maui Mountains, upper northwest branch of Pohakea Gulch, *Oppenheimer & Perlman H100702* (UC), (17) West Maui Mountains, northwest branch of Pohakea Gulch, KRW 11567 (PTBG), **East Maui**, (18) west of Wai-kamoi Stream at Olinda Flume, *BGB 779-2* (UC), (19) upper Honomanu Stream, *Oppenheimer H11004* (UC), (20) headwaters of Kano and Waiokamilo streams, *Oppenheimer H100717* (UC), (21) KRW 12608 (PTBG), (22) KRW 12609

(PTBG), **Hawai'i** (Big Island), (23) Kohala Mountains, Kawainui Stream above canyon rim, *BGB, Bainbridge & Roderick 1214* (UC), (24) west of Southwest Rift Zone (Mauna Loa), near Pahoehoe (Second) 1950 Lava Flow, *BGB, Bainbridge & Perry 1508-1* (UC), (25) *BGB, Bainbridge & Perry 1508-8* (UC). ***Dubautia platyphylla*** (A. Gray) D.D. Keck: USA, *Hawaiian Islands, East Maui*, (1a) Haleakala, northwest outer slope, *BGB 520* (DAV), clone 4, (1b) clone 5, (2) *BGB 523* (DAV), (3) *BGB 524* (DAV). ***Dubautia raillardioides*** Hillebr.: USA, *Hawaiian Islands, Kaua'i*, (1) southern Kahuama'a Flat, *BGB & Bainbridge 1182* (UC), (2) southwest Kalalau Rim, *Bainbridge s.n.*, 7 June 2011 (UC), (3) southeast Kalalau Rim, near Pihea, *BGB & Bainbridge 1172* (UC), (4) Alaka'i Swamp, between upper Kauaikinana and Kawaikoi streams, *BGB & Bainbridge 1525-1* (UC), (5) north of Mohihi Stream, *KRW 12253* (PTBG), (6) edge of Kanaele Swamp (Wahiawa Bog), *BGB 670* (DAV). ***Dubautia reticulata*** (Sherff) D.D. Keck: USA, *Hawaiian Islands, East Maui*, Haleakala, (1) head of Waikamoi Stream at Pu'u Niania, *Carr & Linney 1037* (HAW), (2) northeastern Ko'olau Gap, *BGB 664* (DAV), (3) Kuiki, *Medeiros 799* (HALE). ***Dubautia scabra*** (DC.) D.D. Keck subsp. ***leio-phylla*** (A. Gray) G.D. Carr: USA, *Hawaiian Islands, Moloka'i*, (1) upper East Fork Kawela Gulch, *BGB & Bainbridge 1267* (UC), (2) Ohi'alele vicinity, *KRW 11366* (PTBG), (3) **Lana'i**, near Lana'ihale crest, *Perlman 5074* (BISH), **West Maui**, (4) West Maui Mountains, Lihau, *Welton & Haus 1081* (BISH), (5) West Maui Mountains, head of Waihe'e River (Wall of Tears), *Oppenheimer H110803* (UC), **East Maui**, (6) Ha'ipua'ena Stream along Olinda Flume, *BGB & Bainbridge 778* (DUKE), (7) upper Opana Gulch, *Oppenheimer H70910* (UC), (8) headwaters of Kano and Waiokamilo streams, *KRW 12590* (PTBG), (9) Kopili'ula Stream, *Oppenheimer H71010* (UC), (10) *KRW 12692* (PTBG), (11) *KRW 12699* (PTBG), (12) **Hawai'i** (Big Island), Kohala Mountains, western rim of Alakahi Stream gorge, *BGB, Bainbridge & Roderick 1213* (UC). ***Dubautia scabra*** (DC.) D.D. Keck subsp. ***scabra***: USA, *Hawaiian Islands, East Maui* (1) Haleakala, northeast of Ko'olau, *Oppenheimer H71004* (UC), (2) Haleakala, above Lake Wai'anapanapa, *BGB, Bainbridge & Medeiros 1184* (UC), **Hawai'i** (Big Island), (3) Humu'ula Saddle, Pu'u Huluhulu, *BGB 526* (DAV), (4) west of Southwest Rift Zone (Mauna Loa), Pahoehoe (Second) 1950 Lava Flow, *BGB, Bainbridge & Perry 1509* (UC), (5) Mauna Loa, Kipuka Nene, *BGB, Bainbridge & Robichaux 1514* (UC), (6) Kilauea, *BGB 530* (DAV). ***Dubautia sherffiana*** Fosberg: USA, *Hawaiian Islands, O'ahu*, Wai'anae Range, (1) Ka'ala, *Carr 837-5* (HAW), (2) *Carr 837-12* (HAW), (3) *Carr 837-13* (HAW), (4) Kamaile'una Ridge, *BGB 515* (DAV). ***Dubautia syndetica*** G.D. Carr & Lorence: USA, *Hawaiian Islands, Kaua'i*, headwaters of Wahiawa Stream, (1) *Morden et al. 1382* (HAW), (2) *Morden et al. 1383* (HAW), (3) *Perlman 22323* (UC). ***Dubautia waialealae*** Rock: USA, *Hawaiian Islands, Kaua'i*, Wai'ale'ale, (1) *Caraway et al. Waialeale #5* (UC), (2) *Caraway et al.*

*Waialeale #7*, February 2003 (UC), (3) *Caraway et al. Waialeale #8*, February 2003 (UC), (4) *KRW 12504* (PTBG), (5) *KRW 12522* (PTBG), (6) *KRW 12526* (UC), (7) *KRW 13349* (PTBG). ***Dubautia waianapanapaensis*** G.D. Carr: USA, *Hawaiian Islands, East Maui*, Lake Wai'anapanapa, *BGB, Bainbridge & Medeiros 1183a* (UC). ***Hulsea algida*** A. Gray: USA, *California*, Sierra Nevada, Carson Range, *BGB 678* (DAV). ***Kyhosia bolanderi*** (A. Gray) B.G. Baldwin: USA, *California*, Sierra Nevada, Lake Tahoe, west shore, *BGB 509* (DAV). ***Raillardella pringlei*** Greene: USA, *California*, Trinity Mountains, Toad Lake, *BGB 608* (DAV). ***Wilkesia gymnoxiphium*** A. Gray: USA, *Hawaiian Islands, Kaua'i*, (1) between Awa'awapuhi and Nu'alolo valleys, *BGB & Bainbridge 1444* (UC), (2) Waimea Canyon rim, *Char 76.022* (HAW), (3) head of Kahoana Valley near Waimea Canyon rim, *BGB & Bainbridge 1388* (UC). ***Wilkesia hobdyi*** H. St. John: USA, *Hawaiian Islands, Kaua'i*, Polihale Ridge, *Carr 1150* (HAW).

## APPENDIX 2

*Dubautia* hybrids verified by nuclear ribosomal DNA sequences. Vouchers are deposited at the National Tropical Botanical Garden (PTBG) unless otherwise indicated by *Index Herbariorum* (Thiers, 2020) abbreviations (in parentheses) after collector name and number. *BGB* = Bruce G. Baldwin. *KRW* = Kenneth R. Wood.

***Dubautia imbricata*** subsp. ***acronaea*** × ***D. laxa*** subsp. ***hirsuta***: USA, *Hawaiian Islands, Kaua'i*, Wai'ale'ale, *KRW & BGB 13332, 13333, 13339, 13346*. ***Dubautia imbricata*** subsp. ***acronaea*** × ***D. paleata***: USA, *Hawaiian Islands, Kaua'i*, Wai'ale'ale, *KRW & BGB 13335, 13338, 13340, 13341, 13342, 13344, 13347*. ***Dubautia imbricata*** subsp. ***acronaea*** × ***D. waialealae***: USA, *Hawaiian Islands, Kaua'i*, Wai'ale'ale, *KRW 12530, KRW & BGB 13334*. ***Dubautia imbricata*** subsp. ***imbricata*** × ***D. laxa*** subsp. ***hirsuta***: USA, *Hawaiian Islands, Kaua'i*, Kamo'oloa Gulch, off ridge crest between Kapalaoa and Wai'ale'ale, *Perlman 22311* (UC). ***Dubautia laxa*** subsp. ***hirsuta*** × ***D. paleata***: USA, *Hawaiian Islands, Kaua'i*, Wai'ale'ale, *KRW 12532, KRW & BGB 13350*. ***Dubautia laxa*** subsp. ***hirsuta*** × ***D. waialealae***: USA, *Hawaiian Islands, Kaua'i*, Wai'ale'ale, *KRW 12505, KRW 12529, KRW & BGB 13343*. ***Dubautia laxa*** subsp. ***laxa*** × ***D. scabra*** subsp. ***leiophylla***: USA, *Hawaiian Islands, West Maui*, ridge northeast of 'Eke Crater, between Mananole and Kahakuloa streams, *Oppenheimer H91110* (UC), Kaho'olewa Ridge, *Perlman & KRW 15794* (BISH). ***Dubautia paleata*** × ***D. raillardioides***: USA, *Hawaiian Islands, Kaua'i*, north edge of Alaka'i Swamp, below Pihea, *BGB & Bainbridge 1491* (UC). ***Dubautia paleata*** × ***D. waialealae***: USA, *Hawaiian Islands, Kaua'i*, Wai'ale'ale, *KRW 12527, KRW 12296, KRW & BGB 13336*. ***Dubautia plantaginea*** subsp. ***plantaginea*** × ***D. scabra*** subsp. ***leiophylla***: USA, *Hawaiian Islands, Moloka'i*, Kawela Gulch, *BGB & Bainbridge 1217* (UC), *Perlman 6624*. ***Dubautia reticulata*** × ***D. scabra***: USA, *Hawaiian Islands, East Maui*, *Oppenheimer H101001* (UC).