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Temperate Eurasian Origins of Hawaiian *Chenopodium* (Amaranthaceae), Plus Description of a New Subspecies Endemic to Molokaʻi

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Abstract—Hawaiian taxa of *Chenopodium* are tetraploids and are distinguished from other members of the circumglobally distributed genus by minute morphological characters. Because of these reasons, the geographic origin of Hawaiian *Chenopodium* has remained unclear. Across the Hawaiian Archipelago, taxa of *Chenopodium* are morphologically variable and grow in highly disparate xeric habitats, especially in terms of precipitation, temperature, wind, salt spray, and solar irradiation. Habitats include dry subalpine shrublands, sandy beach strands on atolls in the Northwest Hawaiian Islands, dry to mesic forests, and precipitously tall sea cliffs of northwestern Molokaʻi. From the Molokaʻi sea cliffs, which are battered by high energy winds, salt spray, and strong seasonal precipitation, we describe *C. oahuense* subspecies *ilioensis* as segregated from the widespread Hawaiian *C. oahuense* sensu lato. Morphometric analyses distinguish *C. oahuense* subsp. *ilioensis* by its strongly prostrate to scandent habit, thick succulent leaves, smaller average leaf size, limited lobing of the laminar margins, and smaller seeds. Phylogenetic analyses using two DNA regions (the plastid gene *rpl32-trnL* and nuclear ITS) of newly sequenced individuals of *C. oahuense* s. l. and *C. oahuense* subsp. *ilioensis* plus outgroup taxa support the monophyly of Hawaiian *Chenopodium* and reveal a geographic origin of temperate Eurasia. Two equivocal hypothetical scenarios are discussed regarding the likely sequence of events leading to the arrival of *Chenopodium* in Hawaiian Islands followed by possible in situ speciation of the Molokaʻi endemic *C. oahuense* subsp. *ilioensis*.

Keywords—Chenopodioideae, *Chenopodium oahuense*, *Chenopodium oahuense* subsp. *ilioensis*, genome downsizing, halophyte, speciation.

The flora of the Hawaiian Islands is notable for its significant endemism (> 90% of species) as well as an uneven taxonomic assemblage of higher order plant taxa due to an unbalanced representation of colonizing lineages from different continental source areas. (Carlquist 1966; Whittaker and Fernández-Palacios 2007; Keeley and Funk 2011). Geographic source areas of plant lineages arriving to the Hawaiian Islands has long been a topic of research, stretching as far back as the late 19th Century. Hillebrand (1888) postulated numerous long-distance dispersals connecting Hawaiian taxa to distant continental sources. The most recent review of this topic clarifies that while geographic origins of most Hawaiian taxa are well understood, 18.5% of colonizing lineages are still undetermined due to complicating factors like hybridization or an insufficient understanding of phylogenetic relationships (Price and Wagner 2018).

Among the Hawaiian lineages of undetermined origins is *Chenopodium* L., which has been considered in Hawaiʻi to be monotypic, with *C. oahuense* (Meyen) Aellen as the sole taxon in the archipelago. Previous work employing Sanger sequence based phylogenetic analyses of nuclear ITS, the third intron of two *FLOWERING LOCUS T LIKE* (*FTL*) paralogs *F1L1* and *F1L2*, and three intergenic plastid (cpDNA) spacer regions (*rpl32-trnL*, *ndhF-rpl32*, *psbJ-petA*), has not resolved the relationships of *C. oahuense* from non-Hawaiian diploid and polyploid taxa (Kadereit et al. 2010; Mandák et al. 2018). Despite being unable to disentangle the relationships among these taxa, these first phylogenetic analyses were able to determine that *C. oahuense* is closely related to several diploid and polyploid taxa of *Chenopodium* occurring circumglobally, particularly *C. acuminatum* Willd., *C. suecicum* Murr., *C. ficifolium* Sm., and *C. acerifolium* Andr., among others. Perhaps further obscuring these relationships was the fact that this group of taxa includes the hexaploid and worldwide weedy species, *C. album* L. (Krak et al. 2016).

The origins of *C. album* itself have eluded understanding in part due to its global distribution as a weed linked to historical

human agricultural activities and its invasive nature. Recently, Mandák et al. (2018) were able to better determine relationships within this closely related species group through a combination of cytological and phylogenetic analyses. Their work suggests that Hawaiian *C. oahuense* is an allopolyploid taxon originating from two diploid parental taxa: 1) temperate central and east Asian *C. acuminatum* and 2) an ancestor of Eurasian boreal *C. suecicum* plus Eurasian temperate *C. ficifolium*. Additionally, the tetraploid *C. acerifolium* from eastern Europe and boreal Siberia was determined to have the same parental diploid species as *C. oahuense*. Although not explicitly stated by Mandák et al. (2018), *C. acerifolium* could be the result of a separate allopolyploid event leading to its formation, or it could be the progenitor taxon that dispersed to the Hawaiian Islands and then, in its insular isolation, diverged into the endemic Hawaiian lineage. Regardless of which scenario is correct, there is evidence of genome size reduction supporting a divergence of both *C. oahuense* and *C. acerifolium* from their diploid parents. The authors (Mandák et al. 2018) also showed that *C. oahuense* maintains a 6.3% reduction and *C. acerifolium* a 6.0% reduction in size when compared to the combined genome sizes of their diploid parents *C. acuminatum* and ancestral descendant *C. suecicum* (note: the genome size of the parental descendant *C. ficifolium* was not determined). Finally, Mandák et al. (2018) estimated a molecular clock using cpDNA, which indicated the *C. oahuense* haplotype diverged approximately 2.5 MYA, at a time when Oʻahu was the youngest island, joining the many atolls and islands that preceded its emergence to the northwest (Clague et al. 2010).

Chenopodium oahuense is known from all the main Hawaiian Islands and many of the Northwest Hawaiian Islands including Lisianski, Laysan, French Frigate Shoals, Necker, and Nihoa (Fig. 1). The initial descriptions rendered the taxon as heterotypic for 93 yr, as it was first described as *Atriplex oahuensis* Meyen (Meyen 1834) and shortly thereafter separately described as *Chenopodium sandwicheum* Moq. (Moquin

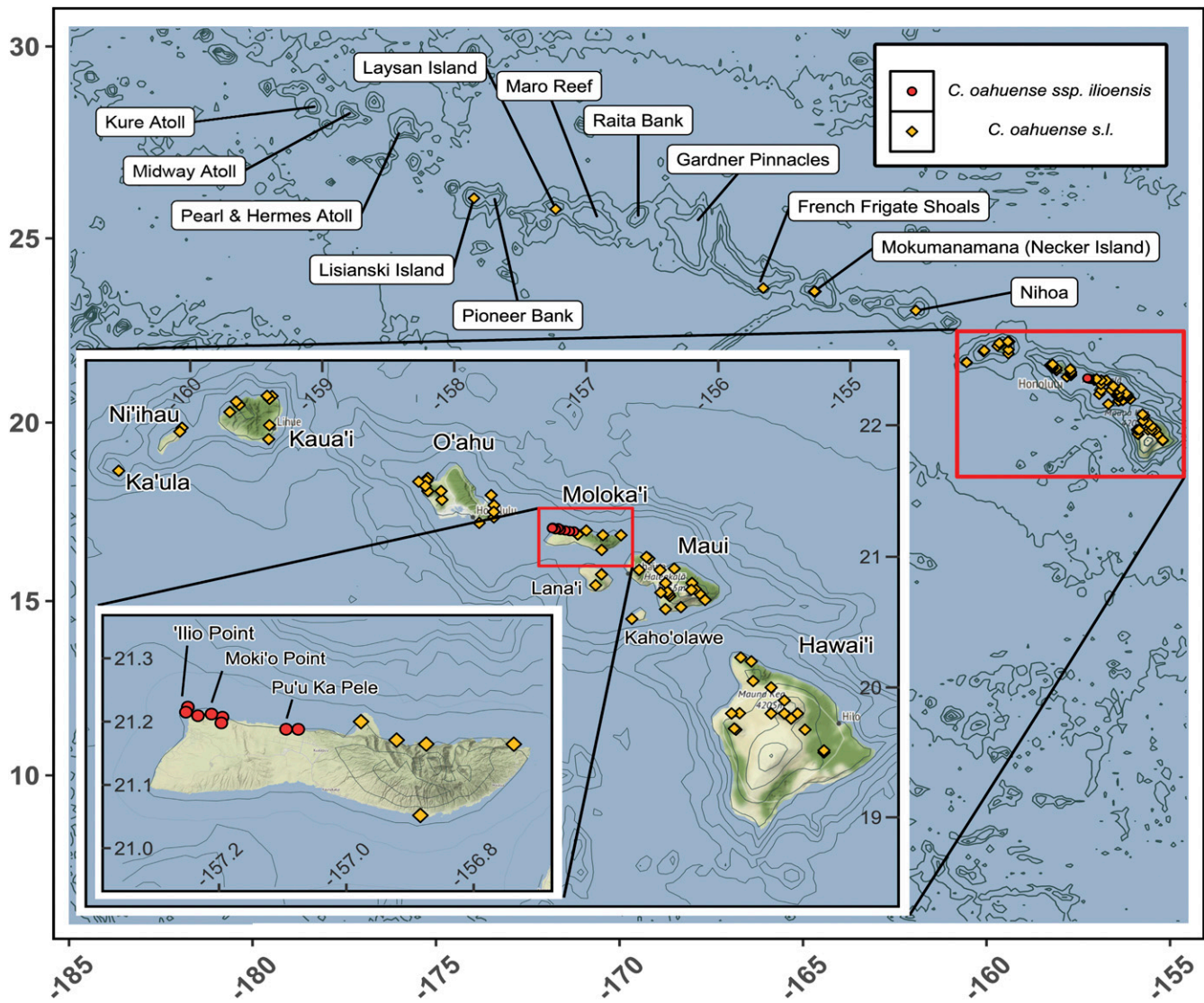


FIG. 1. Map indicating the distribution of *C. oahuense* s. l. and *C. oahuense* subsp. *ilioensis* across the Hawaiian Archipelago.

Tandon 1840). It was not until Aellen (1933) rectified this issue by formalizing the taxon *C. oahuense* and recognizing two lower forms: *C. oahuense* f. *macrospermum* Aellen and *C. oahuense* f. *microspermum* Aellen. Fosberg (1962) then described an additional erect taxon, *C. oahuense* var. *discospermum* Fosberg, from Haleakalā, Maui, with apparently disc-like seeds, while Degener et al. (1962) described a closely related species *C. pekeloii* O.Deg., I.Deg. & Aellen as restricted to the island of Molokai and differing primarily from *C. oahuense* in its 'faintly sculptured seeds' and its location on the dry forested ridges and shrublands to the north and east of Kaunakakai. Wagner et al. (1999) sank all native chenopods into *C. oahuense*, noting that the taxon across the archipelago is "quite variable in habit, leaf size and margins, and to some extent in the inflorescence; However, it seems best treated as a single species without further subdivision." *Chenopodium oahuense* sensu Wagner et al. (1999) is found in a variety of conditions, particularly xeric habitats including coastal strand, dry forest, and subalpine shrubland from 0 to 2520 m in elevation.

Through previously formed relationships of the authors with Butch Haase, Director of the Molokai Land Trust, it became evident that the on-island botanists commonly refer

to a well-known form of *Chenopodium* from northwestern Molokai as a distinct entity. The careful observations of the local botanists over many years suggest that it may have evaded extinction from invasive ungulates only because it grows and reproduces on inaccessible and precipitously tall sea cliffs (William Garnett pers. comm.). Affectionately, these botanists refer to the variant as 'aweoweo papa, a combination of 'aweoweo, which is a Hawaiian name for *C. oahuense*, and papa, translating roughly in Hawaiian language to "flat surface" or "to occur close together." This vernacular name alludes to the taxon's prostrate habit, which positions it closely to its rocky sea cliff habitat substrate. Papa is often used to describe native plants with a prostrate form such as naio papa for a prostrate form of naio (*Myoporum sandwicense* A.Gray) that occurs on Hawaii Island. To the local botanists, the prostrate habit, characteristically small succulent leaves, and a unique Molokai sea cliff habitat immediately distinguish it from *C. oahuense* forms occurring elsewhere in the archipelago (Fig. 2). These botanists working in areas under active conservation management also recognize the necessity to keep populations of this previously undescribed taxon, herein named *C. oahuense* subspecies *ilioensis*

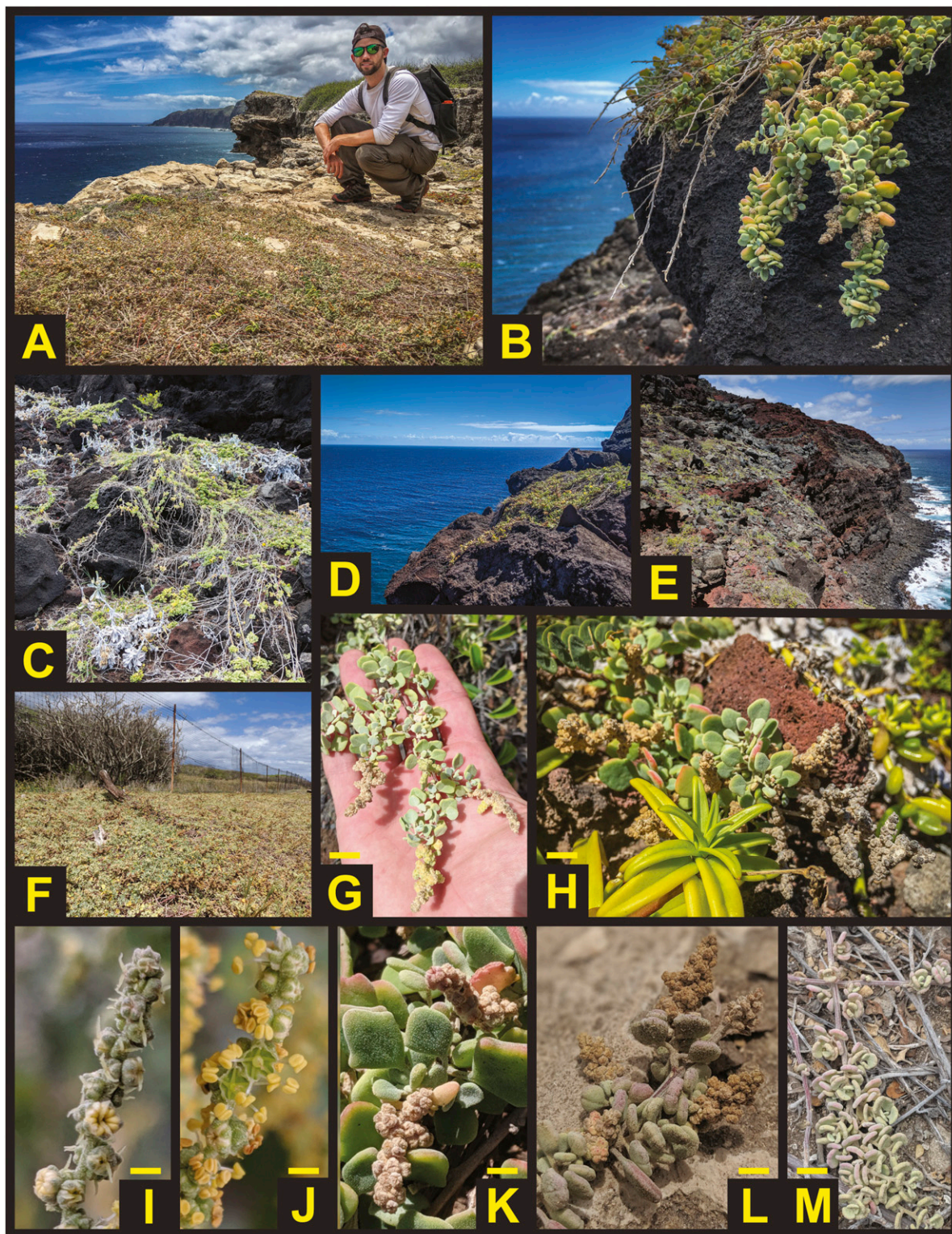


FIG. 2. Habitat and morphology of *Chenopodium oahuense* subsp. *ilioensis*. A. *C. oahuense* subsp. *ilioensis* (foreground) at 'Īlio Point showing prostrate habit with author JTC. B–E. Habitat between Ke Ala Pūpū a Kiha and Pueoao, B and D indicating a scandent habit on basaltic rocks, C and E with co-dominant associates *Pseudognaphalium sandwicense* subsp. *molokaiense*, *Lycium sandwicense*, and *Lipochaeta integrifolia* comprising a unique floristic community. F. Nearly solid ground cover of *C. oahuense* subsp. *ilioensis* (right of fence) at Anapuka Dunes restoration site following removal of invasive kiawe (*Prosopis pallida*). G–H. *Chenopodium oahuense* subsp. *ilioensis* with some associates such as: G. *Jacquemontia sandwicensis* (upper right). H. *Schiedea globosa*, *Euphorbia skottsbergii*, and *Sesuvium portulacastrum*. I–K. Portion of an inflorescence indicating late female (I), male (J), and fruiting phenology (K). L, M. Highlighting prostrate habit, fruiting branches, and succulent leaves at 'Īlio Point. Scale bars are as follows: G, M = 1 cm; H, K, L = 0.5 cm; I, J = 0.25 cm. All photos taken by JTC, except B, D, E by Butch Haase of Moloka'i Land Trust.

J. Cantley & Martine, separate from other *C. oahuense* s. l. populations as part of their best management practices.

In order to assess the validity of the Moloka'i taxon *C. oahuense* subsp. *ilioensis*, a combination of fieldwork, herbarium visits, morphometric and molecular phylogenetic analyses, and common garden experiments in greenhouses were conducted to inform the decision to separate this taxon as a distinct subspecies. Results of our phylogenetic analyses allow us to postulate a temperate Eurasian origin of Hawaiian *Chenopodium* and to infer the monophyly of *C. oahuense*, thus building on recent work of Mandák et al. (2018). We provide detailed hypotheses regarding the geographic location of the allopolyploidization event(s), dispersal(s), and timing of arrival to the Hawaiian Archipelago, and a possible in situ adaptive response of *C. oahuense* subsp. *ilioensis* to the abiotic challenges presented by its novel habitat.

MATERIALS AND METHODS

Molecular Methods and Phylogenetic Analyses—Tissue samples were collected with permission for destructive sampling from three herbaria (BISH, NY, US), and from live material of *C. oahuense* subsp. *ilioensis* from Moloka'i provided by Moloka'i Land Trust. DNA extractions were performed using a modified CTAB protocol (Doyle and Doyle 1987) for 16 individuals of *C. oahuense* s. l. and two of *C. oahuense* subsp. *ilioensis*. Two gene regions, plastid *rpl32-trnL* and nuclear ITS, were amplified using standard polymerase chain reaction (PCR) following Krak et al. (2016). PCR reactions were run out on 1% agarose gels for 1 hr at 100 V. All reactions were optimized to generate a single band of expected size. When this was not possible, bands of expected size were excised from gels. The PCR products were purified using the Wizard SV Gel and PCR clean-up system (Promega, Madison, Wisconsin) prior to Sanger sequencing by Eurofins Genomics (Louisville, Kentucky). Resulting sequences were manually edited and assembled in Geneious (Biomatters Limited, Auckland, New Zealand). The newly generated sequences were combined with 19 sequences from diploid taxa of *Chenopodium*: two samples of putative *C. oahuense* s. l. sequences from unknown Hawaiian locations, and twenty sequences from related outgroup genera. The tetraploid Eurasian *C. acerifolium* was also included. See Appendix 1 for voucher and GenBank accession number information.

Individual loci were aligned using MUSCLE v. 3.8.5 (Edgar 2004) and were adjusted manually. Gene trees for each locus were estimated by maximum likelihood using IQ-TREE v. 1.5.5 (Nguyen et al. 2015; Chernomor et al. 2016) with the optimal substitution models selected by ModelFinder 1.6.10 using a Bayesian information criterion (Kalyaanamoorthy et al. 2017). Clade support was determined by non-parametric bootstrapping using the ultrafast bootstrap with 1000 replicates (Minh et al. 2013).

Morphometric Data Collection and Analyses—Measurements were taken from greenhouse material and herbarium sheets (BISH, NY, US). Field observations have been made over the course of many years from many of the main Hawaiian Islands to assess abundance, take field notes regarding ecology, and shoot digital photos. Seeds were collected with permits during March 2016 from three different locations (*C. oahuense* s. l.: Kanaio Beach Park, Maui and Mauna Kea Recreation Area, Hawai'i Island, *C. oahuense* subsp. *ilioensis*: Pu'u Ka Pele, Moloka'i). Seeds were sown in a growth chamber set to Hawai'i conditions. Following germination, plants were grown in an Integrated Pest Management controlled greenhouse (Bucknell University and San Francisco State University). More than 100 specimens (including type specimens) from BISH, NY, PTBG, RSA, SFSU, UC, and US were examined. Morphological data were collected for 11 characters from 95 herbarium and greenhouse specimens. For measurements of leaves, two size classes of leaves were examined in order to capture a better understanding of existing variability; we speculate that the largest leaves of *C. oahuense* subsp. *ilioensis* may develop during times of higher precipitation post-germination and smaller leaves during drier periods. Thus, leaf variability may be the result of different environmental conditions that correlate with ontogenetic life stages of larger-leaved juveniles versus smaller-leaved adults. Comparison of characters was conducted using JMP Pro 12 (SAS Institute, Inc., Cary, North Carolina). Analyses included one-way ANOVA with Student's *t* test mean comparison at $p = 0.05$ and the all pairs Tukey HSD to compare means and discern which species are different and in what way. All vouchers used,

along with associated GenBank accession numbers, are listed in Appendix 1. A distribution map was generated using *ggmaps* and the *ggplot2* graphics packages in R from georeferenced points, field observations of the authors, and localities gathered from herbarium specimens (Fig. 1).

RESULTS

Phylogenetic Analyses—Alignments of all taxa from both the ITS and *rpl32-trnL* region yielded many parsimony-informative characters (PICs). Most Hawaiian *Chenopodium* sequences were identical to *C. acerifolium* for both DNA regions. In total, 178 PICs were recovered from the ITS region. However, only two PICs were found between *C. oahuense* s. l., *C. oahuense* subsp. *ilioensis*, the diploid parental *C. acuminatum*, and tetraploid *C. acerifolium*. These two PICs were present in only two individuals of *C. oahuense* s. l. individuals from Kilauea Point Kaua'i, which separate them from the aforementioned samples. Comparison of all samples of *C. oahuense* s. l. + *C. oahuense* subsp. *ilioensis* samples to *C. ficifolium* and *C. suecicum* revealed 36 and 38 PICs for the ITS region, respectively. Similarly, the total PICs among all taxa of the *rpl32-trnL* region was 177. Alignments of the *rpl32-trnL* region revealed no PICs between Hawaiian *C. oahuense* s. l. and *C. oahuense* subsp. *ilioensis* and three PICs separating them from *C. acerifolium*. Differing from the ITS alignments, comparison of *C. oahuense* s. l. and *C. oahuense* subsp. *ilioensis* to the diploid parents revealed nine PICs from *C. ficifolium*, nine PICs from *C. suecicum*, and 29 PICs with *C. acuminatum*.

ModelFinder determined that the SYM + G4 and TVM + F + G4 models were the best fit evolutionary model for the ITS and the *rpl32-trnL* DNA regions, respectively. The two gene trees are largely in agreement regarding the placement and support values found for outgroup taxa (Fig. 3). Topologies recovered were not congruent with that of Mandák et al. (2018), but there were only minor differences among relationships of some outgroup taxa. Among the two trees, relationships differed in their placement of [*C. oahuense* s. l. + *C. oahuense* subsp. *ilioensis*] + *C. acerifolium*. In the ITS phylogeny, [*C. oahuense* s. l. + *C. oahuense* subsp. *ilioensis*] + *C. acerifolium* has strong bootstrap support (99) and is most closely related to *C. acuminatum*. However, in the *rpl32-trnL* phylogeny, [*C. oahuense* s. l. + *C. oahuense* subsp. *ilioensis*] + *C. acerifolium* were reconstructed with strong support (100 bootstrap support) as most closely related to a grade containing *C. suecicum* and then *C. ficifolium* as the closest relatives. These results echo the discordant number of PICs determined between the taxa of the two different DNA region alignments. Both trees supported a monophyletic Hawaiian *Chenopodium*, but without enough resolution to confirm or refute a sister relationship of subspecific taxa.

Morphometric Analyses—Observations of plants grown in identical conditions in the greenhouse and field observations indicate that *C. oahuense* subsp. *ilioensis* retains several distinctive morphological characters distinguishing it from *C. oahuense* s. l., including a prostrate to scandent habit, thickened and succulent leaves that are smaller on average, and smaller seeds. However, leaf thickness (i.e. the fleshiness) is reduced, and a less congested inflorescence is notable for *C. oahuense* subsp. *ilioensis* when plants receive a constant/regular input of water from a greenhouse setting. Plants observed in the wild are uniformly succulent with thickened leaves and congested inflorescences. This may be a response to environmental conditions, which were not mimicked in the

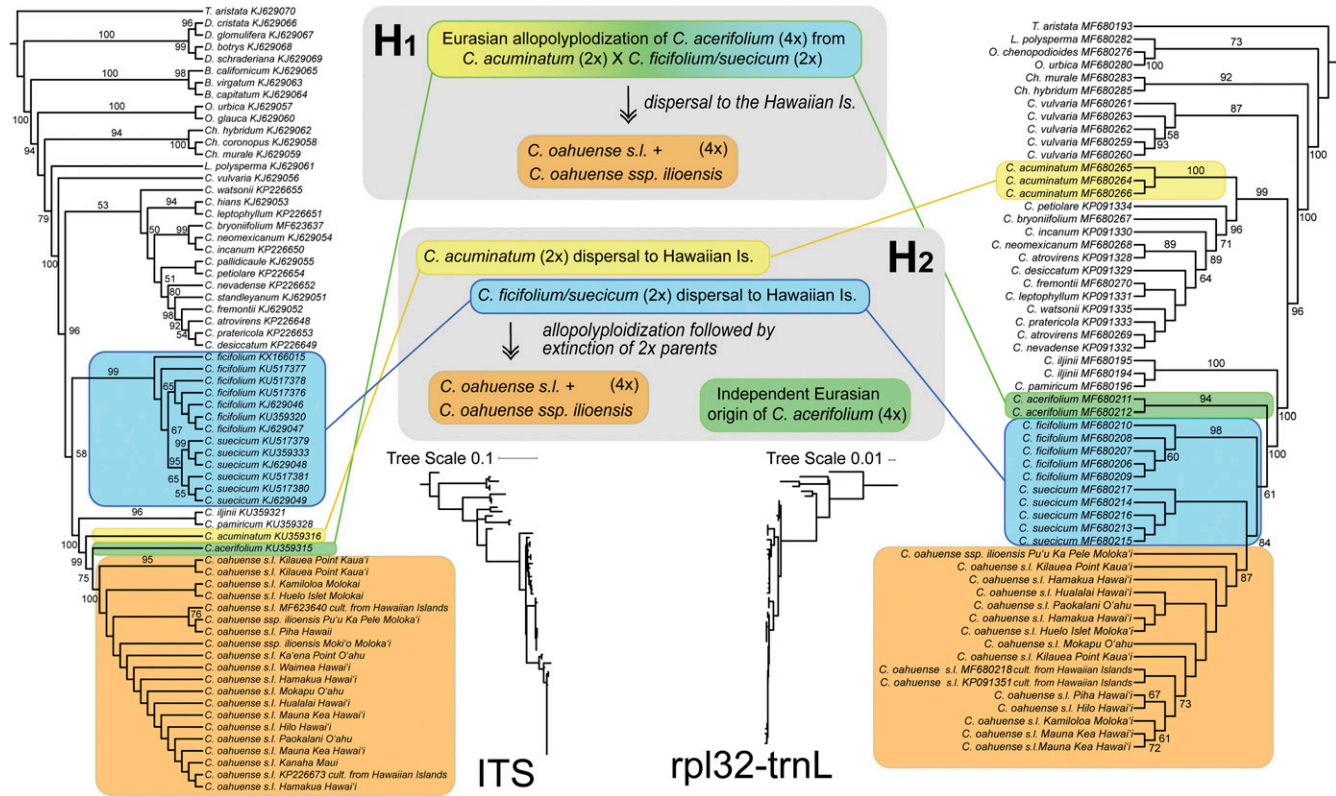


FIG. 3. Maximum likelihood phylogenetic reconstructions of diploid *Chenopodium* species relationships plus tetraploid *C. acerifolium*, *C. oahuense*, and outgroups for the two DNA regions investigated in this study: ITS (left) and *rpl32-trnL* (right). At bottom center, branch lengths are indicated, while numbers at nodes indicate support from 1000 bootstrap replicates. At top center are two equivocal hypotheses of the temperate Eurasian origin of *C. oahuense* in the Hawaiian Islands supported by findings of Mandák et al. (2018).

greenhouse, such as variable periods of precipitation intermittency, salt spray, and wind. Six of eleven measured characters revealed statistically significant differences between *C. oahuense* s. l. and *C. oahuense* subsp. *ilioensis*. Results (Table 1) indicate that leaf length and width, leaf thickness, petiole length, plus seed diameter and seed height significantly differ between the taxa.

Following the collection of morphometric data, an ad hoc assessment of additional herbarium specimens plus field observations helped determine the geographic extent of *C. oahuense* subsp. *ilioensis* as being restricted to the xeric sea cliffs of northwestern Moloka'i and nearby adjacent habitats (Fig. 1). *Chenopodium oahuense* s. l. also occurs on Moloka'i, but in a non-overlapping allopatric distribution. This includes the now synonymous taxon *C. pekeloi* from dry forested ridges of Makaupā'ia (1961, Degener 27509 (holotype: G [barcode: G00379138, photo!]),

which closely matches the typical erect form of *C. oahuense* sensu lato. In fact, inferences from herbarium specimens suggest that *C. oahuense* s. l. occurring on Moloka'i and other islands differs in habitat from *C. oahuense* subsp. *ilioensis*, particularly in a preference for more precipitation as it occurs in similar elevations and temperatures, but at wetter locations subject to frequent orographic precipitation like Makakupa'ia, the Kalaupapa Peninsula, and offshore islets of northeastern Moloka'i.

TAXONOMIC TREATMENT

Chenopodium oahuense subsp. *ilioensis* J. Cantley & Martine, subsp. nov. TYPE: USA: Hawai'i: Moloka'i: Kawaihau Point, West Moloka'i, 22 Feb 1948, F.R. Fosberg 29605 (holotype: US! [barcode 00445659], isotype: PTBG [barcode PTBG1000038425, photo!]).

TABLE 1. Comparison of measured averages in vegetative and reproductive characters of *C. oahuense* and *C. oahuense* subsp. *ilioensis*. All measurements in mm and *p* values from statistical analyses (bold and with an * where significance was found).

Character	<i>C. oahuense</i> s. l.	<i>C. oahuense</i> subsp. <i>ilioensis</i>	<i>p</i> value [<i><</i> 0.05*]
Largest leaf (length from petiole)	23.41	10.3	0.001017*
Largest leaf width	26.37	11.59	0.001682*
Smallest leaf (length from petiole)	6.71	2.94	0.000379*
Smallest leaf width	6.27	3.23	0.015961*
Leaf petiole length	19.31	8.13	0.002467*
Flower diameter	1.33	1.46	0.2304
Flower height	1.16	0.98	0.4663
Seed diameter	1.01	0.6	0.000245*
Seed height	0.82	0.37	0.000608*
Anther length	0.35	0.36	0.5947
Filament length	0.79	0.85	0.2293

Chenopodium oahuense subsp. *ilioensis* is distinguished from *C. oahuense* s. l. in the Hawaiian Islands by its prostrate to scandent habit, smaller leaves that are broadly deltoid ranging from $3.0\text{--}10.3 \times 3.2\text{--}11.6$ mm, and significant leaf thickness at $(1.0\text{--})1.5\text{--}3.5$ mm. The leaf petioles are shorter at $5\text{--}9\text{--}(15)$ mm and laminar lobing of the margin is minimal. *Chenopodium oahuense* subsp. *ilioensis* also differs in its smaller seed size of $0.5\text{--}0.6 \times 0.3\text{--}0.4$ mm.

Scentless to slightly malodorous perennial suffrutescent shrubs; generally less than 0.25 m in height, prostrate or scandent; usually white to light grey-green in overall appearance due to high density of epidermal bladder-cell trichomes. **Stems** striated and ribbed alternating green or red with white due to a mealy pubescence; young growth sometimes whiter and more densely pubescent, sometimes becoming woody proximally, periderm creamy white to light brown, occasionally splitting. **Leaves** alternate; venation palmate, generally with 1 or 3 primary veins; petioles $5\text{--}9\text{--}(15)$ mm; blades $3.0\text{--}10.3 \times 3.2\text{--}11.6$ mm, broadly deltoid to rhombic, usually 3(–5)-lobed, base truncate to cuneate, apex obtuse to emarginate, succulent at $(1.0\text{--})1.5\text{--}3.5$ mm thick; both surfaces mealy pubescent, but upper surface less so and generally greener than lower surface. **Inflorescence** a terminal panicle, branching with hundreds of sessile flowers, generally densely arranged; bracts present at proximal branching nodes, rhombic to lanceolate, decreasing in size distally. **Flowers** protogynous; $1.8\text{--}2.0 \times 1.4\text{--}1.8$ mm; calyx lobes 5, $0.7\text{--}1.0 \times 0.7\text{--}0.9$ mm, margins scarious towards apex, glabrous, crenate, white to translucent, mealy pubescent, completely enclosing (or nearly so) the fruit at maturity; stamens 5, filaments $0.9\text{--}1.1$ mm, opposite calyx lobes, anthers dorsifixed, exstrose, $0.3\text{--}0.5$ mm; gynoecium superior; pistil Y-shaped; stalk $0.2\text{--}0.3$ mm in length, attached at center of ovary disk; branches $0.5\text{--}0.8$ mm in length. Seed discoid, weakly pentagonal, $0.5\text{--}0.6 \times 0.3\text{--}0.4$ mm; red-brown to dark-brown; mealy pubescent. Figures 2, 4, 5.

Distribution—As currently understood, *C. oahuense* subsp. *ilioensis* occurs in two or three natural populations of the northwestern sea cliffs of Moloka'i. The largest and most central population stretches along the cliffs west to east from near Anapuka towards Moki'o Point, with a concentration of individuals occurring between Ke Ala Pūpū a Kiha and Pueoao. The westernmost known population occurs on the northern cliffs of 'Īlio Point approximately 1.5 km to the west, but may be connected to the aforementioned population. The easternmost population occurs approximately 13 km east at Pu'u Ka Pele between the western opening of Mane'opapa Gulch to just east of Pauaia Point. Due to the inaccessible nature of the sea cliffs and private stakeholder ownership, occupation of suitable habitat both east and west of the Pu'u Ka Pele population, as well as the cliffs between Na'ākāhihi to Nēhānaupō is not known. However, a few specimens from the 1960s and 1970s (e.g. *N. Pekelo Jr.* #MR38 & MR39, Mar 1974, BISH) indicate the distribution of a 'crawling form' once occurred one mile east of Pu'u Ka Pele. An observation in 2004 (W. Garnett pers. comm.) noted the taxon at a local fishing spot called Honey Bee between Pu'u Ka Pele and Mo'omomi Bay. *Chenopodium oahuense* subsp. *ilioensis* has not been observed between Moki'o Point to Mo'omomi Bay. In restoration areas of the Moki'o Preserve of Moloka'i Land Trust, *C. oahuense* subsp. *ilioensis* has naturally reseeded itself in areas without human assistance (Butch Haase pers. comm.). The taxon is used successfully as an important native groundcover species

(Fig. 2F) in restoration areas of the Moloka'i Land Trust around Anapuka and other sites where invasive species are removed. *Chenopodium oahuense* s. l. occasionally approaches the prostrate morphology of *C. oahuense* subsp. *ilioensis* on cliffs of Kaua'i (K.R. Wood & Perlman 2477, Mar 1993 [PTBG]) and Maui (H. Oppenheimer H41504, 2 Apr 2015, [PTBG] and C. Trauernicht 417, Mar 2008, [PTBG]), but leaf size and thickness do not match *C. oahuense* subsp. *ilioensis* from Moloka'i, indicating that there is some distinguishing variability within the new subspecies. The Pu'u Ka Pele population exhibits a slightly more erect habit than other populations. It is not clear if this is within the natural variation of the taxon or if, due to the historical presence of the cliff adjacent and now defunct Ho'olehua Dump (i.e. landfill), if introgression has occurred with *C. oahuense* s. l., which occasionally behaves as a ruderal-like weed.

Habitat—Average rainfall across the distribution of *C. oahuense* subsp. *ilioensis* varies from approximately 500–650 mm annually with the highest rainfall occurring from October to January, gradually tapering to nearly zero mm in September, when the lowest monthly average recorded precipitation occurs (Giambelluca et al. 2013). *Chenopodium oahuense* subsp. *ilioensis* occurs in skeletal soils of cinder and basalt origin on northeastern Moloka'i cliffs. At 'Īlio Point, the population occurs in skeletal sandy soils derived from lithified sand dunes. Along the cliffs between Ke Ala Pūpū a Kiha to Pueoao, *C. oahuense* subsp. *ilioensis* is co-dominant with *Pseudognaphalium sandwicense* subsp. *molokaiense* (O. Deg. & Sherff) W.L. Wagner (Asteraceae). *Lycium sandwicense* A.Gray (Solanaceae), *Sesuvium portulacastrum* L. (Aizoaceae), and *Lipochaeta integrifolia* (Nutt.) A.Gray (Asteraceae) also commonly co-occur. This assemblage, characterized by two narrowly endemic taxa, constitutes a unique floristic community occurring nowhere else in the Hawaiian Islands. Additional associates include *Sida fallax* Walp. (Malvaceae), *Scaevola taccata* (Gaertn.) Roxb. (Goodeniaceae), *Portulaca lutea* Sol. ex G.Forst. (Portulacaceae), *Jacquemontia sandwicensis* A.Gray (Convolvulaceae), *Schiedea globosa* H.Mann (Caryophyllaceae), *Euphorbia skottsbergii* C.N.Forbes (Euphorbiaceae), *Panicum fauriei* Hitchc., and *P. torridum* Guadich. (Poaceae) and non-native *Conchus oleraceus* L. (Asteraceae). One of the last remaining individuals of a prostrate form of *Sesbania tomentosa* Hook. & Arn. (Fabaceae) was also recorded for this same community (Butch Haase pers. comm.). At Pu'u Ka Pele and 'Īlio Point, *C. oahuense* subsp. *ilioensis* co-occurs in a different assemblage that additionally includes *Wikstroemia uva-ursi* A.Gray (Thymelaeaceae), *Tetramolopium sylvae* Lowery (Asteraceae), *Fimbristylis* Vahl. sp. (Cyperaceae), *Sporobolus virginicus* (L.) Kunth (Poaceae), *Heliotropium anomalum* Hook. & Arn. (Heliotropiaceae), and a prostrate form of *Heteropogon contortus* Beauv. ex Roem. & Schult. (Poaceae). Several non-native species are also present, including *Prosopis pallida* Kunth (Fabaceae), *Plantago lanceolata* L. (Plantaginaceae), *Cynodon dactylon* (L.) Pers., and *Chloris radiata* (L.) Sw. (Poaceae).

Etymology—*Chenopodium oahuense* subsp. *ilioensis* is named after the well-known location of one of its main populations: 'Īlio Point, Moloka'i. The Hawaiian name 'aweoweo papa is used by local botanists.

Phenology—Flowering is protogynous. In the greenhouse, the female stage of flowering lasted approximately 1–2 wk before anthers fully developed and released pollen. Flowers in female and male stages were noted in March to June, during and shortly after the annual wet season. Fruiting specimens



FIG. 4. Photo of type specimen at US.

were noted year-round, but age of seeds retained on inflorescences is not known.

Conservation Status—*Chenopodium oahuense* subsp. *ilioensis* occurs as scattered individuals that are locally common and occur within two or three populations on one volcano. The recommended conservation category (IUCN 2012) for *C. oahuense* subsp. *ilioensis* is Endangered (EN) under Criteria

B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v). It has an area of occupancy (AOO) < 19 km² and extent of occurrence (EOO) < 15 km², only two or three known subpopulations that are likely fragmented, and an observed continuing decline in habitat quality over much of its distribution primarily due to non-native herbivores, invasive plant species, and variable management on private stakeholder lands. Suitable habitat may

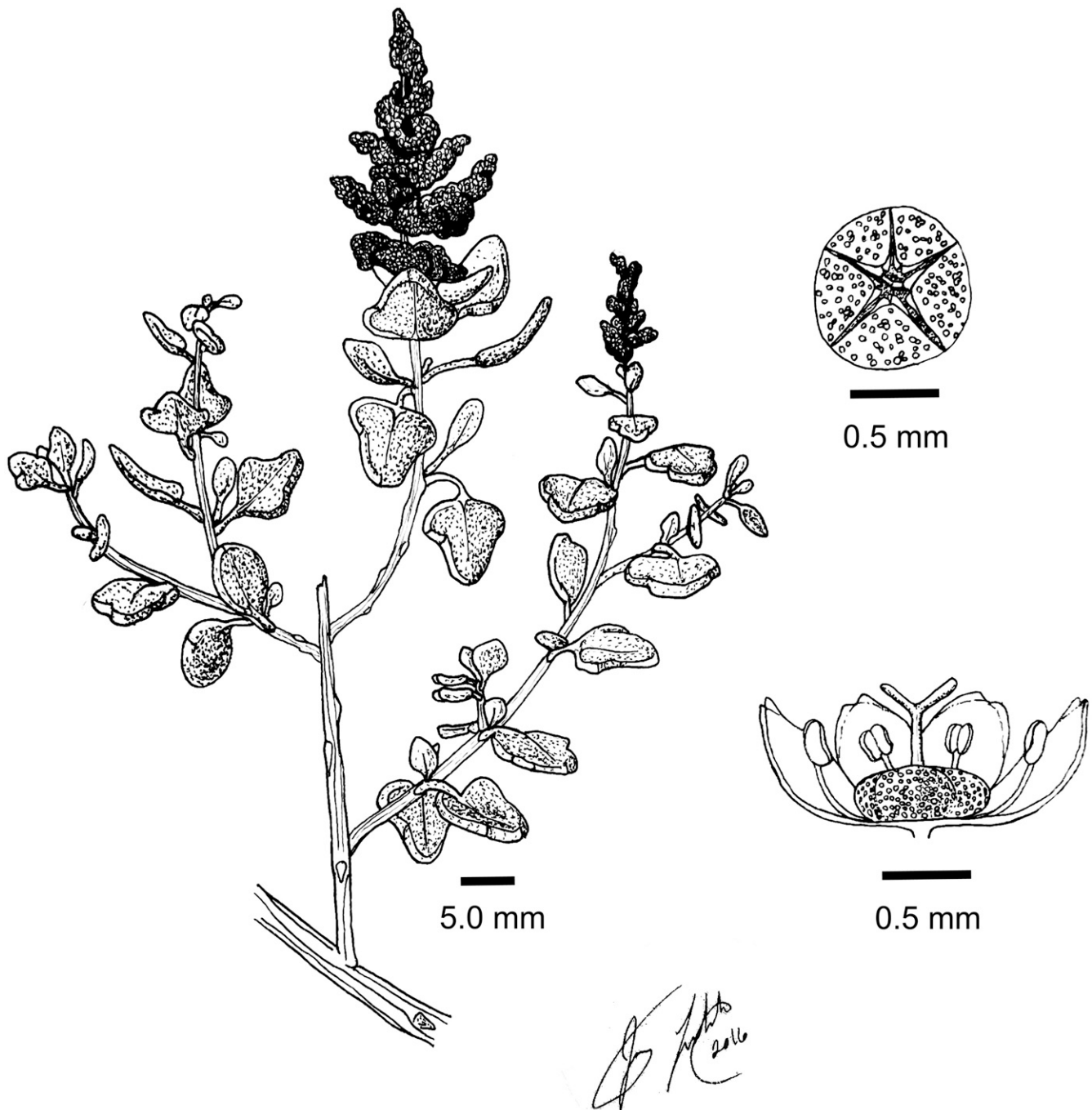


FIG. 5. Illustration of *C. oahuense* subsp. *ilioensis* by Joey Latsha-Gamboa.

continue decreasing in size without active conservation management, particularly in unmanaged areas of private stakeholder ownership. However, in areas where the landscape is actively managed for invasive plants and animals and restoration efforts occur (i.e. primarily by the Moloka'i Land Trust's Moki'o Preserve Anapuka Dune Restoration and Hardpan Restoration Test Plots), *C. oahuense* subsp. *ilioensis* has displayed a marked and largely unaided increase in population size.

Notes—*Chenopodium oahuense* subsp. *ilioensis* has notable plasticity in certain morphological characters in response to environmental conditions. In a greenhouse setting when grown under ideal conditions and a constant water source, leaf

succulence greater than 1.5 mm was not achieved and most inflorescences were widely branched and less congested than those observed in the wild. However, the plasticity of leaf characters did not approach the larger sizes typical of *C. oahuense* sensu lato. Additionally, *Chenopodium oahuense* subsp. *ilioensis* appears to have a higher density of epidermal salt-bladder cells than most populations of *C. oahuense* s. l., except in subalpine habitats like the Saddle Road region of the Big Island. A detailed greenhouse and herbarium investigation of plasticity in these characters is warranted.

Additional Specimens Examined—USA. —HAWAII. —MOLOKAI: Kawaihau Point, West Moloka'i, 22 Feb 1948, F.R. Fosberg 29605 (US, PTBG); Near Anapuka on the coast, 28 Apr 1928, O. Degener 18175 (US); north of

Ho'olehua, West Moloka'i, 26 Aug 1966, S. Carlquist 2235 (US); On cliffs at the edge of Ho'olehua Dump at 300 ft elevation, 21 Jun 1979, R. Gustafson 1271 (RSA); Pu'u Ka Pele, Mar 2016, Cantley et al. 1097 (PTBG, SFSU); Pu'u Ka Pele, 24 Dec 1948, H. St. John et al. 23495 (BISH); 1 mile east of Pu'u Ka Pele at top of sea cliffs, 16 Mar 1974, N. Pekelo Jr. MR38 (BISH); 1 mile east of Pu'u Ka Pele at top of sea cliffs, 11 Mar 1974, N. Pekelo Jr. MR39 (BISH).

Representative Specimens of *C. oahuense* s. l.—**USA.**—**HAWAII:**—**HAWAII ISLAND:** Near Huehue, 24 Jun 1924, C.B. Setchell s. n. (UC); Kōhala road, 1 Sep 1933, F.R. Fosberg 10168 (UC); Saddle Road, 2 mi. southwest of Pu'u Ko'ohi, Hāmakuā district, 26 Dec 1946, Cowan and Rogers 22375 (NY); North Kona, 3 miles west of Pu'u Wa'awa'a, 21 Jun 1948, G.L. Webster and R.L. Wilbur 1854 (UC); Kohala coastal pasture, 6 Aug 1926, O. Degener 18168 (UC); Ka'ūpūlehu Forest Reserve, North Kona District, 24 Mar 1961, F.R. Fosberg 41742 (RSA); Pohakuloa ranger station on slopes of Mauna Kea near Humu'ula Saddle, 5 Sep 1961, R.F. Thorne 3342 (RSA); Above Pu'u Kawaiwai on Kohala coast, 10 Sep 1961, F.R. Fosberg 42129 (RSA); North slope of Hualālai near Highway 1801, 19°48'N, 155°54'W (US), 30 Dec 1961, V.J. Krajina 611230023 (NY); 1 mile south of Kamuela, on road to Pu'u Wa'awa'a, 29 Jul 1966, S. Carlquist 2085 (UC); Humu'ula Saddle Road on lower slopes of Mauna Kea, 31 Jul 1966, S. Carlquist 2104 (RSA); 10 miles southwest on Humu'ula Saddle Road from junction with Highway 19, 18 Aug 1976, R. Gustafson 616 (RSA); Humu'ula Saddle Road, Pohakuloa area near Mauna Kea State Park, 13 Jul 1978, R. Gustafson 1084 (RSA); Humu'ula Saddle Road, 2 mi. west of Hale Pohaku, 13 Jul 1978, R. Gustafson 1087 (BISH); North Kona District, 28 miles south of Waimea on highway 190 at 2,000 ft elevation, 19 Jun 1979, R. Gustafson 1238 (RSA); Hāmākua District 1 mile southeast of Mauna Kea State Park off Saddle Road, 8 July 1982, R. Gustafson 2741 (RSA); *Acacia koa* Preserve 3 miles northwest of Waimea on Highway 250 in South Kohala District, 29 Feb 1984, R. Gustafson 2891 (RSA); 18 miles east of Saddle Road junction on Pohakuloa military training area, 25 Jun 1985, T.G. Lammers 5769 (RSA); 12.3 miles from Saddle Road at 5,800 ft elevation, 28 Jan 1987, G.D. Wallace and R. Gustafson 2600 (RSA); Paokalani islet, 150 m offshore of Honokea Stream, 14 Apr 2005, K.R. Wood 11212 (NY). —**KAUAI:** On Kaholuamonoa above Waimea, 2 Sep 1895, A.A. Heller 2788 (UC); Kīlauea Point National Wildlife Refuge, Hanalei district, 22°8.22'N, 159°14.388'E (PTBG), 27 Jul 2001, T. Flynn and R. Langdon 6912 (NY); Hipalau in lowland forest on cliffs, 30 Mar 1993, K.R. Wood & Perlman 2477 (PTBG). —**KAHOOLAWE:** Honokanaia near basecamp [new island record after restoration efforts], 8. Feb 2005, Starr et al. 0502080-1 (PTBG);

—**LANA'I:** Lower Maunalei Valley, 20 Aug 1957, S. Deshas and R. Holiday 24243 (NY); Floor of Maunalei at 1000 ft, 17 Aug 1963, O. Degener 30108 (NY). —**MAUI:** West Maui, Aug 1910, C.N. Forbes (s.n.) (UC); Haleakalā National Park, 15 Aug 1927, O. Degener 17902 (UC); Haleakalā Crater near bubble cave, 22 Aug 1933, F.R. Fosberg 9934 (RSA); Nakaohu, 100 m off coast, 23 Dec 1948, O. Degener 19307 (UC); East of La Perouse Bay, 20°36'N, 156°24'W (DDM), 16 Apr 1962, V.J. Krajina 620416008 (NY); West of Haleakalā off highway 378 at 5500 ft elevation, 19 Dec 1967, D. Herbst 804 (RSA); North side of Pi'ilani Highway 6 miles east of 'Ulupalakua, 30 Dec 1967, D. Herbst 830 (RSA); Haleakalā Crater 2 miles west of Palikū Cabin, 24 Jun 1969, J. Henrickson and R. Vogl 3711b (RSA); Kahakuloa, between Pu'u Koa'e and Pu'u Kahulianapa on windswept cliffs, 20.998583, -156.544306, 2 Apr 2015, H. Oppenheimer H41504 (PTBG); Kahanu on rocky coastline, [20.648333, -156.082778], 5 Mar 2008, C. Trauernict & N. Tangalin 417 (PTBG). —**MOLOKAI:** Kamilolola, (ca. 305 m), 3 Jun 1983, C.C. Summers (s.n.) (US); Kamilolola Heights Trail at 1500 ft elevation, 3 Mar 1989, R. Gustafson 3556 (RSA); Huelo islet, 2-3 km east of Kalaupapa, 21°6'N, 157°33'E (DDM), 9 Nov 1999, K.R. Wood 8037 (US); Mokapu, offshore islet east of Kalaupapa Peninsula, 70 m, 21°11'N, 157°55'W (DDM), 8 Mar 2000, K.R. Wood 8315 (US); Ho'olehua, 28 Nov 1961, N. Pekelo 27804 (US). —**NORTHWEST HAWAIIAN ISLANDS:** French Frigate Shoals, Central part of island, 22 Jun 1923, E. Caum 89 (UC); French Frigate Shoals, Center of the islet in coral rubble, 13 Sep 1966, S. Carlquist 2273 (RSA); Necker Island (Mokumanamana), Summit at 100 ft elevation, 11 Sep 1966, S. Carlquist 2262 (UC); Nihoa Island, 10 Jul 1924, E. Christophersen 7 (UC). —**OAHU:** Mauka about 2 miles from Ka'ena Point, 22 Mar 1931, O. Degener 17899 (UC); Pālehua trail, 5 May 1929, D. Topping 3328 (UC); 2 miles west of Kawaihāpai on rocky slope, 3 Nov 1929, H. St. John 9989 (UC); Wai'anae valley near Kolekole Pass, 26 Apr 1931, O. Degener 17907 (UC); Wai'anae Mountains, 30 June 1935, F.R. Fosberg 10944 (UC); Wai'anae mountain range 1500 ft, 1946, R.S. Cowan 175 (NY); Mountainside of Camp Erdman, Kawaihāpai, 9 Jan 1949, H.J. Clap and J. Popenoe 19359 (NY); Ulupau crater at Mokapu Peninsula, 30 Aug 1961, M. Sachet 861 (RSA); Mokuleia beach, 22 Oct 1961, O. Degener 27788 (NY); Kealia Trail, Kawaihāpai, 30 Mar 1962, O. Degener 27897 (NY); Slope of Ulupau Head, 10 Dec 1978, F.R. Fosberg 58838 (NY); Waianae District, Waianae Mountains on Kealia trail above Dillingham Air Base, 2 Mar 1984, R. Gustafson 2912 (RSA). Popoia Island: Kailua beach park, 1932, F.R. Fosberg 8892 (UC).

KEY TO THE SUBSPECIFIC TAXA OF *CHENOPodium* OAHUENSE

1. Prostrate or scandent shrubs, generally less than 0.25 m in height, leaves 3.0–10.3 × 3.2–11.6 × (1.0–)1.5–3.5 mm thick, often thickened and succulent, marginal lobes few and generally not prominent, seeds disk-like, 0.5–0.6 × 0.3–0.4 mm, weakly pentagonal; northwestern sea cliff habitats of Moloka'i *C. oahuense* subsp. *ilioensis*
2. Erect shrubs, generally greater than 0.5 m in height, leaves (15–)20–42(–100) × (6–)13–25(–55) × 0.5–1.0 mm thick, somewhat fleshy but generally not thickened, marginal lobes sinuate-dentate to weakly prominent, seeds 1.0 × 0.8 mm, sometimes appearing misshapen or wrinkled; on Lisianski, Laysan, French Frigate Shoals, Necker, Nihoa, and all main Hawaiian Islands (excluding northwestern sea cliffs of Moloka'i) *C. oahuense* s. l.

DISCUSSION

Eurasian Origin of Hawaiian Taxa of *Chenopodium*—Building upon analyses of Mandák et al. (2018), this study corroborates a temperate Eurasian origin of Hawaiian *Chenopodium* (Fig. 3). Our work suggests that Hawaiian *Chenopodium* is likely monophyletic, as the taxa form well-supported clades in phylogenetic analyses and the gene region sequences are identical for nearly all *C. oahuense* samples. However, it is unknown if Hawaiian *Chenopodium* are the result of either one or more colonization events, given that the ploidy level of *C. oahuense* subsp. *ilioensis* was not formally investigated and the geographic location where allopolyploidization occurred was undetermined. Molecular clock estimates from the cpDNA analyses (Mandák et al. 2018; Kadereit et al. 2010) suggest Hawaiian *Chenopodium* arrived 2.5 MYA or 9–1 MYA, respectively. This window of time suggests long distance dispersal to the Hawaiian Archipelago from temperate Eurasia could have occurred any time since the late Miocene, and therefore arrival location may have been equally likely on any of the numerous low-lying atolls of northwestern islands or the more recently emergent high islands of Kaua'i, O'ahu, or Maui Nui.

If a monophyletic Hawaiian *Chenopodium* can be assumed, then the phylogenetic results suggest two plausible scenarios of its arrival in the Hawaiian Islands (Fig. 3). The first involves an allopolyploid formation of tetraploid *C. acerifolium* in Eurasia from the hybridization of its two diploid parents, *C. acuminatum* and an ancestor of *C. sueticum* and *C. ficifolium*. Following tetraploid formation, *C. acerifolium* would next have had to disperse to the Hawaiian Archipelago and subsequently diverge in situ into endemic *C. oahuense*. The second scenario is less parsimonious, but may still be plausible in which there could have been two events of long-distance dispersal of both diploid parents to the Hawaiian Islands followed by in situ allopolyploid formation of Hawaiian *Chenopodium* via hybridization. Extinction of the parental taxa in Hawai'i and a separate allopolyploid event in Eurasia would have to occur to form *C. acerifolium*.

Eurasian floristic origins are one of the least common continental sources comprising the Hawaiian flora (Price and Wagner 2018). The most recent investigation of Price and Wagner (2018) estimates that only 3.9% of the flora, just eight of 259 colonizing lineages, arrived from Eurasia. This study

confirms a ninth colonizing lineage from Eurasia for Hawaiian *Chenopodium*. The dispersal mechanism to the Hawaiian Islands appears to be most commonly correlated to external adhesion by birds where five of the nine lineages, now also including *Chenopodium*, have morphology suggesting this mode of dispersal as most plausible (Price and Wagner 2018). Seed/fruit adhesion is thought to occur when they become stuck among feathers or to the feet in a mud mixture. The other four endemic Hawaiian lineages with external adhesion via birds are three Poaceae, including *Calamagrostis* Adans. (Soreng et al. unpubl.), *Festuca* L. (Catalán et al. 2009), and *Poa* L. (Soreng et al. unpubl.), and an additional Amaranthaceae genus, *Nototrichium* Hillebr. (McCauley & Havran unpubl.). Three other lineages are assumed to have arrived via internal bird ingestion: *Hydrangea* L. (Hydrangeaceae, De Smet et al. 2015), *Zanthoxylum* L. (Rutaceae, Appelhans et al. 2018), and *Nothocestrum* A.Gray (Solanaceae, Olmstead et al. 2008). The sole lineage with arrival via floating from Eurasia is *Lysimachia* L. (Primulaceae, Yan et al. 2018).

Diversification of Hawaiian *Chenopodium*—Multiple lines of evidence, i.e. morphology, distribution, and ecology, support the separation of *C. oahuense* subsp. *ilioensis* as a distinct subspecies from *C. oahuense* s. l. per the general lineage concept of species (De Queiroz 1998). However, the authors have chosen not to elevate the taxon to the rank of species as we have 1) no evidence of any partial reproductive isolating mechanisms beyond a current allopatric distribution of *C. oahuense* subsp. *ilioensis* and other populations, and 2) inconclusive genetic evidence. Nevertheless, it is plausible that results of future studies sampling at the population level across the archipelago may further an argument that this taxon is better represented at the rank of species, especially given the known morphological and ecological variability of Hawaiian *Chenopodium*.

Morphometric analyses revealed several statistically significant characters of *C. oahuense* subsp. *ilioensis* as divergent from *C. oahuense* s. l.: leaf size, leaf width, leaf thickness, petiole length, seed diameter, and seed height. These characters, plus the non-overlapping distribution of *C. oahuense* subsp. *ilioensis* along northwestern sea cliffs of Moloka'i, the qualitative characters of minimal leaf margin lobing, and a prostrate habit all speak to the biology of this taxon that is uniquely adapted to its extreme habitat. It is a succulent and small-leaved prostrate plant with small seeds that is well adapted to a dry, windy sea cliff environment with significant amounts of atmospheric salt spray.

Succulent leaves and the prostrate habit of *C. oahuense* subsp. *ilioensis* are novel characters not shared with *C. oahuense* s. l., *C. acerifolium*, or the diploid parental taxa of Hawaiian *Chenopodium*. Leaf succulence is a well-known adaptive feature of many species of Amaranthaceae (Kadereit et al. 2003) and may be adaptive for *C. oahuense* subsp. *ilioensis*. Field observations suggest a positive correlation between leaf succulence (i.e. thickness) of *C. oahuense* subsp. *ilioensis* and periods of no significant precipitation. Individual plants observed during these drought-like conditions have smaller, thicker, and more congested leaves than after periods of precipitation in which leaves are larger, thinner, and have longer internodes. However, these observations omit the potential role of salt spray in the induction leaf succulence. Plants grown in a greenhouse environment without salt spray and at the Moloka'i Land Trust Baseyard in Kualapu'u, Moloka'i have not been observed to produce succulent leaves. For many other taxa of Amaranthaceae, which includes 44% of

all known halophytic species (Flowers et al. 1986), induction of leaf and stem succulence is known to occur in response to elevated Na^+ and Cl^- ions on leaves or in the soil (De Villiers et al. 1996). Since *C. oahuense* subsp. *ilioensis* is a member of Amaranthaceae, it is possible the same mechanism of succulence induction is in response to Na^+ and Cl^- ions, but this has not been tested.

While it is possible that leaf succulence of *C. oahuense* subsp. *ilioensis* might be a response to salt spray and or limited precipitation, a different abiotic factor, high energy wind, might play a role in selecting for the prostrate to scandent habit and smaller seeds. For much of the year, northeast trade winds blow unobstructed towards the nearly vertical sea cliffs of Moloka'i at an average of 19 km per hour for greater than 12 hr a day during their peak in summer months (see Sanderson 1993). These winds slam into the sea cliffs and then flow up and over or around the island. Concerning seed sizes, the smaller seeds of *C. oahuense* subsp. *ilioensis* are likely additionally lighter in weight, which could allow strong winds to more readily lift seeds into the air carry them upwards to a higher point on the cliff. To a field scientist exploring these cliffs, this explanation seems plausible as it is common for localized sand and other small particles to be blown abrasively across their body for hours on end. However, while this logically makes sense, as lighter seeds may have a selective advantage over heavier seeds to be carried to higher elevation habitat, this hypothesis remains untested. An alternate dispersal mechanism would involve birds as vectors. Seabirds, non-native pigeons, and vagrants utilize the Moloka'i cliffs. A prostrate habit may lend an advantage in windy conditions through reduced mechanical damage of branches and leaves blown in the wind and an increased water retention through reduced evaporative transpiration (see Lloyd 1981). The same argument extends to smaller leaves as a mechanism to reduce the overall evaporative surface area, which can also increase water retention (Shabala et al. 2014).

Chenopodium oahuense subsp. *ilioensis* is not alone among the Hawaiian flora in adopting a prostrate habit in a high-energy windy coastal habitat. For instance, sympatrically occurring with *C. oahuense* subsp. *ilioensis* are three other Hawaiian endemic taxa, *Sesbania tomentosa* (Fabaceae), *Sida fallax* (Malvaceae), and *Heteropogon contortus* (Poaceae) (Butch Haase and J. Cantley pers. obs), which exhibit a prostrate to scandent habit. Populations of these taxa elsewhere in their distribution are not prostrate, except perhaps occasionally for *S. fallax*. Succulent leaves of endemic Hawaiian taxa that are derived from non-succulent colonists occur in similar coastal habitats such as *Lipochaeta integrifolia* and *L. succulenta* (Hook. & Arn.) DC. (Asteraceae), *Scaevola coriacea* Nutt. (Goodeniaceae), and *Bidens mauiensis* (A.Gray) Sherff (Asteraceae) (Wagner et al. 1999). Succulence also occasionally occurs for just a few leaves of a single individual such as in *Santalum ellipticum* Gaudich. (Santalaceae), *Sida fallax*, *Scaevola taccada* (Goodeniaceae), and *Myoporum sandwicense* (Scrophulariaceae) (J. Cantley pers. obs), but in other taxa, succulence is a diagnostic character separating it from close relatives.

Throughout the Hawaiian Islands, the habit of *C. oahuense* s. l. is variable. Both *C. oahuense* s. l. and *C. oahuense* subsp. *ilioensis* are perennial, which is different from their annual parental species. The perennial life history is possibly a key adaptation, which has allowed for secondary growth of wood of Hawaiian *Chenopodium* followed by adaptation to novel habitats. The most extreme example of this occurs in high

elevation dry subalpine habitats on the Big Island, where *C. oahuense* s. l. is a dominant taxon and forms shrublands. Individuals here can reach upwards of > 3 m in height (Hillebrand 1888). Height is achieved through a single thickened primary woody stem of several centimeters in diameter (J. Cantley pers. obs). Additionally, this subalpine environment is subjected to high solar irradiation, and individuals of *C. oahuense* there have a higher density of epidermal bladder cell trichomes, which give young leaves a silvery white coloration and may play a protective role in solar irradiation reflectance. A silvery pubescence involved in solar irradiation reflectance is known to occur for some members of the Hawaiian silversword alliance in similar habitats nearby (Robichaux et al. 1990). The most typical form of *C. oahuense* s. l. is exemplified by the habit occurring in dry forests along the North Shore of O'ahu in the Waianae Mountains. Here, the habit of *C. oahuense* s. l. is a loosely branching shrub to 2 m in height with more or less large and widely branching panicles. The different habits exhibited by Hawaiian taxa of *Chenopodium* may be adaptive responses to variance in the abiotic factors mentioned above: precipitation, high energy winds, oceanic salt spray, and solar irradiation.

A final point of discussion in the evolution of Hawaiian *Chenopodium* is the polyploid nature of the taxa. Many studies have speculated on the advantageous nature of polyploidy, wherein polyploids are likely to have a flexible genetic background allowing them to expand their geographic ranges or invade into new environments (Levin 2002; Parisod et al. 2010; Soltis et al. 2010). A study on *Achillea borealis* (Ramsey 2011) was among the first to demonstrate that polyploidy can indeed confer an ecologically adaptive fitness advantage of individuals in a novel habitat. Many subsequent studies on this topic and a recent review of the evolutionary significance of polyploidy suggests that evidence to date does not provide a concise answer (Madlung 2013; Van de Peer et al. 2017). Arguments for the adaptive evolutionary significance of polyploidy may apply to Hawaiian *Chenopodium* as it has adapted to and diversified across xeric environments in the archipelago. *Chenopodium* certainly is not the only known Hawaiian taxon to share this potential fitness benefit (Carr 1978, 1998; Kiehn 2005). Polyploidy among the lineages of the Hawaiian flora include the two colonizing lineages of *Coprosma* J.R.Forst. & G.Forst. (Cantley et al. 2014, 2016), *Viola* L. (Ballard and Sytsma 2000), Hawaiian mints (Lindqvist and Albert 2002), *Cuscuta* L. (Stefanovic and Costea 2008), *Santalum* L. (Harbaugh 2008), *Lipochaeta* DC. (Gardner 1976), *Euphorbia* (see Baldwin and Wagner 2010), and others. Polyploid lineages may even exhibit less inbreeding depression, a helpful adaptation in overcoming founder effects when colonizing novel environments (Soltis and Soltis 2000) in places such as isolated oceanic islands.

Regardless of the unknown ecological and evolutionary benefits that Hawaiian *Chenopodium* may gain from their polyploid origins, it is clear that the taxa are successful in having colonized xeric habitats across the archipelago after arrival from temperate Eurasia. The diversity of xeric habitats in the Hawaiian Archipelago provide numerous opportunities for differential divergence of *Chenopodium* across habitats primarily differing in abiotic factors such as precipitation, winds, solar irradiation, and salt spray. In at least one instance, the combination of these factors may in part help to explain the evolution of the endemic *C. oahuense* subsp. *ilioensis* among the sea cliffs of Moloka'i. Moreover, Hawaiian *Chenopodium* constitutes a prime lineage to assess the evolutionary and

ecological effects of polyploidization and differential genome downsizing, adaptive features across different xeric habits, and the investigation of morphological plasticity in certain adaptive traits. Noted variants across the archipelago suggest that additional taxa may reside within the morphologically diverse *C. oahuense* sensu lato. Further work on Hawaiian *Chenopodium* should likely include population level genetic studies and greenhouse experiments to better understand the unique evolutionary history of this lineage.

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AUTHOR CONTRIBUTIONS

JTC conducted all analyses, photographs, phylogenetic figure design, and was the primary manuscript author. JTC, CTM, and AJM provided mentoring of undergraduate students on molecular bench work, phylogenetic and morphometric analyses, growth and care of cultivated plants at both Bucknell University and San Francisco State University, and assisted with manuscript drafting. SRH and AJM conducted molecular bench work for newly generated sequences. JB and JK helped with morphometric data collection, and generation of figures, tables, and GenBank sequence submission. WG provided field assistance, specimen collection, and invaluable insights into the ecology and distribution of *C. oahuense* subsp. *ilioensis*. All authors provided editorial feedback on manuscript drafts.

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APPENDIX 1. Voucher information and GenBank numbers for sequences used in molecular phylogenetic analyses. Information is ordered by species, voucher location information, herbarium code (for specimens newly used in this study), and the ITS and *rpl32-trnL* GenBank accession number, respectively. GenBank numbers for sequences newly generated by the authors are in bold. Generic abbreviations are as follows: B. = *Blitum*, C. = *Chenopodium*, Ch. = *Chenopodiastrum*, D. = *Dysphania*, L. = *Lipandra*, O. = *Oxybasis*, T. = *Teloxys*.

Ingroup: *C. acerifolium* Andr., Asia (temperate) + Europe, KU359315, —. *C. acerifolium* Andr., Asia (temperate) + Europe, —, MF680211. *C. acerifolium* Andr., Asia (temperate) + Europe, —, MF680212. *C. acuminatum* Willd., Asia + Europe, KU359316, —. *C. acuminatum* Willd., Asia + Europe, —, MF680266. *C. acuminatum* Willd., Asia + Europe, —, MF680264. *C. acuminatum* Willd., Asia + Europe, —, MF680265. *C. atrovirens* Rydb., North America, KP226648, —. *C. atrovirens* Rydb., North America, —, MF680269. *C. bryoniifolium* Bunge, Asia (temperate), MF623637, —. *C. bryoniifolium* Bunge, Asia (temperate), —, MF680267. *C. desiccatum* A. Nelson, North America, KP226649, —. *C. desiccatum* A. Nelson, North America, —, KP091329. *C. ficifolium* Sm., Asia + Europe, —, MF680206. *C. ficifolium* Sm., Asia + Europe, —, MF680209. *C. ficifolium* Sm., Asia + Europe, KX166015, —. *C. ficifolium* Sm., Asia + Europe, KU517377, —. *C. ficifolium* Sm., Asia + Europe, KU359320, —. *C. ficifolium* Sm., Asia + Europe, KU517376, —. *C. ficifolium* Sm., Asia + Europe, KJ629046, —. *C. ficifolium* Sm., Asia + Europe, KJ629047, —. *C. ficifolium* Sm., Asia + Europe, KU517378, —. *C. ficifolium* Sm., Asia + Europe, —, MF680210.

C. ficifolium Sm., Asia + Europe, —, MF680207. *C. ficifolium* Sm., Asia + Europe, —, MF680208. *C. fremontii* S.Watson, North America, KJ629052, —. *C. fremontii* S.Watson, North America, —, MF680270. *C. hians* Standl., North America, KJ629053, —. *C. iljinii* Golosk., Asia (temperate), KU359321, —. *C. iljinii* Golosk., Asia (temperate), —, MF680194. *C. iljinii* Golosk., Asia (temperate), —, MF680195. *C. iljinii* Golosk., Asia (temperate), —, MF680195. *C. incanum* (S.Watson) A.Heller, North America, —, KP091330. *C. leptophyllum* (Moq.) Nutt. ex S.Watson, North America, KP226651, —. *C. leptophyllum* (Moq.) Nutt. ex S.Watson, North America, —, KP091331. *C. neomexicanum* Standl., North America, KJ629054, —. *C. neomexicanum* Standl., North America, —, MF680268. *C. nevadense* Standl., North America, KP226652, —. *C. nevadense* Standl., North America, —, KP091332. *C. oahuense* subsp. *ilioensis* J.Cantley & C.T.Martine, Pu'u Ka Pele, Moloka'i, Hawaiian Islands, SFSU, **MK912160**, **MK912151**. *C. oahuense* subsp. *ilioensis* J.Cantley & C.T.Martine, Moki'o, Moloka'i, Hawaiian Islands, US, **MK912161**, —. *C. oahuense* (Meyen) Aellen, Paokalani, O'ahu, Hawaiian Islands, US, **MK912162**, **MK912150**. *C. oahuense* (Meyen) Aellen, Hualalai, Hawai'i, Hawaiian Islands, US, **MK912163**, **MK912149**. *C. oahuense* (Meyen) Aellen, Piha, Hawai'i, Hawaiian Islands, —, US, **MK912155**. *C. oahuense* (Meyen) Aellen, Hamakua, Hawai'i, Hawaiian Islands, US, **MK912164**, **MK912147**. *C. oahuense* (Meyen) Aellen, Waimea, Hawai'i, Hawaiian Islands, US, **MK912165**, —. *C. oahuense* (Meyen) Aellen, Hamakua, Hawai'i, Hawaiian Islands, US, **MK912166**, **MK912146**. *C. oahuense* (Meyen) Aellen, Mauna Kea, Hawai'i, Hawaiian Islands, US, **MK912167**, —. *C. oahuense* (Meyen) Aellen, Hilo, Hawai'i, Hawaiian Islands, US, **MK912168**, —. *C. oahuense* (Meyen) Aellen, Mokapu, O'ahu, Hawaiian Islands, US, **MK912169**, **MK912153**. *C. oahuense* (Meyen) Aellen, Kilauea Point, Kaua'i, Hawaiian Islands, US, **MK912170**, **MK912154**. *C. oahuense* (Meyen) Aellen, Kilauea Point, Kaua'i, Hawaiian Islands, US, **MK912171**, **MK912152**. *C. oahuense* (Meyen) Aellen, Mauna Kea, Hawai'i, Hawaiian Islands, US, **MK912172**, —. *C. oahuense* (Meyen) Aellen, Kanaha, Maui, Hawaiian Islands, US, **MK912173**, —. *C. oahuense* (Meyen) Aellen, Kamiloloa, Moloka'i, Hawaiian Islands, US, **MK912158**, —. *C. oahuense* (Meyen) Aellen, Huelo Islet, Moloka'i, Hawaiian Islands, US, **MK912159**, **MK912148**. *C. pallidicaule* Aellen, South America, KJ629055, —. *C. pamiricum* Iljin, Asia (temperate), KU359328, —. *C. pamiricum* Iljin, Asia (temperate), —, MF680196. *C. petiolare* Kunth, South America, KP226654, —. *C. petiolare* Kunth, South America, —, KP091334. *C. pratericola* Rydb., North America + South America,

KP226653, —. *C. pratericola* Rydb., North America + South America, —, KP091333. *C. standleyanum* Aellen, North America, KJ629051, —. *C. suecicum* Murr, Asia (temperate) + Europe, KJ629048, —. *C. suecicum* Murr, Asia (temperate) + Europe, —, MF680213. *C. suecicum* Murr, Asia (temperate) + Europe, —, MF680217. *C. suecicum* Murr, Asia (temperate) + Europe, —, MF680216. *C. suecicum* Murr, Asia (temperate) + Europe, KU517380, —. *C. suecicum* Murr, Asia (temperate) + Europe, KU517381, —. *C. suecicum* Murr, Asia (temperate) + Europe, KJ629049, —. *C. suecicum* Murr, Asia (temperate) + Europe, KU359333, —. *C. suecicum* Murr, Asia (temperate) + Europe, KU517379, —. *C. suecicum* Murr, Asia (temperate) + Europe, —, MF680214. *C. suecicum* Murr, Asia (temperate) + Europe, —, MF680215. *C. vulvaria* L., Asia + Europe + Africa, KJ629056, —. *C. vulvaria* L., Asia + Europe + Africa, —, MF680263. *C. vulvaria* L., Asia + Europe + Africa, —, MF680262. *C. vulvaria* L., Asia + Europe + Africa, —, MF680260. *C. vulvaria* L., Asia + Europe + Africa, —, MF680261. *C. vulvaria* L., Asia + Europe + Africa, —, MF680259. *C. watsonii* A.Nelson, North America, KP226655, —. *C. watsonii* A.Nelson, North America, —, KP091335.

Outgroup: *B. californicum* S.Watson, California, USA, KJ629065, —. *B. capitatum* L., North America, KJ629064, —. *B. virgatum* L., Asia + Europe, KJ629063, —. *Ch. coronopus* (Moq.) S. Fuentes, Uotila & Borsch, Canary Islands, Africa, KJ629058, —. *Ch. hybridum* (L.) S. Fuentes, Uotila & Borsch, Asia + Europe, KJ629062, —. *Ch. hybridum* (L.) S. Fuentes, Uotila & Borsch, Asia + Europe, —, MF680285. *Ch. murale* (L.) S. Fuentes, Uotila & Borsch, Asia + Europe + Africa, KJ629059, —. *Ch. murale* (L.) S. Fuentes, Uotila & Borsch, Asia + Europe + Africa, —, MF680283. *D. botrys* (L.) Mosyakin & Clemants, Asia + Europe, KJ629068, —. *D. cristata* (F.Muell.) Mosyakin & Clemants, Australia, KJ629067, —. *D. schradleriana* (Schult.) Mosyakin & Clemants, Asia (temperate) + Africa, KJ629069, —. *L. polysperma* (L.) S. Fuentes, Uotila & Borsch, Asia (temperate) + Europe, —, MF680282. *L. polysperma* (L.) S. Fuentes, Uotila & Borsch, Asia (temperate) + Europe, KJ629061, —. *L. polysperma* (L.) S. Fuentes, Uotila & Borsch, Asia (temperate) + Europe, —, MF680282. *O. chenopodioides* (L.) S. Fuentes, Uotila & Borsch, Asia + Europe + Africa + North America, —, MF680276. *O. glauca* (L.) S. Fuentes, Uotila & Borsch, Asia (temperate) + Europe + North America, KJ629060, —. *O. urtica* (L.) S. Fuentes, Uotila & Borsch, Asia (temperate) + Europe + Africa, KJ629057, —. *O. urtica* (L.) S. Fuentes, Uotila & Borsch, Asia (temperate) + Europe + Africa, —, MF680280. *T. aristata* (L.) Moq., Asia (temperate) + Europe, KJ629070, —. *T. aristata* (L.) Moq., Asia (temperate) + Europe, —, MF680193.