



Symposium article

Insights into the Evolutionary History of the Hawaiian *Bidens* (Asteraceae) Adaptive Radiation Revealed Through Phylogenomics

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Abstract

Hawaiian plant radiations often result in lineages with exceptionally high species richness and extreme morphological and ecological differentiation. However, they typically display low levels of genetic variation, hindering the use of classic DNA markers to resolve their evolutionary histories. Here we utilize a phylogenomic approach to generate the first generally well-resolved phylogenetic hypothesis for the evolution of the Hawaiian *Bidens* (Asteraceae) adaptive radiation, including refined initial colonization and divergence time estimates. We sequenced the chloroplast genome (plastome) and nuclear ribosomal complex for 18 of the 19 endemic species of Hawaiian *Bidens* and 4 outgroup species. Phylogenomic analyses based on the concatenated dataset (plastome and nuclear) resulted in identical Bayesian and Maximum Likelihood trees with high statistical support at most nodes. Estimates from dating analyses were similar across datasets, with the crown group emerging ~1.76–1.82 Mya. Biogeographic analyses based on the nuclear and concatenated datasets indicated that colonization within the Hawaiian Islands generally followed the progression rule with 67–80% of colonization events from older to younger islands, while only 53% of events followed the progression rule in the plastome analysis. We find strong evidence for nuclear-plastome conflict indicating a potentially important role for hybridization in the evolution of the group. However, incomplete lineage sorting cannot be ruled out due to the small number of independent loci analyzed. This study contributes new insights into species relationships and the biogeographic history of the explosive Hawaiian *Bidens* adaptive radiation.

Subject areas: Molecular systematics and phylogenetics

Keywords: ancestral range estimation, founder events, island biogeography, phylogenetic dating, priority effects, progression rule.

A hallmark of adaptive radiations is that many diverse species arise within a short time frame (Schluter 2000; Seehausen 2006). Despite the high morphological and ecological diversity that characterizes these rapid radiations, when insufficient time has elapsed between

speciation events to allow for the accumulation of phylogenetically informative nucleotide substitutions, this impedes reconstruction of their evolutionary history. Classical genetic studies that employ a small number of loci to understand evolutionary relationships are

often hampered by the low level of genetic divergence among species and may be further complicated by processes that produce conflicting phylogenetic signals such as incomplete lineage sorting and introgressive hybridization (e.g., Som 2015). These problems are especially severe for evolutionarily young radiations, often resulting in “comb-like” phylogenetic trees, where little to no information is available to infer species relationships or the processes involved in species formation (e.g., Lowrey et al. 2001; Hughes and Eastwood 2006; Valente et al. 2010; Knope et al. 2012a). However, it is precisely these young radiations that stand to provide valuable insight into the origins of adaptive radiation, as they can be studied while the early stages of diversification are occurring.

The Hawaiian Islands have emerged as one of the world's premier natural laboratories for the study of speciation and adaptive radiation (e.g., Funk and Wagner 1995; Ziegler 2002) in part due to their well-understood and relatively simple geologic history (e.g., Price and Clague 2002; Clague et al. 2010; Obbard et al. 2012), isolation from the nearest mainland source pool of immigrant taxa, and high proportion of endemic species. The native Hawaiian flora is comprised of more than one thousand native angiosperm species of which ~90% are endemic, all descended from an estimated 259 original colonists (Price and Wagner 2018) that in some cases underwent explosive adaptive radiations (e.g., Wagner et al. 1999; Price and Wagner 2004). However, despite many decades of intense research on the evolutionary history of Hawaiian plant radiations, researchers have often been stymied by an apparent paradox: while Hawaiian plant radiations often have exceptionally high species richness and exhibit extraordinary ecological and morphological differentiation, they typically display extremely low levels of genetic variation compared to their mainland relatives, hindering the ability to use classic DNA markers to infer their evolutionary history. Many of the iconic Hawaiian plant radiations show this pattern, including silverswords (Baldwin and Robichaux 1995; Baldwin and Sanderson 1998), lobeliads (Givnish et al. 1995, 2004, 2009; Pillon et al. 2013), mints (Lindqvist and Albert 2002; Lindqvist et al. 2003; Welch et al. 2016), *Cyrtandra* (Cronk et al. 2005; Clark et al. 2009; Johnson et al. 2017, 2019), *Plantago* (Dunbar-Co et al. 2008), *Scaevola* (Howarth et al. 2003), *Schiedea* (Soltis et al. 1996; Sakai et al. 1997), *Tetramolopium* (Lowrey 1995; Okada et al. 1997, 2000; Lowrey et al. 2001) and *Bidens* (Helenurm and Ganders 1985; Ganders et al. 2000; Knope et al. 2012a).

The Hawaiian Islands are formed over a stationary magma hotspot as the Pacific tectonic plate continuously moves to the northwest, creating an exceptionally clear linear chronosequence of islands that are each progressively older as one moves to the northwest from Hawai'i Island, currently the youngest island at the southeast end of the archipelago (Price and Clague 2002; Clague et al. 2010; Obbard et al. 2012). The progression rule was first proposed in an attempt to explain general patterns of the origins of biological diversity in the Hawaiian Islands by Funk and Wagner (1995) and is a testable hypothesis that predicts that cladogenetic events generally coincide with interisland dispersal events from older to younger islands. Indeed, many Hawaiian plant groups including silverswords (Baldwin and Robichaux 1995; Landis et al. 2018), *Schiedea* (Nepokroeff et al. 2003), *Psychotria* (Ree and Smith 2008), lobeliads (Givnish et al. 2009), and *Cyrtandra* (Johnson et al. 2017, 2019) show evidence for the progression rule. Moreover, many animal groups in Hawai'i also display evidence for the progression rule including *Megalagrion* damselflies (Jordan et al. 2003), *Laupala* crickets (Mendelson and Shaw 2005), Banza katydids (Shapiro et al. 2006), *Hypsomocoma* moths (Haines et al. 2014), picture-winged *Drosophila* (Bonacum

et al. 2005; Magnacca and Price 2015), succineid land snails (Rundell et al. 2004), *Orsonwelles* spiders (Hormiga et al. 2003), and flycatcher birds (VanderWerf et al. 2010). In contrast, Hawaiian *Tetramolopium* (Lowrey 1995), *Geranium* (Funk and Wagner 1995; Pax et al. 1997), and *Hylaeus* bees (Magnacca and Danforth 2006) do not show evidence for the progression rule in Hawai'i and appear to have first colonized younger islands before subsequently colonizing older islands.

Bidens (Ko'oko'olau, Asteraceae) is generally considered, alongside the silverswords and lobeliads, to be among the best examples of adaptive radiation in the Hawaiian flora (Carr 1987; Givnish et al. 1995, 2004, 2009; Baldwin and Sanderson 1998). In fact, the 19 *Bidens* species endemic to the Hawaiian archipelago display greater ecological and morphological diversity than the remaining *c.* 230 currently described species in the genus, which are distributed across 5 continents (Sherff 1937; Ganders and Nagata 1984; Kim et al. 1999). Endemic Hawaiian *Bidens* species occur on all 8 main islands (Ni'ihau, Kaua'i, O'ahu, Maui, Lāna'i, Moloka'i, Kaho'olawe, and Hawai'i Island), spanning an elevational range from sea level to over 2200m. Species occupy a wide variety of habitats including sand dunes, lava flows, deserts, scrublands, mesic forests, rainforests, and wetland bogs (Figure 1; Ganders and Nagata 1984; Wagner et al. 1999). Most taxa are single-island endemics (~70%; Figure 1), and many are restricted to individual habitat types within or among islands (Ganders and Nagata 1984). In addition, many species and subspecies are of pressing conservation concern, listed as threatened, endangered, or critically endangered (see Table 1; Wagner et al. 1999; Knope et al. 2013; IUCN 2017).

The Hawaiian *Bidens* radiation has long fascinated biologists, as the clade is thought to display adaptive shifts in pollination syndrome, seed (achene) dispersal mode, and growth form. Additionally, high levels of hybridization have been inferred among taxa based on the formation of hybrid swarms at contact zones on multiple islands (e.g., Carlquist 1974; Gillett 1975; Ganders and Nagata 1984; Carr 1987). However, despite considerable effort by researchers across many decades, the evolutionary history of Hawaiian *Bidens* has largely remained a mystery. Starting in the 1980's researchers began using molecular techniques to understand how this iconic adaptive radiation unfolded, but Helenurm and Ganders (1985) found little genetic divergence in isozymes across the 15 endemic Hawaiian species surveyed as compared to their mainland relatives. Ganders et al. (2000) then surveyed 5 endemic Hawaiian *Bidens* species and 2 hybrids and found them all to be identical at the nuclear ribosomal internal transcribed spacer (nrITS; for critical review of this approach see Álvarez and Wendel [2003]), suggesting that colonization and radiation was recent and rapid. With the inclusion of several putative outgroup taxa, this study also provided evidence that the most recent common ancestor of the Hawaiian radiation may have originated from Central America. Knope et al. (2012a) sequenced all 19 endemic Hawaiian species, 2 putative Hawaiian hybrids, and multiple outgroup species at 4 chloroplast and 2 nuclear markers and found evidence for a single colonization event possibly from Central America to the Hawaiian Islands ~ 2.1 Mya (1.3–3.1 Mya, 95% highest posterior density). With this single colonization event giving rise to the 19 extant endemic Hawaiian species, the radiation is considered among the fastest documented in plants globally, particularly when considered on a per-unit-area basis (Knope et al. 2012a). However, the phylogeny remained completely unresolved for the Hawaiian taxa because the paucity of DNA sequence divergence precluded inference of species relationships or biogeographic history.

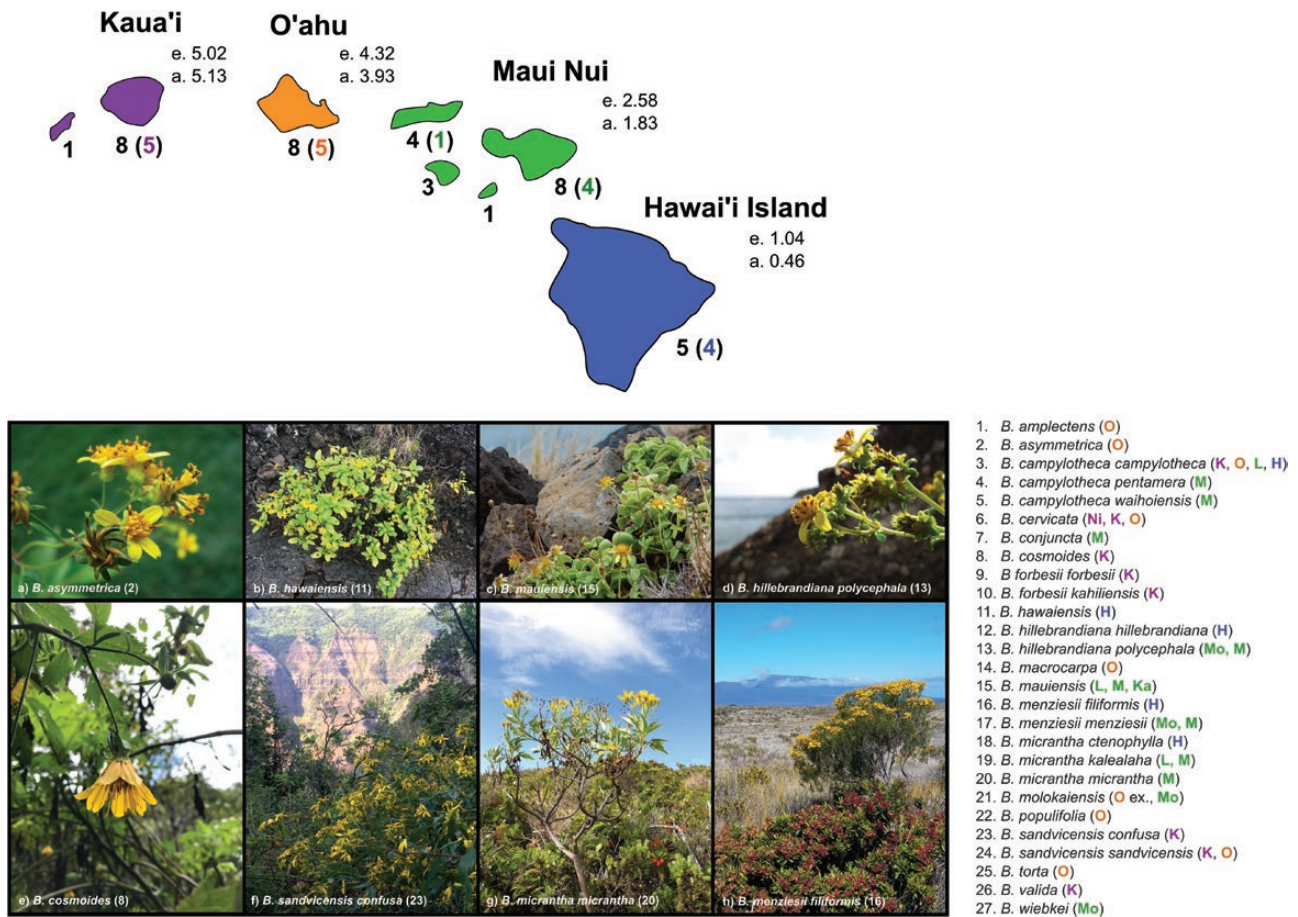


Figure 1. Map of the Hawaiian Islands showing the number of *Bidens* taxa (species and subspecies) per island (with number of island endemic taxa in parentheses), age estimates of islands based on first emergence (e) and oldest surface rocks (a), and the distribution of each taxon by island (northwest to left and southeast to right: Ni = Ni'ihau, K = Kaua'i, O = O'ahu, M = Maui, L = Lāna'i, Mo = Moloka'i, H = Hawai'i; ex. = extinct) with representative photographs of taxa showing examples of growth habit, floral morphology, and habitat differences. Taxa in photographs are as follows: a) O'ahu endemic *B. asymmetrica* (D. Eickhoff); b) Hawai'i Island endemic *B. hawaiiensis* (J. Stallman); c) Maui Nui endemic *B. mauiensis* (F. and K. Starr); d) Maui Nui endemic *B. hillebrandiana* subsp. *polycephala* (F. and K. Starr); e) endangered Kaua'i endemic *B. cosmoides* (T. Kroessig); f) critically endangered Kaua'i endemic *B. sandvicensis* subsp. *confusa* (M. Clark); g) vulnerable Maui Nui endemic *B. micrantha* subsp. *micrantha* (F. and K. Starr); h) Hawai'i Island endemic *B. menziesii* subsp. *filiformis* (J. Latsha).

Here we develop a phylogenomic hypothesis for the Hawaiian *Bidens* based on the entire chloroplast genome (plastome) and the nuclear ribosomal DNA complex obtained from next-generation sequencing data. The resulting phylogenetic framework from individual and concatenated sequence datasets are applied to: 1) evaluate relationships among the Hawaiian *Bidens* taxa and outgroups, 2) estimate the timing and patterns of colonization across the Hawaiian archipelago, and 3) infer the biogeographic processes that may have led to the present-day distribution of species.

Materials and Methods

Taxon Sampling

Total genomic DNA from 29 previously collected samples (Knope et al. 2012a) representing 23 *Bidens* species (including 18 of 19 endemic Hawaiian species and 11 subspecies in total), 1 interspecific hybrid, and 4 outgroup taxa were sequenced on an Illumina HiSeq 3000 or 4000 platform (Table 1). One additional Hawaiian endemic, *B. populifolia*, failed to produce usable data during library preparation and was excluded from the sequencing run. For outgroup taxa, we sequenced two invasive congeners (*B. alba* var. *radiata* and

B. pilosa) from Central America that are naturalized in Hawai'i, and belong to the lineage that is hypothesized to be sister to the Hawaiian radiation (Ganders et al. 2000; Knope et al. 2012a). Two more distantly related *Bidens* species (*B. pachyloma* and *B. schimperii*) from Africa were also included as outgroups (Kim et al. 1999; Knope et al. 2012a). To root the tree, we used GenBank genomic data for the common sunflower, *Helianthus annuus*, which alongside *Bidens* is also a member of the Helianthodeae supertribe in the Asteroideae subfamily of the Asteraceae family, but well-established to be outside of the *Bidens* focal clade (Mort et al. 2008).

Library Construction and High-Throughput Sequencing

DNA accessions from Knope et al. (2012a) were originally extracted by Qiagen DNeasy plant mini kits (Qiagen, Valencia, California) or a modified CTAB/CsCl banding extraction protocol (Morden et al. 1996). For next-generation library preparation, all laboratory-grade pipettes, plastics, and water were subjected to additional UV sterilization to minimize potential contaminants. Shotgun whole-genome sequencing libraries were prepared using the Nextera XT protocol and TruSeq indices (Illumina Inc.). Samples were quantified

Table 1. Sample and voucher information for the 29 *Bidens* specimens used for phylogenomic analyses

Species	Authority	Herbarium number (HPDL)	Collector	Year	Conservation status	Geography
<i>Bidens alba</i> var. <i>radiata</i>	(Sch. Bip.) R.E. Ballard ex Melchert	None	J. Knope & K. McMillan	2008	None	Central America; Widespread Invasive
<i>Bidens amplexans</i>	Sherff	BISH 739530	M. Knope	2007	Vu	O'ahu ^a
<i>Bidens asymmetrica</i>	Sherff	BISH 739247	M. Knope	2008	None	O'ahu ^a
<i>Bidens campylotheca</i> subsp. <i>pentamera</i>	(Sherff) Ganders & Nagata	None	M. Knope	2008	Cr	Maui ^a
<i>Bidens campylotheca</i> subsp. <i>campylotheca</i>	Sch. Bip.	BISH 739538	M. Knope	2008	Vu	Kaua'i, O'ahu ^a , Lāna'i, Hawai'i
<i>Bidens cervicata</i>	Sherff	UBC V206886	K. Wood	1992	None	Ni'ihau, Kaua'i ^a , O'ahu
<i>Bidens conjuncta</i>	Sherff	UBC V206887	S. Perlman	1991	Vu	Maui ^a
<i>Bidens cosmoides</i>	Sherff	None (3787)	M. Harbinirman	—	En	Kaua'i ^b
<i>Bidens forbesii</i> subsp. <i>forbesii</i>	Sherff	BISH 739568	M. Knope	2007	En	Kaua'i ^a
<i>Bidens forbesii</i> subsp. <i>kahiliensis</i>	Ganders & Nagata	UBC V245595	K. Wood	2008	En	Kaua'i ^a
<i>Bidens hawaiiensis</i>	Pilg.	None	M. Knope & S. Hinard	2008	None	Hawai'i ^a
<i>Bidens hillebrandiana</i> subsp. <i>hillebrandiana</i>	O. Deg.	UHM (5248)	C. Morden	2005	None	Hawai'i ^a
<i>Bidens macrocarpa</i>	Sherff	UHM (608)	C. Morden	1995	None	O'ahu ^a
<i>Bidens mauiensis</i>	Sherff	BISH 739567	M. Knope & S. Hinard	2008	None	Lāna'i, Maui ^a , Kaho'olawe
<i>Bidens menziesii</i> subsp. <i>filiformis</i>	Sherff	UHM (384)	C. Morden	1995	None	Hawai'i ^a
<i>Bidens menziesii</i> subsp. <i>menziesii</i>	Sherff	None (4733)	C. Morden	—	None	Moloka'i ^a , Maui
<i>Bidens menziesii</i> filiformis	N/A	None	M. Knope & P. Aldrich	2008	None	Hawai'i ^a
<i>X micrantha ctenophylla</i>	(Sherff) Nagata & Ganders	UHM (2513)	C. Morden	2000	Cr	Hawai'i ^a
<i>Bidens micrantha</i> subsp. <i>ctenophylla</i>	Nagata & Ganders	None (5207)	C. Morden	—	En	Lāna'i, Maui ^a
<i>Bidens micrantha</i> subsp. <i>kalealaha</i>	Sherff	None	M. Knope & S. Hinard	2008	Vu	O'ahu (ex), Moloka'i ^a
<i>Bidens pachyloma</i>	(Oliv. & Hiern) Cufod.	None	D. Crawford	—	None	East Africa
<i>Bidens pilosa</i>	L.	BISH 739532	M. Knope	2008	None	Central America; Widespread Invasive
<i>Bidens sandvicensis</i>	Less.	None (3786)	M. Harbinirman	—	None	Kaua'i, O'ahu ¹
<i>Bidens sandvicensis</i> subsp. <i>confusa</i>	Nagata & Ganders	None	M. Knope	2008	Cr	Kaua'i ^a
<i>Bidens sandvicensis</i> subsp. <i>sandvicensis</i>	Less.	BISH 739535	M. Knope	2008	None	Kaua'i ^a , O'ahu
<i>Bidens schimperi</i>	Sch. Bip. ex Walp.	None	D. Crawford	—	None	East Africa
<i>Bidens torta</i>	Sherff	UHM (1675)	C. Morden	1998	None	O'ahu ^a
<i>Bidens valida</i>	Sherff	UBC 725966	K. Wood	2004	En	Kaua'i ^a
<i>Bidens wiebkei</i>	Sherff	BISH 739536	M. Knope & S. Hinard	2008	Cr	Moloka'i ^a

HPDL = Hawaiian Plant DNA Library at the University of Hawaii at Mānoa; BISH = Bernice Pauahi Bishop Museum, Honolulu, HI; UHM = University of Hawaii at Mānoa, Joseph F. Rock Herbarium, Honolulu, HI voucher numbers pending; UBC = University of British Columbia Herbarium, Vancouver, BC, Canada. International Union for the Conservation of Nature (IUCN) redlist conservation status: Critically endangered = CR; endangered = En; vulnerable = Vu; least concern = LC; none = not assessed. Distribution of *Bidens* taxa taken from the Smithsonian Flora of the Hawaiian Islands website (Wagner et al. 2005); (ex) = extirpated.

^aIsland where samples were collected.

^bSamples collected from Hui Kū Maoli Ola Native Plant Nursery, Kaneohe, HI.

using High Sensitivity qubit fluorometry (ThermoFisher Scientific) and visualized on a 2100 Bioanalyzer (Agilent), and then pooled at equimolar concentrations. Libraries were sequenced in single

lanes on Illumina HiSeq 3000 (n = 13 *Bidens* samples, Oregon State University Center for Genome Research) or 4000 (n = 16 *Bidens* samples, Biocomputing Lab and the University of California,

Berkeley, Vincent J. Coates Genomics Sequencing Lab) platforms to produce 150 bp paired-end reads. Reads were quality controlled using Trimmomatic version 0.3.2 (Bolger et al. 2014) to remove adapter sequences, trim low-quality bases (< 20 phred) from beginnings and ends of reads, and to apply a 4bp sliding window trimming approach with average phred quality score >20, retaining sequences with a minimum length of 75 nucleotides (nt).

Assembly of the *Bidens* Reference Plastome and Nuclear Ribosomal Complex

An initial draft *Bidens* reference plastome was assembled by mapping reads of *B. asymmetrica* to the phylogenetically closest available reference, *Helianthus annuus* (NCBI Accession KU315426.1 plastome) using the Geneious mapper (Kearse et al. 2012). This reference was iteratively improved through manual edits to correct mis-assemblies due to short repeats, and insertions and deletions in *Bidens* that were not present in the *Helianthus* reference. This process was repeated several times until all mis-assemblies were resolved. Finally, poly A and T regions were manually edited to favor reads that sequenced through these regions over reads that ended in poly A's or T's. We identified a 24,624 bp inverted repeat in the plastome reference sequence; this repeat region was retained in the reference for read-mapping, but removed for phylogenetic analyses. To recover the complete nuclear ribosomal complex (ETS, 18S, ITS1, 5.8S, ITS2, 26S, NTS) we also iteratively remapped reads until the draft *Bidens* reference sequence extended beyond the initial *Helianthus* reference (NCBI Accession KF767534.1). This was continued until coverage on both the 5' and 3' ends of the sequence dropped to < 50% of the average overall coverage, after which reads that mapped to the region were non-homologous. We interpreted this pattern to indicate the boundaries of the ribosomal complex (Baldwin et al. 2005).

Chloroplast Genome and Nuclear Ribosomal Complex Sequences

The chloroplast genomes for the remaining samples were constructed by mapping demultiplexed, quality-controlled reads to the plastome reference to generate a genotype file for sequence extraction. The genotypes were called using the samtools 1.4.1/bcftools-1.2 pipeline with setting “multiallelic-caller” (Li 2011), applying map and base quality score thresholds of 20 and 30. Reads were mapped to the reference with Bowtie2 (Langmead and Salzberg 2012) using the end-to-end alignment mode, a maximum mapping distance of 1000 nt, and custom effort search options -D 40 -R 10 -N 1 -L 15 -i S,1,0.50. Only concordantly mapped, paired-end reads, identified with the program samtools view (setting -F 2316), were used for genotype calling. We tested other mapping parameters and shorter read lengths, but found these parameters minimized the number of fixed heterozygous positions for each sample, which should be 0 assuming uniparental inheritance of plastomes. The draft set of consensus sequences for each taxon was extracted from the genotype file using the vcfutils.pl utility script vcf2fq. Next, the draft sequences were polished in Geneious (Kearse et al. 2012) by re-mapping each sample's concordantly mapped, paired-end reads back to its own draft consensus sequence in order to manually check for mis-assemblies and rectify ambiguous positions. Heterozygous positions in the final set of sequences were masked by changing the IUPAC code to “N”. The nuclear ribosomal complex sequences were generated by mapping paired-end reads in Geneious, extracting consensus sequences, and polishing as described above. The sequence data sets were aligned in MAFFT v.7.388 (Katoh and Standley 2013),

stripping alignment columns with more than 10% gaps from the final alignments.

Phylogenetic Analyses and Divergence Time Estimates

PartitionFinder v. 2.1.1 (Guindon et al. 2010; Lanfear et al. 2016) was used to determine the appropriate data-partitioning scheme for the nuclear rDNA dataset, while the plastome dataset was not partitioned and treated as a single super locus (e.g., Lin et al. 2019; Table 2). For the nuclear rDNA dataset, we used the greedy algorithm (Lanfear et al. 2012) and the corrected Akaike Information Criterion (AICc) to find the optimal partition scheme from 7 partitions that were determined a priori based on coding and non-coding regions. The best scoring partition scheme (Supplementary Table S1) was used for Maximum Likelihood (ML) and Bayesian inference (BI). Model testing and ML analyses were conducted using the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The relative fit of the different models of nucleotide evolution was assessed using jModelTest v. 2.1.6 (Darriba et al. 2012; see Supplementary Table S1) and the Akaike Information Criterion (AIC). ML analyses were conducted using RAxML v. 8.2.6 (Stamatakis 2006, 2014; Stamatakis et al. 2008). The best scoring ML tree estimated from 1000 alternative maximum parsimony starting trees was annotated with the results from 1000 bootstrap replicates using the GTRGAMMA nucleotide substitution model. Separate ML analyses were conducted for the plastome and nuclear regions to assess congruence between trees, with areas of conflict determined by examining the placement of individual taxa on tree tips (Supplementary Figure S1). Relationships were considered incongruent if the placement of taxa varied among the individual trees and exhibited BS values $\geq 80\%$. An additional ML analysis was conducted on the concatenated (plastome + nuclear) partitioned dataset, with the same parameters used as above.

Phylogeny and clade divergence times were estimated separately for the nuclear, plastome, and concatenated datasets using BI implemented in BEAST2 v. 2.5.1 (Ronquist et al. 2012) in the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). As with the ML analyses, separate Bayesian analyses were conducted for the plastome and nuclear regions to assess congruence, with areas of conflict determined by examining the placement of individual taxa on each gene tree. The trees were modeled under a Yule process using a random starting tree, the GTR+I+G substitution model, and a relaxed clock with a lognormal distribution. We placed a calibration point at the crown of the Hawaiian *Bidens* clade based on the previous dating analysis by Knope et al. (2012a), which estimated the crown age of Hawaiian *Bidens* to be 2.1 Mya (1.3–3.1, 95% highest probability density, HPD). We used a lognormal distribution and set the mean to 2.1 Mya, with the standard deviation set to include the 95% HPD. For a second calibration point we used the age of Hawai'i Island to constrain the crown of the endemic Hawai'i Island clade (*B. micrantha* subsp. *ctenophylla*, *B. hawaiiensis*, *B. menziesii* subsp. *filiformis*, *B. micrantha* subsp. *ctenophylla* x *B. menziesii* subsp. *filiformis*). Estimates for the age of Hawai'i Island were based on a combination of K–Ar or Ar–Ar dates and a growth model for typical Hawaiian volcanoes (see Obbard et al. 2012 and references therein). For this second calibration point, we used a uniform distribution, with the upper bound set to the age of shield emergence (1.04 Ma) and no lower bound. Although the age of the oldest surface rocks or the age of shield completion have been used for island age calibrations in other dating analyses of Hawaiian genera (Obbard et al. 2012; Magnacca and Price 2015), we opted to use the age of shield

Table 2. Summary statistics for the nuclear ribosomal loci and plastome, including models of molecular evolution, the length of each region in base pairs, and the number and percentage of variable and parsimony-informative sites

Locus	Model of molecular evolution	Length (bp)	Variable sites	Parsimony informative sites	Variable sites (%)	Parsimony informative sites (%)
ETS	TPM2uf+I+G	2867	471	201	16.43	7.01
18S	TIM1+I	1811	18	4	0.99	0.22
ITS1	SYM+G	271	101	24	37.27	8.86
5.8S	TrNef	159	9	3	5.66	1.89
ITS2	TPM2uf+G	253	82	29	32.41	11.46
26S	GTR+I+G	3349	58	13	1.73	0.39
NTS	TPM3uf+G	3327	472	289	14.19	8.69
Plastome	TIM+I+G	128,025	6139	1198	4.80	0.94

emergence as the maximum age estimate for colonization, given that extant species of *Bidens* on Hawai'i Island can be found on very young volcanic soils (e.g., *B. hauaiensis*), and could theoretically colonize the island prior to the development of mature soils and forests (Seijmonsbergen et al. 2018). Two independent Markov Chain Monte Carlo (MCMC) analyses were run for 200 million generations, sampling every 5,000 generations. Independent runs were merged in LogCombiner v. 2.5.0 (Bouckaert et al. 2014), and chain convergence was checked in Tracer v. 1.6 (Rambaut et al. 2014) by examining log-likelihood plots and ensuring that Effective Sample Size (ESS) values were well above 200. After discarding 20% of the trees as burn-in, a maximum clade credibility tree with mean node heights was constructed using TreeAnnotator v. 2.5.0 (Bouckaert et al. 2014). For ML and BI analyses, tree topology and node support were examined in Figtree v. 1.4.3.

Analysis of Nuclear-Plastome Conflict

Conflict within each dataset and in the combined nuclear/plastome dataset was visualized using the NeighborNet method in SplitsTree v. 4.14.8 (Huson and Bryant 2006). We also visualized incongruence between the plastome and nuclear ribosomal phylogenies using a tanglegram. A maximum pseudo-likelihood approach implemented in PhyloNet 3.6.10 (Than et al. 2008; Yu and Nakhleh 2015) was used to infer the phylogenetic history of the Hawaiian *Bidens* lineage under the assumption that both incomplete lineage sorting (ILS) and hybridization may be responsible for the observed incongruences. We pruned the non-Hawaiian *Bidens* taxa from the plastome and nuclear ribosomal trees in order to reduce computation time. The 2 trees were used as input to compute hybridization networks with between 0–5 hybridization events. We note that the method is more robust when comparing many loci (often >500), and that we are unlikely, given only 2 independent loci, to recover the true evolutionary network. However, we opted to use this method to identify potential cases of hybridization, which will require verification with subsequent analysis based on multiple nuclear loci.

Ancestral Range Estimation

BioGeoBEARS (BioGeography with Bayesian (and likelihood) Evolutionary Analysis in R Scripts'; Matzke 2013a) was used to estimate ancestral ranges under the DEC (Dispersal-Extinction-Cladogenesis; Ree et al. 2005; Ree and Smith 2008), DIVA (Dispersal-Vicariance Analysis; Ronquist 1997) and BayArea (Bayesian Inference of Historical Biogeography for Discrete Areas; Landis et al. 2013) models in the R statistical environment (R Core Team 2014). Given that these models are implemented in a ML framework and thus differ from the original parsimony-based

DIVA method and Bayesian BayArea method, we follow Matzke (2013b) in referring to these models as DIVA-like and BayArea-like. We also included 2 free parameters that are likely important for understanding biogeographic patterns on islands: founder events (“*j*” parameter; Matzke 2014) and dispersal distance (“*x*” parameter; Van Dam and Matzke 2016). The ultrametric Bayesian tree for each of the 3 datasets (nuclear, plastome, and concatenated) was pruned to remove all non-Hawaiian taxa and used to reconstruct ancestral ranges. Given that they were connected for most of their history, the islands of Maui, Moloka'i, Kaho'olawe and Lāna'i were treated as a single island region, referred to as Maui Nui (e.g., Funk and Wagner 1995). Four geographic areas were defined (Kaua'i, O'ahu, Maui Nui, and Hawai'i Island), and each taxon was assigned to an area based on distributions listed in the Flora of the Hawaiian Islands website (Wagner et al. 2005). Distances between areas were determined using ArcGIS software (ESRI 2011), with *x* defined as the distance between the centers of 2 areas measured in kilometers. Distances were then rescaled (dividing by the smallest distance) to ensure that units of measurement would not influence the outcome of likelihood searches. The maximum number of areas (i.e., the allowed number of geographic ranges that can be explored together in state space) was set to 4 to reflect the multi-island distribution of *B. campylothecha* subsp. *campylothecha*, which occurs in all 4 geographic areas. Lastly, we used an ‘areas allowed’ constraint that permitted taxa to colonize Hawai'i Island only after its emergence at 1.04 Mya (Obbard et al. 2012). Models were compared for statistical fit using the AIC and a Likelihood Ratio Test (LRT). Lastly, we used BioGeoBEARS to perform a biogeographic stochastic mapping (BSM) analysis (Matzke 2016; Dupin et al. 2017). We conducted 500 stochastic mapping simulations on the best model to get event counts for biogeographic processes involving anagenesis and cladogenesis, as well as to infer the directionality of dispersal events.

Results

Nucleotide Variability

For the 29 samples included in this study, we obtained full high coverage (>1000x sequencing depth) plastome sequences that were ~150,600 bp each including the 24,624 bp inverted repeat. After gap stripping the plastome data set and removing the inverted repeat, the number of variable sites was 6139 for all samples considered (including *H. annuus*), 3281 excluding *H. annuus*, and 392 for Hawaiian *Bidens* taxa only. The number of parsimony informative sites was 1,198 across all taxa (including *H. annuus*), 1054 excluding *H. annuus*, and 108 for Hawaiian *Bidens* taxa only.

We also obtained high coverage (>1000x sequencing depth) for all samples for the nuclear ribosomal complex. The sequence length of the Hawaiian *Bidens* and the 2 Central American *Bidens* species was ~11,570 bp, whereas the sequence length for the 2 African *Bidens* was 6650 bp. During assembly the African outgroup taxa reached a non-homologous flank, indicating the edge of the complex was reached, sooner than the Central American and Hawaiian *Bidens* taxa. Rather than shortening the alignment to the length of the African taxa, we retained these flanking regions found in all the Hawaiian and Central American taxa for our analyses. After gap stripping the number of variable sites in the nuclear ribosomal complex was 1,031 for all samples considered (including *H. annuus*), 847 excluding *H. annuus*, and 325 for Hawaiian *Bidens* taxa only. The number of parsimony informative sites was 457 across all taxa (including *H. annuus*), 428 excluding *H. annuus*, and 138 for Hawaiian *Bidens* taxa only (see Table 2 for breakdown by locus).

Phylogenetic Relationships

We found significant conflict between the nuclear ribosomal and plastome trees (Figure 2), and therefore present results for the nuclear, plastome, and concatenated datasets separately. The ML tree based on the nuclear ribosomal data indicates the 2 African taxa are sister (100 BS) and are in turn sister to all remaining *Bidens* taxa (100 BS; Supplementary Figure S1). The 2 Central American taxa are sister to one another (100 BS), but their relationship to the Hawaiian taxa is unresolved (Supplementary Figure S1). The Hawaiian taxa are reconstructed as monophyletic (100 BS), but branch lengths within the Hawaiian clade are short and node support is highly variable (ranging from 13–100 BS; Supplementary Figure S1). Thirteen nodes within the Hawaiian clade are well-supported (≥ 82 BS; Supplementary Figure S1). Similarly, in the Bayesian tree based on the nuclear data the 2 African taxa are sister (1.00 PP), the 2 Central American taxa are sister (1.00 PP), and the Hawaiian taxa are strongly supported as monophyletic (1.00 PP; Figure 2). Node support for relationships within the Hawaiian clade is highly variable (ranging from 0.27–1.00 PP), but 11 nodes are strongly supported (1.00 PP; Figure 2). In contrast to the ML tree, the Central American

taxa are reconstructed as sister to the Hawaiian clade (1.00 PP) in the BI tree (Figure 2).

The ML tree based on the plastome data shows strong support for the monophyly of the Hawaiian taxa (100 BS) and for the node that unites the Central American taxa (100 BS), but support is weak for the sister relationship between the Central American and African taxa (61 BS) and the sister relationship between the African taxa (62 BS; Supplementary Figure S1). As in the nuclear trees, branch lengths within the Hawaiian clade are short, and node support is highly variable (ranging from 11–100 BS), but 12 nodes display strong support (≥ 85 BS; Supplementary Figure S1). The BI tree based on the plastome data shows strong support for a sister relationship between the two African taxa (1.00 PP) and the two Central American taxa (1.00 PP), and these two clades are in turn strongly supported as sister to one another (0.98 PP; Figure 2). The monophyly of the Hawaiian taxa is also strongly supported (1.00 PP; Figure 2). In contrast to the nuclear tree reconstructed using BI, the sister group to the Hawaiian clade is unresolved (Figure 2). Branch lengths within the Hawaiian clade are exceedingly short and node support is highly variable (ranging from 0.15–1.00 PP), but 12 nodes are strongly supported (1.00 PP; Figure 2).

The concatenated BI (Figure 3) and ML trees (inset Figure 3) resulted in nearly identical topologies, although branch lengths were again generally short among the Hawaiian taxa. The 2 African *Bidens* taxa were placed sister to all remaining *Bidens* in the Bayesian analysis, although with weak support (0.56 PP; Figure 3); additionally, there was no support for this relationship in the ML analysis (< 50 BS; Figure 3, node 1). The two Central American *Bidens* taxa were strongly supported in the ML analysis as sister to the Hawaiian radiation (94 BS; Figure 3), although BI support was weaker for this relationship (0.78 PP; Figure 3, node 2). The monophyly of the Hawaiian species was strongly supported in both the ML and BI analyses (100 BS, 1.00 PP; Figure 3, node 3), although our study does not include taxa from the South Pacific islands (see Discussion).

The trees based on the concatenated dataset for the Hawaiian taxa show that two primary clades diverged early in the evolutionary history of the group, a Maui Nui/Hawai'i Island clade

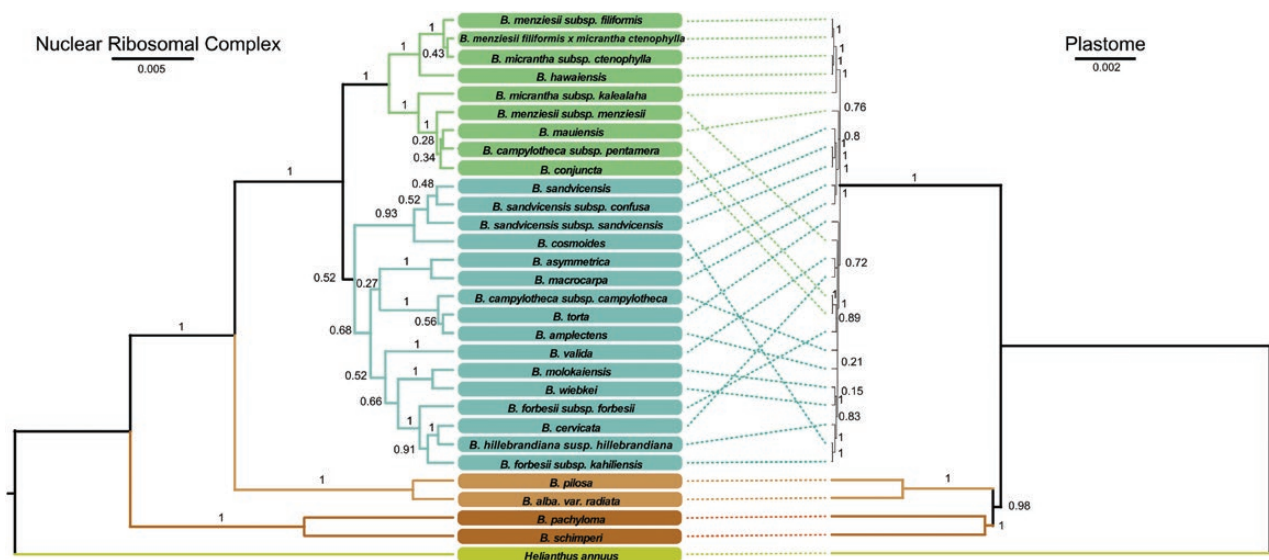


Figure 2. Tanglegram comparing the *Bidens* phylogeny estimate based on the nuclear ribosomal complex (left) and the complete plastome (right). Bayesian trees with posterior probabilities at each node are shown. Scale bars indicate the number of nucleotide substitutions per site.

(Figure 3; node 13) and a Kaua'i/O'ahu/Maui Nui/Hawai'i Island clade (Figure 3; node 4). The Maui Nui/Hawai'i Island clade is strongly supported (89 BS, 1.00 PP; node 13) and further bifurcates into two smaller clades: 1) a Maui Nui clade (98 BS, 1.00 PP; node 14) comprising *B. mauiensis*, *B. menziesii menziesii*, *B. conjuncta*, and *B. campylothea pentamera*, and 2) a Hawai'i Island clade (100 BS, 1.00 PP; node 18) comprising *B. micrantha ctenophylla*, *B. menziesii filiformis*, *B. hawaiiensis*, and the natural hybrid *B. menziesii filiformis* x *B. micrantha ctenophylla*. This interspecific hybrid is strongly supported as sister to *B. menziesii filiformis* (90 BS, 1.00 PP; node 19), confirming the status of this species as one of the two parental taxa involved in the formation of this hybrid. The second purported parental species is supported as belonging to the sister clade comprising *B. micrantha ctenophylla* and *B. hawaiiensis* (100 BS, 1.00 PP; node 20). The two subspecies of *B. menziesii* do not form a natural species group according to the phylogenetic hypothesis presented here, but are supported as belonging to the larger Maui Nui/Hawai'i Island clade (node 13). Three morphologically similar species from Kaua'i and O'ahu were placed sister to

the remaining taxa in the Kaua'i/O'ahu/Maui Nui/Hawai'i Island clade but not supported (52 BS, 0.68 PP; node 4). *Bidens macrocarpa* (O'ahu) and *B. asymmetrica* (O'ahu) are strongly supported as sister species (100 BS, 1.00 PP; node 22), and are in turn strongly supported as sister to *B. sandvicensis* (Kaua'i/O'ahu) (91 BS, 1.00 PP; node 21). The *B. sandvicensis* individual that was not identified to the subspecies level was strongly supported as sister to the two subspecies of *B. sandvicensis* (*B. sandvicensis sandvicensis* and *B. sandvicensis confusa*; 91 BS, 1.00 PP; node 23), which are in turn supported as sister to one another (100 BS, 1.00 PP; node 24). The clade comprising *B. amplexens* (O'ahu), *B. torta* (O'ahu) and *B. campylothea campylothea* (the only taxon found throughout the island chain) is strongly supported as sister to the remaining taxa (84 BS, 1.00 PP; node 5). We note that *B. campylothea pentamera* (Maui) is not recovered as sister to *B. campylothea campylothea* (O'ahu, Lāna'i, Hawai'i Island) but instead is strongly supported as sister to *B. conjuncta* in the Maui Nui clade (100 BS, 1.00 PP; node 16). *Bidens valida* from Kaua'i is strongly supported as sister to the remaining taxa in the clade (84 BS, 1.00 PP; node 6). *Bidens*

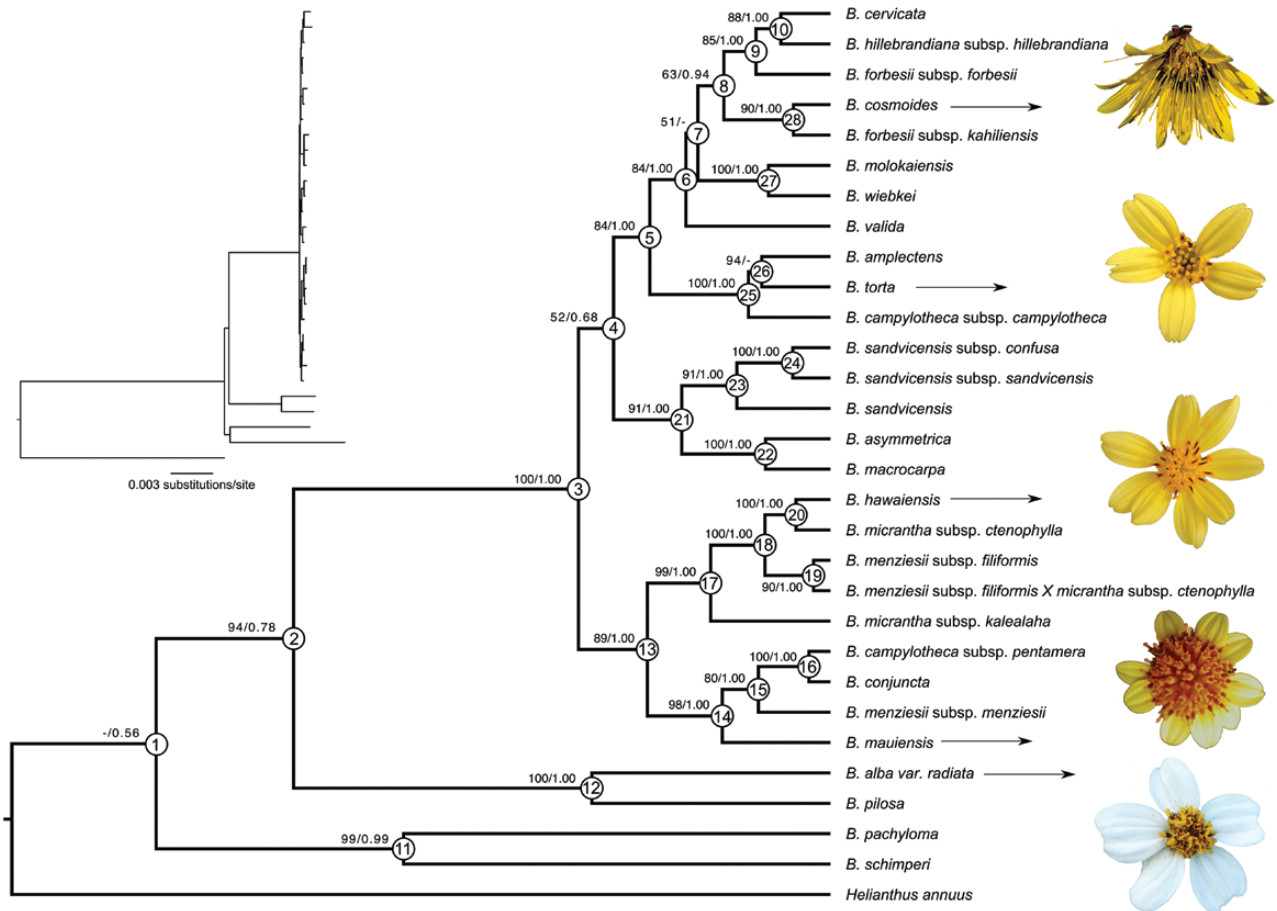


Figure 3. Maximum clade credibility tree of Hawaiian *Bidens* and outgroups obtained from a BEAST analysis of concatenated whole plastome and nuclear ribosomal complex sequences. Support values for each node are indicated as maximum likelihood bootstrap (BS; left) or Bayesian posterior probabilities (PP; right). A dash indicates that the branch was not supported (< 50 % BS or < 0.50 PP). The inset depicts branch lengths for the maximum likelihood tree obtained from RAxML. Photographs from top to bottom display representative variation in floral morphology: the endangered Kaua'i endemic *B. cosmoides* (G. Daida) which grows at high-elevations and is bird-pollinated, the O'ahu endemic *B. torta* (E. Guinther) which has a tree growth habit and can grow to heights of >10 m and is likely insect pollinated, the Hawai'i Island endemic *B. hawaiiensis* (E. Datlof) which is often found on new lava flows and is likely insect pollinated, the Maui Nui endemic *B. mauiensis* (D. Eickhoff) which can have succulent leaves on Maui and Kaho'olawe and is likely insect pollinated, and the Central American outgroup species *B. alba* var. *radiata* (E. Datlof), recently introduced and invasive in Hawai'i (Knope et al. 2013) and is also likely insect pollinated.

molokaiensis, which is endemic to southwest O‘ahu (although this population is believed to be locally extinct) and west Moloka‘i, and *B. weibkei* endemic to northeast Moloka‘i are recovered as sister species with strong support (100 BS, 1.00 PP; node 27), although their relationship as sister to the remaining taxa is not supported (51 BS, < 0.50 PP; node 7). The high-elevation Kaua‘i endemic species *B. cosmoides* and the adjacent ranging *B. forbesii kabiliensis* are also strongly supported as sister taxa (90 BS, 1.00 PP; node 28). Lastly, *B. forbesii forbesii* (Kaua‘i) is recovered as sister to *B. hillebrandiana* subsp. *hillebrandiana* (endemic to Kohala mountain on Hawai‘i Island; 85 BS, 1.00 PP; node 9), which is in turn sister to *B. cervicata* (endemic to northwest O‘ahu, Ni‘ihau, and northwest Kaua‘i) with strong support (88 BS, 1.00 PP; node 10).

Reticulation Network Analysis

The phylogenetic hypotheses derived from the nuclear ribosomal complex were largely incongruent with those based on the plastome (Figure 2) and the PhyloNet analyses suggest potential hybridization events at both shallow and deeper nodes within the Hawaiian radiation (Supplementary Figure S2). The addition of hybridization events sequentially increased the log-likelihood of the reticulation network models until the maximum number of 5 events was run (due to computational time constraints). With one allowed reticulation, the clade consisting of *B. campylotheca* subsp. *pentamera* (Maui Nui), *B. conjuncta* (Maui Nui) and *B. menziesii* subsp. *menziesii* (Maui Nui) was found to be derived from a hybridization event between ancestors of *B. forbesii* subsp. *forbesii* (Kaua‘i) and *B. mauiensis* (Maui Nui). This hybridization event was also recovered in the analysis with 3, 4 and 5 reticulations allowed. *Bidens torta* (O‘ahu) was found to be a hybrid taxon when 2, 4, and 5 reticulations were allowed. In each case, one parent was from an unsampled lineage that is sister to all other taxa and the other was an ancestor of either *B. campylotheca* subsp. *campylotheca* (O‘ahu, Lāna‘i, Hawai‘i Island), *B. amplexens* (O‘ahu), or *B. forbesii* subsp. *kabiliensis* (Kaua‘i). With 4 and 5 reticulations allowed, *B. cosmoides* (Kaua‘i) was inferred to be a hybrid taxon. The parents were either the ancestors of *B. sandwicensis* (Kaua‘i, O‘ahu) and those of *B. amplexens* (O‘ahu), or the ancestors of *B. amplexens* (O‘ahu) and an unsampled taxon sister to *B. molokaiensis* (Maui Nui) and *B. weibkei* (Maui Nui). Also in the analysis with 4 and 5 reticulations allowed, the clade consisting of *B. forbesii*, *B. valida*, *B. cervicata*, *B. weibkei*, *B. molokaiensis*, *B. hillebrandiana* subsp. *polycephala*, *B. forbesii* subsp. *kabiliensis*, *B. amplexens* and *B. campylotheca* had a parent derived from an ancestor of a clade consisting of the remaining Hawaiian taxa except *B. torta* and *B. cosmoides*.

The SplitsTree results show that the concatenated dataset produces a tree-like network structure that is consistent with the individual plastome and nuclear datasets, but with a strong signal of data conflict, particularly involving several O‘ahu and Kaua‘i taxa, including *B. cosmoides*, *B. valida*, and *B. forbesii* (both subsp.) (Figure 4A). The nuclear ribosomal and plastome datasets each have low to moderate levels of internal data incongruence as indicated by the relative tree-like structure of the resulting networks (Figure 4B, C). The nuclear ribosomal network shows the greatest amount of conflict involving these same four taxa.

Divergence Time Estimates

Estimated divergence times for Hawaiian *Bidens* were highly similar across the nuclear, plastome, and concatenated datasets. In general, node ages were slightly older in the analyses based on the plastome

and concatenated dataset than the ages based on the nuclear ribosomal complex. For example, the crown group of Hawaiian *Bidens* was estimated to have emerged 1.82 Mya based on both the plastome and concatenated datasets and 1.76 Mya based on the nuclear dataset (Figure 5, Supplementary Figure S3). Given the similarity in these estimates, we present results from the concatenated dataset for simplicity. Hawaiian *Bidens* is estimated to have split from its closest sampled relatives in Central America ~3.88 Mya (1.43–7.36, 95% HPD; Figure 5). The crown group of Hawaiian *Bidens* began diverging ~1.82 Mya (1.11–2.55, 95% HPD) during the Pleistocene. The 2 major Hawaiian clades began diverging ~1.57 Mya (Kaua‘i/O‘ahu/Maui Nui/Hawai‘i Island) and ~1.32 Mya (Maui Nui/Hawai‘i Island), with most speciation events in both clades occurring within the past one million years (see Supplementary Table S2 for age ranges at all nodes in Figure 5). We also note that we examined different island age calibrations for Hawai‘i Island (0.26, 0.46, and 1.04 Mya) and different distributions (uniform, log normal, normal) and found that these had little effect on age estimates for the crown node (0.02–0.12 My of one another) or internal nodes (0.01–0.15 My of one another; data not presented here).

Ancestral Range Estimation and Patterns of Island Colonization

Of the 12 biogeographical models evaluated for each of the concatenated (Supplementary Table S3), nuclear (Supplementary Table S4), and plastome datasets (Supplementary Table S5), 8 models were plausible (Δ AIC values < 7; Burnham et al. 2011) in the nuclear and concatenated analyses and included all variations of the DEC and DIVA-like models, while 6 models were plausible in the plastome analysis and included the “+j” and “+j+x” variations in each of the DEC, DIVA-like and BayArea-like models. All plausible models for each respective dataset were broadly congruent and varied only slightly in node probabilities. Therefore, for each dataset, we present results from the best model according to the AIC (Δ AIC = 0). For both the plastome and concatenated datasets, DEC+j was the best model. In the analysis based on the plastome dataset, the anagenetic dispersal rate was similar to that estimated in the concatenated analysis, and both analyses recovered an extinction rate of zero. However, in the concatenated analysis the cladogenetic dispersal rate (i.e., founder event rate) was considerably lower than the rate estimated in the plastome analysis. In contrast, DEC was the best model for the nuclear dataset, with higher rates of anagenetic dispersal and extinction relative to that estimated in the plastome and concatenated analyses.

The initial colonization point for *Bidens* in the Hawaiian Islands was largely unresolved, with the highest probability for a combined Kaua‘i/O‘ahu/Maui Nui area in the concatenated and nuclear analyses (Figure 6A, 6B), and an O‘ahu/Maui Nui area in the plastome analysis (Figure 6C). The ancestral range for the earliest diverging clade comprising species primarily from Kaua‘i, O‘ahu, and Hawai‘i Island (but with a few nested members from Maui Nui) was unresolved, but with the highest probability for either a combined Kaua‘i/O‘ahu area (nuclear and concatenated analyses) or O‘ahu (plastome) (Figure 6). In contrast, the ancestral range for the Maui Nui/Hawai‘i Island clade was reconstructed as either Maui Nui (concatenated and plastome) or Maui Nui/Hawai‘i Island (nuclear) (Figure 6).

The BSM analysis for the concatenated dataset also recovered ~30 events, with 24 of these involving cladogenesis (Table 3). Of the 24 cladogenetic events, 65% involved speciation within islands, 15%

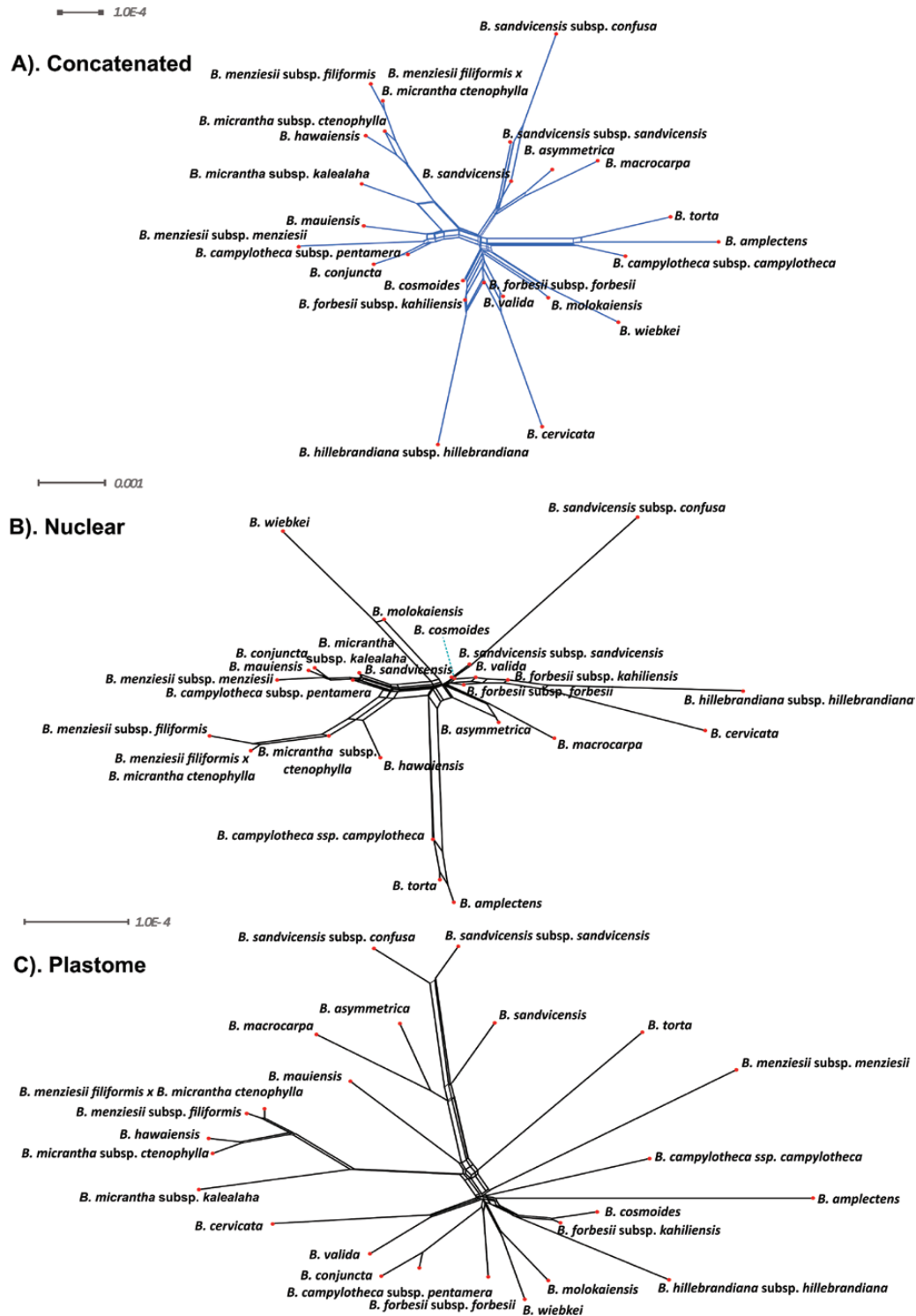


Figure 4. Splits graph networks of the concatenated, nuclear and plastome sequence alignments calculated using the NeighborNet method in Splitstree v. 4.15.1.

involved founder events (i.e., speciation following colonization of a new area), 14% involved subset sympatric speciation (the ancestral range is widespread, and one daughter species inherits the ancestral range and the other inherits a subset of the ancestral range), and 7% involved vicariance (the ancestral range is split, and each daughter species inherits a portion of the ancestral range). The 4 founder events followed progression rule dispersal, with one dispersal from Kaua'i to O'ahu, one from

Kaua'i to Maui Nui, one from Kaua'i to Hawai'i Island, and one from Maui Nui to Hawai'i Island. All 6 anagenetic dispersal events involved range expansion of the 4 multi-island taxa, with 4 being from older to younger islands and 2 from younger to older islands. Kaua'i was the main source of dispersal (6 events), followed by O'ahu and Maui Nui (2 each). O'ahu and Hawai'i Island were the main areas of immigration, receiving 4 and 3 dispersals, respectively (Figure 6A).

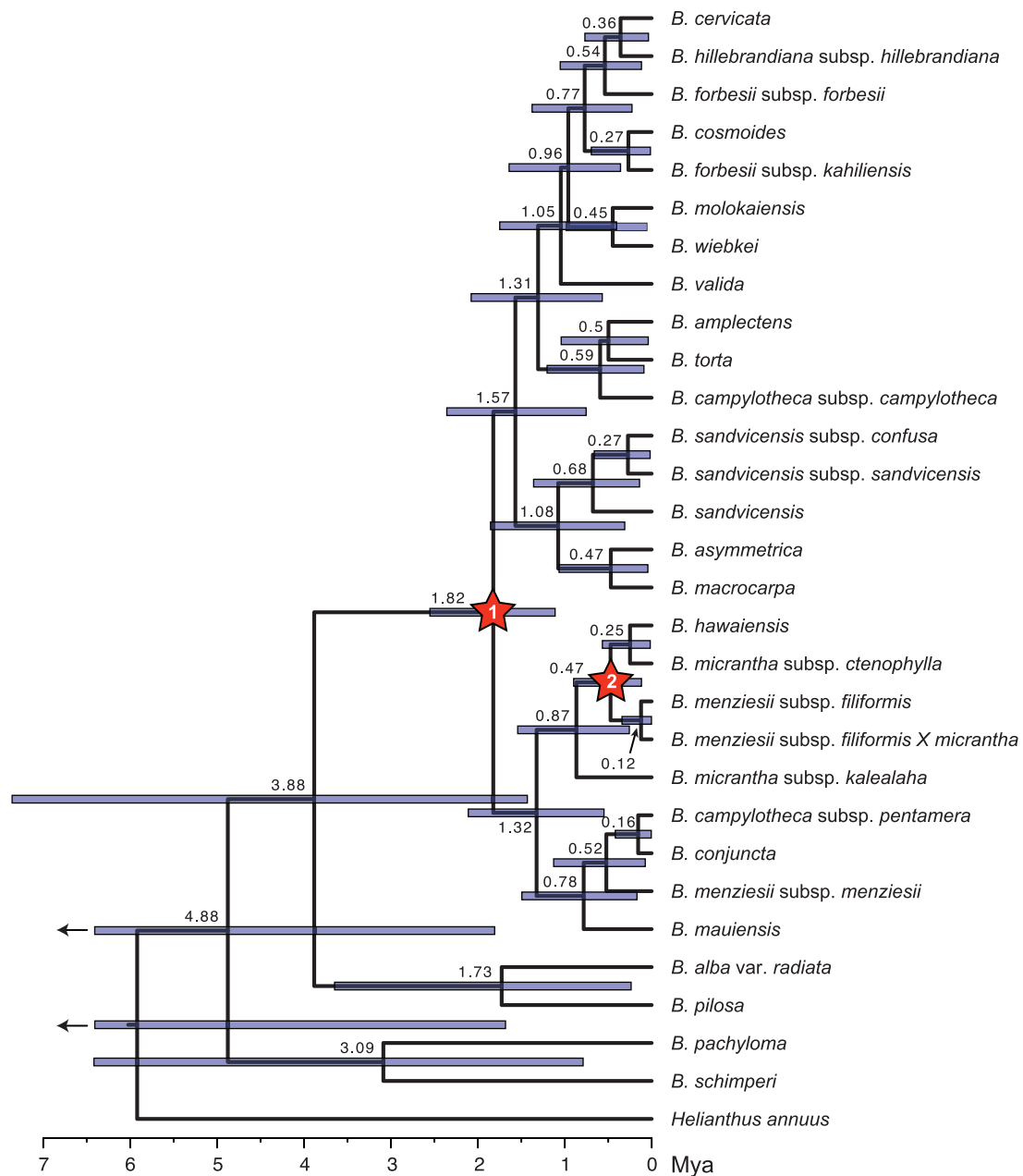


Figure 5. Maximum clade credibility tree based on a BEAST analysis of concatenated whole plastome and nuclear ribosomal complex sequences. Mean divergence estimates are shown at nodes with 95% HPD as blue bars. Stars indicate calibration points: 1) crown age of Hawaiian *Bidens* of 2.1 Mya (Knoppe et al. 2012a) and 2) maximum age for shield emergence of Hawaii Island at 1.04 Mya (Obbard et al. 2012).

The BSM analysis recovered a total of ~33 biogeographic events from the nuclear dataset, with 24 of the 33 events involving cladogenesis (Table 3). Of these 24 events, 66% involved speciation within islands, 22% involved subset sympatric speciation and 12% involved vicariance. Of the 9 range expansion events, 6 followed progression rule dispersal from older to younger islands, while 3 were from younger to older islands. O'ahu was the main source of dispersal (4 events), followed by Kaua'i (3) and Maui Nui (2), while O'ahu and Hawaii Island were the main areas of immigration, receiving 3 dispersals each (Figure 6B).

For the plastome dataset, 24 of the ~30 events recovered in the BSM analysis involved cladogenesis (Table 3). Of these 24 events, 45% involved speciation within islands, 37% involved founder

events, 12% involved subset sympatric speciation, and 6% involved vicariance. Of the 9 founder events, only 5 followed progression rule dispersal, with the remaining 4 being from younger to older islands. The 6 anagenetic dispersal events followed a similar trend with only half of the dispersals following the progression rule. Maui Nui was the main source of dispersal (5 events), followed by Kaua'i and O'ahu (4 each). Kaua'i and O'ahu were the main areas of immigration, receiving 4 and 5 dispersals, respectively (Figure 6C).

Discussion

The present study provides the first generally well-resolved phylogenetic hypothesis for the Hawaiian *Bidens* adaptive radiation. We

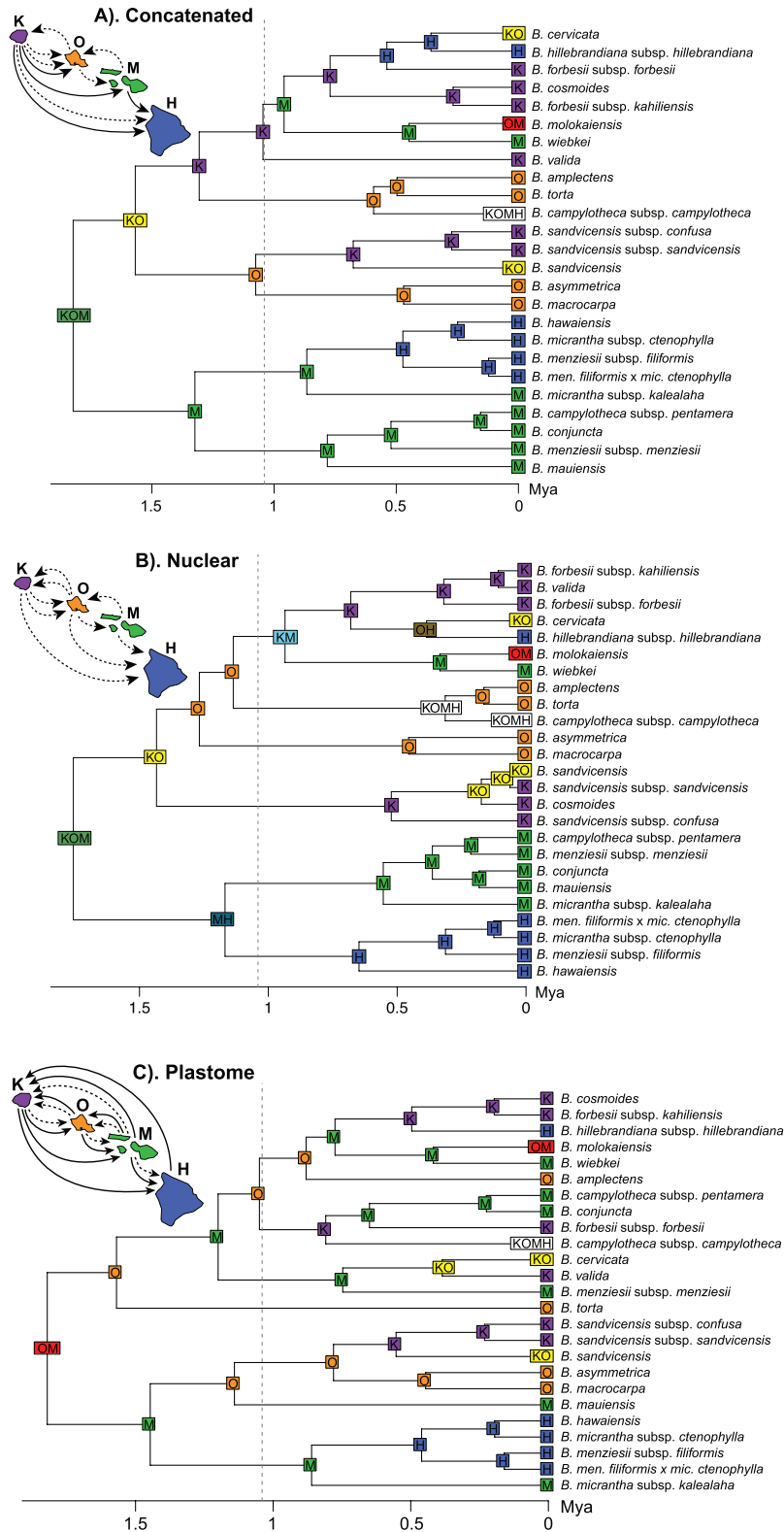


Figure 6. Ancestral range estimation for Hawaiian *Bidens* based on the best biogeographical model ($\Delta AIC = 0$) as determined in BioGeoBEARS using A) the concatenated dataset (DEC+), B) the nuclear ribosomal complex (DEC), and C) the plastome (DEC+). Areas are color-coded for the main Hawaiian Islands, as well as combinations of these islands. The vertical dashed line indicates the areas allowed constraint for Hawai'i Island, with taxa only being permitted to colonize the island after its emergence (1.04 Mya). Solid arrows on the inset map depict the hypothesized direction of founder events between islands and the dashed lines depict the hypothesized direction of range expansion events, as determined in the biogeographic stochastic mapping analysis.

Table 3. Event counts (mean and standard deviations) for Hawaiian *Bidens* from 500 biogeographic stochastic mappings in BioGeoBEARS

Biogeographical event	Nuclear	Plastome	Concatenated
Range switching	0	0	0
Range expansion	9.31 ± 1.16	6.56 ± 0.90	6.33 ± 1.04
Narrow sympatry	15.71 ± 1.72	10.94 ± 1.37	15.60 ± 1.25
Subset sympatry	5.43 ± 1.97	2.89 ± 1.90	3.27 ± 1.68
Vicariance	2.86 ± 0.94	1.36 ± 1.02	1.57 ± 1.00
Founder events	0	8.79 ± 1.6	3.57 ± 1.13
Total events	33.31 ± 1.16	30.56 ± 0.90	30.31 ± 1.04

Mapping was performed using parameters from the best model of biogeography according to the Δ AIC. Results are presented for the nuclear ribosomal complex (DEC model), the plastome (DEC+j model) and the concatenated dataset (DEC+j model).

recover support for the monophyly of the radiation but emphasize the need for greater taxonomic sampling outside of Hawai'i and additional genome-wide analyses. Dating analyses based on the nuclear, plastome, and concatenated datasets resulted in nearly identical estimates for the timing of diversification within the clade, and biogeographic analyses based on both the nuclear and concatenated datasets indicate that colonization patterns within Hawai'i generally followed the progression rule, with 67–80% of events from older to younger islands (whereas 53% of colonization events followed the progression rule in the plastome analysis). Our analyses also revealed a history of hybridization events and/or incomplete lineage sorting leading to significant nuclear-plastome conflict. In addition, the role of hybridization without subsequent concerted evolution in the nuclear ribosomal DNA could be contributing to the observed nuclear-plastome phylogenetic conflict (e.g., Doyle 1992). We primarily focus our discussion on the results obtained from the concatenated dataset, given that node support, the number of phylogenetically informative sites, and branch lengths are all greater than in either of the 2 independent datasets alone (but see Figures 2, 3, 6 and Supplementary Figure S1 for a detailed presentation of the similarities and differences obtained from each).

Phylogenetic Relationships

While our results appear to suggest that the ancestral clade that gave rise to the original colonist of the Hawaiian Islands was likely from Central America (Figure 3), more detailed outgroup sampling is necessary to confidently determine the ancestral range of the most recent common ancestor (MRCA) of the Hawaiian radiation. Monophyly of the Hawaiian species appears to be strongly supported (100 BS, 1.00 PP in the nuclear, plastome, and concatenated trees; Figures 2, 3 and Supplementary Figure S1), but our study does not include any taxa from the South Pacific Islands (~25 native *Bidens* species currently described). In the phylogenetic analysis by Knope et al. (2012a), 2 undescribed *Bidens* species from the Marquesas were nested within the Hawaiian radiation based on nrITS, although that node lacked support. A more comprehensive phylogeny that is inclusive of *Bidens* taxa from across the Pacific Basin, as well as potential source regions, will be necessary to better understand relationships and colonization patterns in this genus (Knope et al., in preparation). Regardless of the exact location of the MRCA outside of Hawaii, long-distance dispersal to the Hawaiian Islands may have been facilitated by sea birds, with the dry fruits (achenes) generally having barbed awns that are thought to easily attach to feathers (ectozoochory; Carlquist 1974).

Our phylogenetic analysis of the Hawaiian *Bidens* radiation recovered only one of the 5 species comprised of 2 or more subspecies included in the study as monophyletic in the concatenated data (Figure 3).

Bidens sandvicensis subsp. *sandvicensis* and *B. sandvicensis* subsp. *confusa*, as well as a *B. sandvicensis* individual not identified to the subspecies level, are recovered as a monophyletic taxon with strong support (91 BS, 1.00 PP; node 23). *Bidens campylotheca* subsp. *pentamera* from West Maui was not recovered as sister to the more widespread *B. campylotheca* subsp. *campylotheca*, but instead is strongly supported as sister to *B. conjuncta* also found on West Maui (100 BS, 1.00 PP; node 16). Similarly, *B. forbesii* subsp. *forbesii* (endemic to lowland areas on Kaua'i) was not recovered as sister to *B. forbesii* subsp. *kahiliensis*, but rather was recovered as sister to *B. hillebrandiana* subsp. *hillebrandiana* (endemic to Kohala Mountain on Hawai'i Island) and *B. cervicata* (endemic to northwest O'ahu, Ni'ihau, and northwest Kaua'i near populations of *B. forbesii* subsp. *forbesii*) with strong support (85 BS, 1.00 PP; node 9). *Bidens forbesii* subsp. *kahiliensis* is known only from Kahili Mountain on Kaua'i and is recovered as sister (90 BS, 1.00 PP; node 28) to the more widespread high-elevation Kaua'i endemic *B. cosmoides* (the only known bird-pollinated species, having radically different floral architecture from other Hawaiian *Bidens*; Figure 3). *Bidens micrantha* subsp. *kalealaha* is supported as sister to the clade of Hawai'i Island endemics (99 BS, 1.00 PP; node 17), within which *B. micrantha* subsp. *ctenophylla* is supported as sister to *B. hawaiiensis* (100 BS, 1.00 PP; node 20). Lastly, *B. menziesii* subsp. *menziesii* is nested within a clade comprised of other Maui Nui endemics with strong support (80 BS, 1.00 PP; node 15), and *B. menziesii* subsp. *filiformis* is supported in a clade with other Hawai'i Island endemics (90 BS, 1.00 PP; node 19).

Several non-mutually exclusive possibilities exist for why we recovered some species as polyphyletic, including hybridization, introgression, and/or incomplete lineage sorting. For example, while ~70% of Hawaiian *Bidens* taxa are single island endemics (Figure 1; Ganders and Nagata 1984; Wagner et al. 1999), when species do co-occur on islands, hybridization and hybrid swarms are not uncommon as intrinsic reproductive barriers amongst the Hawaiian taxa do not exist (Gillett and Lim 1970; Ganders and Nagata 1984; Knope et al., 2013), which may have led to genetic introgression in one or both parent species. For example, *B. forbesii* subsp. *forbesii* and *B. cervicata* are thought to hybridize and possibly inter-grade on the Nāpali coast of Kaua'i (Wagner et al. 1999), which may account for the close affinity recovered here among these taxa. Further, *B. torta* is known to hybridize naturally with *B. amplexans* where their ranges overlap (Ganders and Nagata 1984), and they are recovered as sister taxa in the concatenated phylogeny. In addition, the recent and rapid nature of the radiation may have resulted in some taxa being in an ongoing state of separating from one another, resulting in incomplete lineage sorting (Maddison and Knowles 2006), and more genome-wide, unlinked markers may generate a clearer picture and greater confidence in the observed patterns.

Comparison to Previous Taxonomic and Evolutionary Assessments

Sherff (1937) recognized 43 species and more than 20 varieties of Hawaiian *Bidens*, but had little understanding of the level of intra-specific variation in the clade. Much of his species level diagnostics were based on leaf shape characters that are now known to be highly variable (both within and among populations) and thus unreliable for the purposes of taxonomic delineation (Ganders and Nagata 1984). At the other extreme, Gillett (1975) proposed that all Hawaiian *Bidens* be considered just 2 species, with perhaps 7 subspecies, based on their genetic compatibility and natural hybridization. However, Ganders and Nagata (1984) asserted that if inter-fertility is to be used as a criterion for species delimitation in Hawaiian *Bidens*, then all Hawaiian *Bidens* would be a single species with greater morphological and ecological variability than the remaining ~230 species in the genus distributed across 5 continents. Helenurm and Ganders (1985) found evidence in a survey of isozyme diversity that Hawaiian *Bidens* species are as similar genetically as are populations of a single species in most plants, but all species grown in common garden experiments have the growth phenotypes of the species found in the wild (Gillett and Lim 1970; Knope et al. 2013), indicating morphological differences are not the result of phenotypic plasticity. The delineation of the 19 currently recognized species in Hawaiian *Bidens* is based on morphology, ecology, and biogeography (Ganders and Nagata 1984), despite the general lack of genetic differentiation (Helenurm and Ganders 1985; Ganders et al. 2000; Knope et al. 2012a; Stallman et al. 2019). Additionally, the combination of polyacetylenes in the roots and leaves of both naturally occurring and greenhouse populations distinguishes almost all of the currently recognized species and subspecies from one another and supports the currently recognized species based on morphology and ecogeography (Marchant et al. 1984). Despite the previous difficulties in understanding the relationships of Hawaiian *Bidens*, several affinities have been postulated by previous workers. For example, based on geographic relationships and morphological similarities, Ganders and Nagata (1984) suggested that the following species are sister taxa: *B. micrantha* and *B. conjuncta*; *B. cervicata* and *B. forbesii*; and *B. molokaiensis* and *B. mauiensis*. In contrast to these hypothesized sister relationships, we find that these species are generally resolved in distant clades (Figures 2 and 3; Supplementary Figure S1). Ganders and Nagata (1983; also see Wagner et al. 1999) provided a key for identification of species but emphasized the difficulty in creating it. The non-concordance between the phylogeny in the present study and views expressed in previous taxonomic works likely reflect the explosive nature of the morphological and ecogeographic divergence in this rapid adaptive radiation.

Initial Colonization, Early Divergence Time Estimates, and Geologic History

In the present study, we estimate the divergence time for the crown group of the Hawaiian *Bidens* radiation at ~1.76–1.82 Mya (1.11–2.55, 95% HPD). The age estimate we derive here places *Bidens* among the youngest of the Hawaiian plant radiations investigated to date. However, we note that the initial colonization of the Hawaiian archipelago may have taken place prior to our estimate of 1.76–1.82 Mya, and could conceivably date as far back as the split with the ancestor that led to *B. alba* and *B. pilosa* (1.43–7.36 Mya) (Figure 5). Our estimate for the crown group age is similar to, although slightly younger than the earlier estimate of ~2.1 Mya (1.3–3.1, 95% HPD) from Knope et al. (2012a). The discrepancy between the 2

estimates may stem from the difference in the genetic/genomic data used for the dating analysis (nrITS marker only in the previous study vs. whole plastome plus nuclear ribosomal DNA complex in the current study), differences in taxon sampling, and/or the use of different calibration points in the dating analysis. Knope et al. (2012a) based their age estimates on mean rates of nrITS nucleotide substitution for herbaceous angiosperms (Kay et al. 2006) and used the estimated age of Kure Atoll (~29 Mya, the oldest of the Northwestern Hawaiian Islands) as a single calibration point to constrain the maximum age for the Hawaiian *Bidens* crown. In the present study, we combined a secondary calibration point (the mean age of the crown group estimated at 2.1 Mya from Knope et al. (2012a)) with a geological age calibration (the maximum age of shield emergence for Hawai'i Island at 1.04 Mya (Obbard et al. 2012)).

The ancestral area analysis indicates that the crown ancestor of Hawaiian *Bidens* occupied Kaua'i, O'ahu, Maui Nui or some combination of these areas (Figure 6). Our estimate of 1.76–1.82 Mya for the mean age of the crown ancestor suggests that the initial split may have occurred after the shield emergence of West Moloka'i (2.58 Mya) and West Maui (2.15 Mya), but before shield emergence of Hawai'i Island (1.04 Mya; Obbard et al. 2012). During this time, O'ahu may have been connected to West Moloka'i by Penguin Bank, a now-submerged volcano to the southeast of O'ahu (Carson and Clague 1995; Price and Elliot-Fisk 2004). The O'ahu-Maui Nui connections may have lasted from ~2.0 to 1.7 Mya (Price and Elliot-Fisk 2004), which coincides with our estimate of the age of the crown divergence of Hawaiian *Bidens*. Price and Elliot-Fisk (2004) hypothesized that the direct connections between O'ahu and Maui Nui early in the islands' history could have facilitated dispersal from the older to younger volcanoes, and that the subsequent periodic direct connections between the islands comprising Maui Nui likely not only facilitated dispersal, but also vicariant speciation during times of separation via ocean barriers. It is also possible that the newer volcanoes of the Maui Nui complex coalesced with the older ones, creating a single large landmass above sea level (Price and Elliot-Fisk 2004). The area of this landmass was likely larger than the current size of Hawai'i Island before ultimately subsiding and submerging the saddles between its volcanoes, thus the speciation rate-area effect (Losos and Schluter 2000; Gillespie and Baldwin 2010; Knope et al. 2012a) may have been an important determinant of diversification for Hawaiian *Bidens* during this time interval. In addition, Asquith (1995) suggested that concurrent with these events, changes in eustatic sea level periodically reconnected the fragments of Maui Nui above sea level. While a detailed understanding of the exact size, timing, and configuration of the island chain during the initial divergence of the *Bidens* crown group is not possible at this time, the geologic evidence suggests that the buildup and separation of O'ahu Nui (O'ahu and West Moloka'i) from Maui Nui, and of Maui Nui itself, may have played an instrumental role in facilitating early cladogenesis in the nascent adaptive radiation.

Historical Biogeography and the Progression Rule

Given that a well-supported phylogenetic hypothesis for Hawaiian *Bidens* has not been proposed to date, there have been no prior attempts to reconstruct the historical biogeography of this iconic adaptive radiation. We combined the phylogenetic hypotheses obtained from the nuclear ribosomal, plastome, and concatenated datasets with present-day range information (Wagner et al. 2005), and employed BioGeoBEARS to discriminate among 12 alternative biogeographic models via LRT and AIC scores, with the assumption that

taxa could not disperse to Hawai'i Island before shield emergence. For simplicity, we focus our discussion on the single model with the lowest Δ AIC score, although 6–8 of the 12 models investigated for each dataset were deemed plausible.

The parameter estimates for the best models (Supplementary Tables S3–S5) indicate that extinction has not yet been an important factor in determining the biogeographic relationships of extant Hawaiian *Bidens* taxa. This suggests that the radiation is likely still in an expansion phase and has not yet reached any ecological limits to clade diversity (e.g., Gillespie 2004; Rabosky 2009), or begun long-term evolutionary decline as appears to be the case for many of the older radiations in Hawai'i (Lim and Marshall 2017). Knope et al. (2012a) proposed that evolutionarily young radiations, such as Hawaiian *Bidens*, have likely not yet reached any ecological limits on clade diversification, and would thus be the least impacted by extinction and possible island “saturation” dynamics in estimates of diversification rates (Rabosky 2009). These young radiations, therefore, provide a unique opportunity to gain insight into the early stages of adaptive radiation and, in particular, can lead to a broader understanding of patterns of colonization and factors affecting rates of diversification on islands.

For the plastome and concatenated analyses, we also found that incorporating jump dispersal (*j*) significantly improved model fit, emphasizing the role of founder events in island cladogenesis. The DEC+*j* model has recently been criticized as being a poor model for founder-event speciation by parameterizing the mode, but not the rate of speciation, and in some cases may have a tendency to inflate the contribution of cladogenetic events while underestimating anagenetic events (Ree and Sanmartín 2018). However, in the present study, we find reasonable rate estimates for both founder events and anagenetic dispersal events and do not see any obvious departures from intuitive patterns of dispersal, given present-day ranges of extant taxa.

In the analysis based on the concatenated data, we recovered 6 anagenetic dispersal events leading to range expansion of the 4 multi-island taxa with 4 of the 6 following progression rule dispersal (Table 3; Figure 6A). Progression rule dispersal was most clearly evident in the founder event from Maui Nui that gave rise to the monophyletic clade on neighboring Hawai'i Island (Figure 6A). This dispersal event from Maui Nui appears to have occurred very close to the age of the oldest surface rocks on Hawai'i Island at 0.46 mya (Obbard et al. 2012). We also find evidence for progression rule dispersal in the founder events from Kaua'i to O'ahu, Kaua'i to Maui Nui, and Kaua'i to Hawai'i Island (Figure 6A), suggesting an important role for Kaua'i as a source area for cladogenetic dispersal throughout the island chain. While this result may provide some indication that Kaua'i could have been the initial colonization point for *Bidens* in Hawaii, the incongruencies among datasets, the similar numbers of species among islands, and the recent age estimate for crown-group divergence make it difficult to rule out the possibility of O'ahu or Maui Nui as ancestral areas for the group. Additional sampling of multi-island taxa, the inclusion of *B. populifolia* from O'ahu (the only Hawaiian species not included in the present study), and possibly additional data from the nuclear genome will be needed to resolve the ancestral area for *Bidens* in Hawaii.

Given that the initial divergence of the Hawaiian *Bidens* crown group appears to coincide with the formation of Maui Nui (the second youngest of the current high islands), this presents an interesting twist on the progression rule hypothesis. While the majority of dispersal events recovered in the present study followed

the progression rule, we also recovered 2–7 dispersal events in each of the 3 BSM analyses that occurred from younger to older islands. Successful establishment of taxa on older islands may be prevented as a consequence of evolutionary niche preemption (i.e., an evolutionary priority effect), whereby the first taxa to colonize an island diversify and occupy the full range of niche space, inhibiting closely related taxa that are ecologically similar from successfully establishing at later times (e.g., Silvertown 2004; Fukami et al. 2007; Knope et al. 2012b). Although infrequent, the results presented here suggest that *Bidens* has the ability to thrive in previously established communities. The somewhat stochastic dispersal pattern seen in the present study may reflect the availability of multiple islands as targets for colonization during the initial divergence of the group (as observed in Hawaiian *Tetramolopium*; Lowrey 1995). We note that other Hawaiian genera that colonized the archipelago within the last 2 My tend to exhibit a stochastic pattern of inter-island dispersal, rarely following the progression rule (Pax et al. 1997; Magnacca and Danforth 2006; Magnacca and Price 2015).

Dispersal probability appears to be inversely related to the distance among islands, with neighboring islands being likely source areas for dispersal events (both in terms of cladogenetic founder events and anagenetic range expansions), as evidenced by the negative parameter estimate for the role of dispersal distance. Our BSM results suggest that the source area for 8 out of ten dispersal events were neighboring islands, with the 2 exceptions being the founder events from Kaua'i to Maui Nui, and Kaua'i to Hawai'i Island (Figure 6). However, it is important to note that we did not sample across the full range of the 4 *Bidens* taxa that occur on multiple islands (only accessions from a single island were included for each taxon). For example, we sampled *B. hillebrandiana* subsp. *hillebrandiana* (endemic to Kohala Mountain, which is the northwestern-most peak on Hawai'i Island and closest to Maui Nui), but did not sample *B. hillebrandiana* subsp. *polycephala* from Maui Nui. It is possible based on the importance of dispersal distance that future inclusion of *B. hillebrandiana* subsp. *polycephala* may reveal that the dispersal event that gave rise to *B. hillebrandiana* subsp. *hillebrandiana* on Hawai'i Island was actually from Maui Nui and not directly from Kaua'i. In addition, increased sampling for these taxa could provide important insight into the possible role of population differentiation and/or cryptic speciation, hybridization with multiple taxa, and/or incomplete lineage sorting on the limits of species and their potential impact on inference of historical biogeography.

Lastly, the BSM results from the concatenated dataset indicate that the Hawaiian *Bidens* taxa sampled in the present study are the result of 24 cladogenetic events, with 65% of these events involving narrow sympatry, 15% involving founder events, 14% involving subset sympatry, and 7% involving vicariance (Table 3). Although narrow sympatry refers to within-area speciation, we note that because we defined areas as individual islands (or in the case of Maui Nui, several islands that were once connected), it is likely that some *Bidens* taxa may, in fact, have diverged in allopatry, especially when considering the heterogeneous topography of the main Hawaiian Islands. Finer-scale biogeographical analyses are needed to further elucidate the geographic conditions under which speciation events took place within islands. A more detailed population-level sampling within islands would permit further inference not only into the geography of speciation, but also the drivers involved in species divergence including the role of hybridization and introgressive gene flow (e.g., Stacy and Sakishima 2019).

Future Directions

Phylogenomic approaches are increasingly being used to unravel the evolutionary histories of recent plant radiations (e.g., Mort et al. 2015; Welch et al. 2016). Our use of the complete plastome and the nuclear ribosomal complex allowed further insight into phylogenetic relationships and the biogeographical history of the recent and rapid Hawaiian *Bidens* radiation. However, our data consisted of essentially 2 independent loci, which prevented us from using methods that would allow the signature of incomplete lineage sorting vs. hybridization to be distinguished (Yu et al. 2011). These methods require data from 100's or 1000's of unlinked loci across the nuclear genome (e.g., Meier et al. 2017). Those types of data are tractable for taxa with diploid genomes, but producing such data for Hawaiian *Bidens* spp., which are hexaploid species whose genome sizes are unknown, but may exceed 10 Gb (based on *Helianthus* ploidy patterns [Kallamadi and Mulpuri 2016]), presents a challenge at the limits of current sequencing technology. Further, the lack of a reference genome from a closely related taxon and short-read data for SNP-typing thus constrains identifying a suitable set of loci for phylogenomic purposes at this point in time. The use of a distantly related genome reference for genotyping biases results because only regions conserved in both the focal taxa and the reference will be considered in SNP calls. Although *Helianthus annuus* and *Bidens* are members of the same supertribe in the Asteroideae (Mort et al. 2008), their nuclear genomes are moderately divergent, as evidenced by the overall low mapping rates of *Bidens* sequences (~26%) to the *Helianthus annuus* genome (data not shown). We did identify an additional >9,000 genome-wide SNP loci with the inclusion of outgroup taxa, of which 487 SNPs were un-linked (data not shown), but these data are likely of limited phylogenetic utility because they represent highly conserved regions only. Generating a Hawaiian *Bidens* reference genome (in progress) will support producing a genome-wide analysis of nucleotide variability within *Bidens*, which we anticipate will enable further disentanglement of ancient (or ongoing) interspecific gene flow, incomplete lineage sorting, and the possible role of hybridization in speciation and adaptive radiation (e.g., Marques et al. 2019).

Conclusions

The present study has contributed the first generally well-resolved phylogenetic hypothesis for the evolution of the Hawaiian *Bidens* adaptive radiation, refined estimates of initial colonization and divergence times, and provided the first estimates for the historical biogeography of the group. We found that the Hawaiian *Bidens* radiation likely occurred within the last 2 million years. The crown age of the radiation is estimated to be approximately coincident with the possible separation of O'ahu Nui and repeated build-up and separation of the islands of Maui Nui. Further investigation into the complex geologic history of the main Hawaiian Islands during this time period and its role in the speciation and adaptive radiation of Hawaiian taxa is warranted. We also found that dispersal patterns among islands largely support the progression rule hypothesis, with the direction of dispersal events being primarily from older to younger islands. Departures from this hypothesis in the form of dispersal events from younger to older islands may reflect the availability of multiple islands for colonization during the group's initial divergence. Lastly, the majority of cladogenetic events appear to have occurred within single islands after colonization, with both allopatric and sympatric phases being plausible. While these results suggest that a phylogenomic approach can help to resolve

the evolutionary history of recent adaptive radiations, many questions remain, and further work is needed to better understand the roles of hybridization, introgressive gene flow, and incomplete lineage sorting in this radiation. While evolutionarily young lineages present challenges for phylogenetic reconstruction, they also stand to provide unprecedented insight into the origins of adaptive radiation, as the obscuring effects of time have likely not yet compromised the ability to unravel both the patterns and drivers of species diversification.

Supplementary Material

Supplementary materials are available at *Journal of Heredity* online.

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Data Availability

We have made the primary data underlying these analyses available as follows:

- DNA sequences: Genbank accession numbers: ribosomal MN535766-MN535794; plastomes MN433088-MN433116;
- Next-generation sequencing data BioProject PRJNA555637; SRA SRR9727165-SRR9727193.

Final DNA sequence assembly available upon request from the corresponding author.

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