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Evolution and Biogeographic Origins of the Endemic Hawaiian Genus *Hesperomannia* (Asteraceae)

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Abstract

The endemic Hawaiian genus *Hesperomannia* was investigated to examine the relationships among species and to test the hypotheses of dispersal to the islands over 17 MYA. Both nuclear ITS sequences and RAPD markers were used to assess genetic divergence among populations and species. PAUP, Neighbor-Joining and Bayesian phylogenetic trees were generated to examine species boundaries and relationships. Principal coordinates analysis was used to examine the relationships among individuals within populations and genetic distances among populations. Analyses suggest that four species should be recognized: *H. lydgatei*, *H. oahuensis*, *H. swezeyi*, and *H. arborescens*. Sequence analysis is consistent with arrival to Hawaii as recently as the last 2.3 MY, after the three main islands groups (Kaua‘i, O‘ahu, and Maui Nui) had emerged, followed by rapid dispersal among them. O‘ahu species are more closely related to each other than either is to the species of Maui Nui as was previously hypothesized. In contrast, Maui Nui plants are not genetically distinct enough to warrant separate species as previously recognized. Long-distance dispersal is evoked for dispersal among distantly situated island groups, but there is no evidence that colonization followed the progression rule model of dispersal among the islands and may have occurred from younger to older islands. Vicariance is probable within O‘ahu and among the islands of Maui Nui following erosion and subsidence of these islands, and may also explain the distribution of species among O‘ahu and Maui Nui. A revised key to and diagnostic descriptions of the species of *Hesperomannia* are provided.

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INTRODUCTION

The Hawaiian Islands are renowned for examples of evolution of many unique plant species from single ancestral colonization events. Taxonomic classifications based solely on morphological characters can sometimes be problematic in species complexes that have evolved through adaptive radiation. The Hawaiian flora is replete with examples where our understanding of relationships among endemic taxa based on morphological analysis has shifted tremendously following analysis with molecular markers. Notable examples include the radiations of lobeliads (Givnish et al. 1995, 2008), *Cyrtandra* (Cronk et al. 2005), *Rubus* (Howarth et al. 1997, Morden et al. 2003), *Viola* (Ballard and Sytsma 2000), and *Chamaesyce* (Morden and Gregoritza 2005). Moreover, convergence toward a similar morphology by distantly related congeners has also been demonstrated in this flora (Morden et al. 2003). Biogeographical studies based on molecular markers have also demonstrated that the origin of the founding colonists for some Hawaiian radiations were markedly different than previously predicted (Kim et al. 1998, Ballard and Sytsma 2000). Thus, having an accurate taxonomic classification is a vital component to understanding the biogeographic patterns among populations and species. Additionally, as many Hawaiian taxa are endangered, accurate taxonomic circumscription is essential for effective conservation and management to eliminate erroneous decisions based on incorrect identifications or species circumscriptions (Frankham et al. 2002).

Hesperomannia A. Gray (Vernonieae; Asteraceae) is an endemic Hawaiian genus with the original colonist to Hawaii putatively of African origin (Kim et al. 1998, Keeley et al. 2007). The species occur in mesic to wet forest sites at approximately 500 to 700 m elevation and are small trees or sprawling shrubs. The flowering heads are up to 5 cm long containing 25-40 disk florets and are subtended by 4-8 series of involucral bracts. The floret corollas are up to 3 cm long and brilliantly colored yellow or with a tinge of purple.

Although variously classified in the past, Wagner et al. (1990) recognized three species: *H. arborescens* A. Gray, *H. arbuscula* Hillebrand, and *H. lydgatei* C. Forbes in the Hawaiian flora. All species are listed as U.S. federally protected endangered species and are considered critically endangered by the IUCN. *Hesperomannia arborescens* is the type species of the genus having been first collected and described from Lanaʻi. The population of the type locality is now believed extinct (Wagner et al. 1990). As currently circumscribed, it occurs in wet forests of Northern Molokaʻi and in the Koʻolau Mountain Range of Oʻahu with the exception of a recently discovered population (Palikea Gulch) in the Waiʻanae Mountains that has become extinct since its discovery. Several subspecific taxa have been recognized based on morphological variation (Carlquist 1957). *Hesperomannia arbuscula* was collected and described from mesic to wet forests along ridges of deep valleys of west Maui and later from a few, small and scattered populations in the Waiʻanae Mountain Range of Oʻahu. *Hesperomannia lydgatei* is known primarily from the wet forest of Wahiawa/Kanaele Bog drainage basin in south Kauaʻi in several small subpopulations along various stream tributaries and a few scattered individuals recently located on the north side of the island (S. Perlman, National Tropical Botanical Garden, pers. com.).

As we made collections to assess genetic variation within and among populations of these endangered plants, it became apparent to us that the current circumscription of the species was inconsistent. Populations of *H. arbuscula* from West Maui did not share habit and leaf morphology with *H. arbuscula* populations from the Waiʻanae Mountain Range on Oʻahu. Similarly, *H. arborescens* plants from Molokaʻi were not the same as those from the Koʻolau Mountain range of Oʻahu. Instead, the only known population of *H. arborescens* from northern Molokaʻi seemed to share a comparable morphology and habit with *H. arbuscula* from West Maui. This led us to question the taxonomic circumscription of the populations of this genus

throughout the islands. Communications with field biologists working with these species revealed that they similarly found the taxonomy inconsistent with the morphology. Two studies examined anatomical and morphological variation in more detail. Carlquist (1957) was able to identify anatomical distinctions among cells within the involucral bracts and corolla of some species, but a detailed examination among features of vegetative anatomy found no differences. Funk and Wagner (1995a) assessed morphological characters among the three recognized species to examine biogeographic distribution of the species, but did not address issues of species boundaries. Morphological traits identified by authors in earlier classification schemes were not directly applicable to the names as applied by Wagner et al. (1990), and for some taxa neither fit well with what was observed in the field.

An apparent morphological and ecological similarity among *Hesperomannia* populations from Maui and Molokaʻi would be consistent with these islands, along with Lanaʻi and Kahoʻolawe, having shared a linked past as a single contiguous island commonly referred to as Maui Nui (Price and Elliott-Fisk 2004). However, such a relationship would also alter the biogeographic concept of how the species likely dispersed across the islands. *Hesperomannia* is widely divergent from its African ancestors, and a long distance dispersal from Africa to Hawaiʻi is estimated to have occurred at least 17 MYA (Kim et al. 1998, Keeley et al. 2007), much older than the age of Kauaʻi, the oldest of the main islands at 4.7 MY (Clague 1996). The present classification of the species would suggest that, following dispersal to Kauaʻi from a previous high island, dispersal continued following a progression rule model (Hennig 1966, Funk and Wagner 1995b) along two routes: to the Waiʻanae Mountains, Oʻahu and on to west Maui (*H. arbuscula*), and to the Koʻolau Mountains, Oʻahu and on to Molokaʻi and Lanaʻi (*H. arborescens*). A change in the classification among these species would necessarily also alter this hypothesis. The only critical morphological analysis conducted on these species to date also

concluded that colonization was from older to younger islands, but this was aberrantly based on the then accepted assumption that *Hesperomannia* was descended from species in South America in the tribe Mutisieae (Funk and Wagner, 1995a).

This study was undertaken to address the genetic relationships among populations to clarify species boundaries and their biogeographic affinities. In doing so, their distribution was examined to determine if evidence supports the progression rule model of dispersal across the islands or if an alternate hypothesis is supported. Variation among random amplified polymorphic DNA (RAPD) markers and sequence analysis of the DNA encoding the ribosomal RNA internally transcribed spacer (ITS) were used to assess genetic similarities within and among populations, identify species boundaries based on genetic cohesiveness, and identify geographic patterns present among species. A reassessment of the morphological variation of the species was also made and a new key to the species is provided. Analysis of genetic variation within and among populations will not be addressed here, but will be presented in a subsequent publication.

MATERIALS AND METHODS

Plants were sampled from populations of *Hesperomannia* representing the geographic breadth of the species (Figure 1) with one exception. Populations referable to "*H. bushiana*" occur in remote regions of the Koʻolau Mountains and access for this study was unavailable. Because of their endangered status and few individuals per population, collections were restricted to one to two leaves per plant to minimize the impact on individuals and no voucher specimens were collected. However, specimens from most of these populations are on deposit at Bishop Museum Herbarium (BISH) and representative samples are cited in Table 1 where

available. Six of the small subpopulations of *H. lydgatei* from the large population of the Wahiawa/Kanaele Bog were sampled.

<< FIGURE 1 near here >>

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One leaf per healthy individual was sampled from each population for DNA analysis. Mature individuals were primarily sampled although juveniles and seedlings that were visually judged to be healthy were sampled in very small populations. Estimated size and number of plants sampled from each population are listed in Table 1. DNA was extracted from fresh leaf material using the CTAB extraction procedure of Doyle and Doyle (1987) with some modifications (Morden et al. 1996). All DNA samples were accessioned into the Hawaiian Plant DNA Library (HPDL; Morden et al. 1996, Randell and Morden 1999) and are stored at –20° C.

Population Analyses

Approximately 25 ng of DNA were amplified via the polymerase chain reaction for RAPD analyses. RAPD markers were amplified in 25 µl volumes under the following conditions: ca. 0.2 mM each of dATP, dCTP, dGTP, dTTP, 1X *Taq* DNA polymerase buffer [10 mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl and 0.1% Triton X-100, Promega], 1.5 mM $MgCl_2$, 0.25 mg BSA, 0.2 µM random 10-mer oligonucleotide primer (Operon Technologies, Alameda, Calif. USA), and ca. 1 unit of *Taq* DNA Polymerase (Promega, Madison, WI, USA). PCR reactions were performed in a MJ Research DNA thermocycler using the following reaction conditions: an initial denaturation cycle of 94°C for 2 min 15 sec followed by 45 cycles of 95°C for 45 sec, 35°C for 30 sec and 72° C for 2 min, and a final extension at 72°C for 4 min. PCR amplified products were mixed with loading dye and separated on 1.5% agarose gels, stained with EtBr and visualized with a UV light source. Negative control reactions were run without

DNA for all PCR amplifications to ensure reaction components were uncontaminated. Size of amplification products was estimated using either the 100 kb ladder (Promega, Madison, Wisconsin, USA) or a pBS plasmid (Stratgene, La Jolla, CA, USA) digested with restriction enzymes to produce fragments in a size range from 0.448-2.96 kb. Gels were digitally photographed using a Kodak DC 290 camera and digital photos of RAPD gels were analyzed using Kodak ID Image Analysis Software (Eastman Kodak Company 2000, Scientific Imaging Systems, Rochester, NY USA).

A total of 31 RAPD primers (Operon Technology, Alameda, California, USA) kits A-D were screened using a subset of DNA with two individuals from three separate populations. Bands from reproducible amplification phenotypes (determined from replicated analyses) were scored for either presence (1) or absence (0) at each locus (Rieseberg 1996). Other assumptions associated with RAPD marker analysis are described in Lynch and Milligan (1994). A RAPD marker was determined to be polymorphic when found in less than 95% of the individuals of a population sampled. Absence of a marker within a population, although present in others, was assumed to indicate that all individuals of the population were null/null homozygotes rather than there being a loss of the locus.

Population Data Analyses

Percent polymorphic loci for each species and population was calculated using MS Excel. Genetic similarity indices were estimated using both Gower (1971) and Nei and Li (1979) similarity coefficients for populations and species of *Hesperomannia* sampled using MVSP Plus ver. 3.1 (Kovach 2007). Relationships within and among populations and species were projected from the similarity matrixes using principal coordinate analysis (PCO) and cluster analysis with

MVSP Plus ver. 3.1 (Kovach 2007); cluster analyses gave results consistent with the PCO analyses, and are not presented.

Phylogenetic Analyses

A subset of the original *Hesperomannia* DNA samples was chosen for phylogenetic investigations. One sample from each of the *Hesperomannia* populations examined with RAPD markers was employed in the ITS analyses (GenBank accessions to be included upon article acceptance). The ITS region was amplified in 50 µl volumes under the following conditions: 25 ng of DNA, ca. 0.2 mM each of dATP, dCTP, dGTP, dTTP, 1X *Taq* DNA polymerase buffer [10mM Tris-HCl (pH 9.0 at 25° C), 50 mM KCl and 0.1% Triton X-100, Promega], 1.5mM $MgCl_2$, 0.50 mg BSA, 0.2 µM ITS forward and reverse primers (Wendel et al. 1995), and ca. 1 unit of *Taq* DNA Polymerase (Promega). PCR reactions were performed in an MJ Research DNA thermocycler using the following reaction conditions: an initial denaturation cycle of 94° C for 2 min. 15 sec followed by 30 cycles of 95° C for 30 sec, 50° C for 30 sec and 72° C for 2 min, and a final extension at 72° C for 4 min. Size of PCR products were verified on 1.5% agarose gels, and compared to a 1 kb ladder. Negative control reactions were run without DNA for all PCR amplifications to ensure reaction components were uncontaminated. The PCR products for the ITS regions were cleaned using a Qiagen QIAquick PCR Purification Kit (Valencia, California, USA) according to the manufacturer's instructions. Double-stranded PCR products were sequenced in two forward reactions and two reverse reactions using the primers of Wendel et al. (1995) at the University of Hawai'i Biotechnology and Molecular Biology Instrumentation and Training Facility.

Taxa used for outgroup comparison were selected from those that clustered most closely to *Hesperomannia* in the phylogenetic analysis of tribe Vernonieae by Keeley et al. (2007) and

sequences were kindly provided by the authors (Table 1). Sequence data were aligned manually with reasonable confidence and all positions were included in analyses. Alignment was manually adjusted; the Hawaiian taxa contained no gaps and few were present relative to outgroup species; gaps were not coded as additional character states. Neighbor-Joining (using PAUP*; Swofford 2002) was used to construct trees under minimum evolution, using the parameters determined in Modeltest. Parsimony trees were constructed using the branch-and-bound search option. Both parsimony and distance analyses were subjected to bootstrap resampling (1000 replicates) to estimate robustness of nodes (Felsenstein 1985). A Bayesian analysis was conducted with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Four chains were initiated from a random start and run for 10 million generations. Every 100 generations, a tree was sampled from the chain for a total of 176,000 trees sampled. Due to burn-in, 24,000 sample points were discarded. PAUP 4.0b10 was used to calculate a 50% majority rule consensus tree and to report the posterior probability for each clade. Posterior probabilities have been shown to overestimate branch support (Suzuki 2002) and were interpreted with caution.

Morphological Analysis

Following genetic analyses, herbarium specimens from the B. P. Bishop Museum (BISH), Joseph F. Rock Herbarium of the University of Hawai‘i (HAW), and the Harold L. Lyon Arboretum (HLA) were examined to assess morphological traits consistent with data. Character states of vegetative and floral traits were measured from which a key to the species was developed and species descriptions made. Measurements for some characters were taken from published sources (corollas: Degener 1932; involucral bracts in *H. lydgatei*: Wagner et al 1990) to avoid destructive sampling of the limited specimens available. Specimens examined from BISH, HAW, and HLA are listed in Appendix 1.

RESULTS

Population Analyses

Of the 31 primers tested for amplification, nine produced clear and consistent products that were used on all individuals. These nine primers yielded 202 scorable RAPD markers with a range of 12-35 loci (average 22) identified for each RAPD primer (Table 2). Ten of the 202 (4.9%) markers were present in all individuals of all species and 192 (95%) markers were polymorphic across the genus.

<< TABLE 2 near here >>

Principle coordinate analysis (PCO) was performed with all samples for all populations examined in the entire genus. This PCO plot resulted in three distinct groupings that represent populations on Kauaʻi, Oʻahu and the Maui Nui complex (i.e., Maui and Molokaʻi) (Figure 2). The first PCO axis accounts for the distinction of Maui Nui populations from the Oʻahu and Kauaʻi populations. The second axis differentiates populations on Oʻahu and Kauaʻi. These data suggest that *H. arborescens* and *H. arbuscula*, as currently circumscribed, are highly heterogeneous. Populations within island complexes are more closely related to one another than they were to conspecific populations following current taxonomic circumscription.

<< FIGURE 2 near here >>

Genetic variation among Oʻahu populations was not clearly distinguishable in Figure 2 although clustering of populations was evident. Therefore, a separate PCO analysis with only Oʻahu populations was conducted (Figure 3). Individuals aligned into three distinct clusters. The first axis distinguishes populations of the Waiʻanae Mountains from those of the Koʻolau Mountains. One exception to this distribution was the Palikea Gulch population located in the Waiʻanae Mountains, the morphology of these two plants having the typically glabrous leaves of

those from the Koʻolau Mountains. Two observations were evident from examining the second axis: 1) most individuals of the Kaukonahua population were genetically well differentiated from others within the Koʻolau Mountains, and 2) populations in the Waiʻanae Mountains aligned geographically from north (Makaha) to south (Palawai).

<< FIGURE 3 near here >>

Differentiation among the four population clusters was strongly supported by the presence / absence of RAPD markers by the populations. Twenty genetic markers were diagnostic (i.e., private alleles present only in these populations) for the Waiʻanae, Oʻahu populations, the highest number for all population clusters examined. An additional six markers were uniquely absent from these populations (i.e., present in at least one population in each of the other three population clusters). Each of the other clusters had less than half this number of diagnostic markers: six in Kauaʻi populations, five in the Maui Nui populations, and 10 in the Koʻolau, Oʻahu populations. No markers were diagnostic for species based on the presently accepted classification.

Sequence Analysis

Ten outgroup species along with representative members of each *Hesperomannia* population (except from Palikea Gulch) were examined for sequence variation in the ITS region. Analyses based on parsimony, Neighbor-Joining and Bayesian analysis all show outgroup species, the source material for each from Africa, to have the same relationship to *Hesperomannia* species as has been previously demonstrated (Keeley et al. 2007). The two species most closely affiliated to *Hesperomannia* were *Gymanthemum amigdalinum* and *Vernonia humbloti*. As such, outgroup species relations will not be further discussed in this analysis.

Sequence analyses identified three major clades among the *Hesperomannia* populations, these corresponding to each of the island groups: Kauaʻi, Oʻahu, and Maui Nui (Figure 4). The Oʻahu clade further divided to separate the Waiʻanae and Koʻolau populations. These results were consistent with results from RAPD analysis. Variation within each species was very limited and bootstrap support was high (≥95%) except among Koʻolau populations. Koʻolau populations showed considerable variation at the sequence level and corresponding bootstrap support for this grade was low (64%) as nearly each of the populations had one or more unique apomorphies. This was also reflected in the RAPD analysis where these populations demonstrated the greatest level of genetic diversity within a species (data not shown).

<< FIGURE 4 near here >>

The three methods of analysis used to estimate species relationships (parsimony, Neighbor-Joining, and Bayesian analysis) consistently identified the three strongly supported clades as described above. However, the present data do not resolve the branching order of clades associated with the three island groups. Parsimony analysis weakly supported the Maui Nui complex as the most basal among the three clades (bootstrap support = 68%). In contrast, Neighbor-Joining analysis weakly supports the Kauaʻi populations as most basal (bootstrap support = 65%). Bayesian analysis (Figure 4) resulted in a trichotomy of the three island groups (i.e., support for resolution was below 50%) with no clear ancestral clades.

Taxonomy of Hesperomannia

The combined RAPD and sequence analysis results indicate that four species are clearly distinguishable genetically supporting differences observed in the field. Although subspecific delineations have been made in the past, none are recognized here. All individuals from Kauaʻi are genetically and morphologically consistent, and represent *H. lydgatei*. All Maui Nui plants

are genetically cohesive and share morphological similarity, and are referred to *H. arborescens* (this name having priority over *H. arbuscula*).

Two distinct groupings of plants on O‘ahu are genetically and morphologically evident, one restricted to the Wai‘anae Mountains and the other (with the single exception of the Palikea Gulch population pointed out above) to the Ko‘olau Mountains. Plants in the Wai‘anae Mountains have tomentose leaves, are found in mesic habitats, and have green and magenta involucral bracts at anthesis. In contrast, those in the Ko‘olau Mountains have largely glabrous leaves, are found in the wet forests, and have magenta involucral bracts at anthesis. Because the names presently recognized for these two species are based on types from Maui Nui (both of which now recognized as *H. arborescens*), their names must necessarily be changed. Based on the priority of available names, plants from the Ko‘olau Mountains are *H. swezeyi* Degener and those from the Wai‘anae Mountains are *H. oahuensis* (Hillebrand) Degener. The Palikea Gulch population of *H. swezeyi* from the Wai‘anae Mountains has since gone extinct, and thus this species is now known solely from the Ko‘olau Mountains. There was no evidence that any of the populations examined are distinct enough to be recognized at the subspecific level; RAPD analysis shows that many individuals of the Ko‘olau-Kaukonahua population differentiate from other *H. swezeyi* populations along PCO axis 2, but populations were not differentiated based on sequence analysis and they are otherwise similar morphologically. Efforts are underway to access and examine a population of “*H. bushiana*” as it fits closely with the remainder of *H. swezeyi*, but with more elliptic leaves and may represent a distinct variety.

Key to the species of Hesperomannia

Because these species had been variously classified in the past, a clear understanding of their morphological affinities had been difficult. Clarifying the species based on genetic

relationships aided us in developing a morphologically based key that represents the four species now recognized. Diagnostic descriptions of the species are also provided.

1. Flowering heads nodding at anthesis; leaf blades glabrous; involucre white to pink or brown at anthesis; Kaua‘i ..... *H. lydgatei*

1. Flowering heads erect to ascending at anthesis; leaf blades pubescent or nearly glabrous; involucre green and magenta, magenta, or dusty pink at anthesis 2
2. Plants with lower leaf surfaces, petioles, apical buds densely tomentose; leaves ovate to elliptic-ovate, upper surface tomentose to sparsely pubescent; innermost involucral bracts 2.3–2. 5 cm long; involucre green and magenta at anthesis; O‘ahu (Wai‘anae Mountains.) ..... *H. oahuensis*
2. Plants with lower leaf surfaces, petioles, and apical buds nearly glabrous or sparsely pubescent; leaves oblanceolate to obovate or broadly oblanceolate (sometimes elliptic), upper surface glabrous; innermost involucral bracts 2.7–3.0 cm long; involucre dusty pink or magenta at anthesis 3
3. Leaf blades oblanceolate to obovate, lower leaf surface sparsely puberulent, especially along lower 1/3 –1/2 portion of midrib on young leaves, upper surface glabrous; petioles 1/7–1/4 of total leaf length; peduncles 8–13 mm long; middle involucral bracts 4–5 cm wide; involucre dusty pink at anthesis; West Maui, Moloka‘i, Lana‘i ..... *H. arborescens*
3. Leaf blades oblanceolate to broadly oblanceolate, or sometimes elliptic, both leaf surfaces glabrous or nearly so with lower leaf surface of young leaves sometimes sparsely pubescent along 1/2–1/3 of midrib; petioles 1/8–1/7 of leaf total length; peduncles 4–6 mm long; middle involucral bracts 3–3.5 cm wide; involucre magenta at anthesis; O‘ahu (Ko‘olau Mountains.) ..... *H. swezeyi*

***Hesperomannia arborescens* A. Gray**

Hesperomannia arborescens Gray, Proc. Amer. Acad. Arts Sci., 6: 554, 1865. TYPE: Summit of Lanai, *H. Mann & W. T. Brigham 357* (Holotype: GH! [00008996]; Isotype: BISH! [1005806, 1005807], US [US00432531]).

Hesperomannia arbuscula Hillebrand, Flora Hawaiian Islands, 232, 1888. TYPE: W. Maui about 1200 ft. above Lahaina, *E. Bishop s. n.*, May 1871 (Holotype: B [destroyed], fragment: BISH-1005809!; Lectotype: GH-00008997!, Isolectotype: BISH-1005808!).

Hesperomannia mauiensis St. John, Ann. Missouri Bot. Gard., 1983. TYPE: ʻIao Valley, Makalaloa Stream, steep forest slope, West Maui, *Hobdy 859* (Holotype: BISH! [1005814]).

Trees 2–4 m tall, young stems and apical buds pubescent. Leaves oblanceolate to obovate; petioles, apical buds and lower leaf surfaces sparsely puberulent, especially along lower 1/3–1/2 portion of midrib on young leaves, margins entire or slightly crenate/undulate, petioles 1/7–1/4 of total leaf length. Heads on stout puberulent peduncles 8–13 mm long; involucre in 6–7 series, dusty pink at anthesis, inner bracts 2.7–2.9 cm; middle bracts 4–5 cm wide. Corollas 2.5–3 cm long; pappus pale pink to light brown. Occurring occasionally in wet forests on West Maui; one population on the Olokuʻi sea cliffs on Molokaʻi; extirpated from Lanaʻi.

Hesperomannia lydgatei **Forbes**

Hesperomania lydgatei C. Forbes, Bernice P. Bishop Mus. Occas. Paper 4: 220, 1909. TYPE: Wahiawa Mts., Kauai, *Lydgate s. n.*, May 1908 (Holotype: BISH-1005813!).

Small trees 2–3 m tall. Leaves and young stems glabrous; leaf blades obovate-elliptic to broadly oblanceolate; margins entire; petiole 1/10–1/7 of total leaf length. Heads on narrow glabrous peduncles, 2.3–4 cm long, nodding at anthesis; involucre in 4–5 series, white to pink or brown at anthesis, inner bracts 3.7–4.5 cm long; middle bracts 2.6–3.4 cm wide. Corollas 2.3–2.5 cm long; pappus pink to light brown. Rare in wet forest, Kauaʻi.

Hesperomannia oahuensis (Hillebrand) Degener

Hesperomannia arborescens ssp. *oahuensis* Hillebrand, Flora Hawaiian Islands, 232, 1888. TYPE: Puakea, Mt. Ka‘ala, O‘ahu, *Wawra s. n.* (Lectotype: B [destroyed]); Makaleka, Mt. Ka‘ala, Oahu, *Lydgate sn.* (Syntype: BISH-1005805!); *Hesperomannia oahuensis* (Hillebrand) Degener, Flora Hawaiiensis, 1938; *Hesperomannia arbuscula* ssp. *oahuensis* (Hillebrand) Carlquist, Pacific Science 11: 213, 1957. (See discussion by St. John [1978] on effective lectotypification by Degener.)

Hesperomannia arbuscula var. *pearsallii* St. John, Phytologia 40: 241, 1978. TYPE: Southern Wai‘anae, O‘ahu, *Pearsall 500* (Holotype: BISH-1005804!).

Small, sprawling trees/shrubs 2–3 m tall, young branches and apical buds densely tomentose. Leaves ovate to elliptic-ovate, margins entire or dentate, petioles 1/4–1/3 of total leaf length, densely tomentose on lower surface, upper surface tomentose to sparsely pubescent. Heads on stout, puberulent peduncles, 6–8 mm long; involucre in 5–8 series, green at bottom and magenta at top at anthesis; inner bracts 2.3–2.5 cm long; middle bracts 3–3.5 cm wide. Corollas 1.3 cm long; pappus pink to light purple. Highly endangered, restricted to mesic forests in the Wai‘anae Mountain Range of O‘ahu.

Hesperomannia swezeyi Degener

Hesperomannia swezeyi Degener, Flora Hawaiiensis, 1933. TYPE: Pupukea-Kahuku region on Kahuku side, O‘ahu, in rainforest at crest just south of trail, *O. Degener & O. Swezey 4398* (Holotype: BISH-1005815!; Isotypes: B [destroyed], NY-00007532); *Hesperomannia arborescens* ssp. *swezeyi* (Degener) Carlquist, Pacific Science 11: 214, 1957.

Hesperomannia bushiana Degener, Flora Hawaiiensis,1933. TYPE: Along crest of middle Halawa Ridge about 2.5 mi. above Makai boundary of Forest Reserve, O‘ahu, *O. Degener, W. Bush, C. Potter, K. Park 9981* (Holotype: BISH-1005810!; Isotype: B 10 0088463 [3 sheets; ex Gray Herb], BISH-1005811!, M-0031144, MICH-1107453!, NY-00007529 (2 sheets), WIS-0256899WIS); *Hesperomannia arborescens* ssp. *bushiana* (Degener) Carlquist, Pacific Science 11: 214, 1957.

Hesperomannia bushiana var. *fosbergii* Degener, Flora Hawaiiensis, 1933. TYPE: Kalawao Ridge Koolau Mts., O‘ahu, Alt. 540 m., *Fosberg 9470* (Holotype: BISH-1005812!; Isotype: NY-00007531).

Trees 2–5 m tall, young stems and apical buds pubescent. Leaves broadly oblanceolate to obovate, sometimes elliptic (in *H. bushiana* specimens), both leaf surfaces glabrous or nearly so with lower leaf surface of young leaves sometimes being sparsely pubescent along 1/2–1/3 of midrib, margins entire or sometimes crenate, petioles 1/8–1/7 of total leaf length. Heads on stout sparsely puberulent peduncles 4–6 mm long; involucre in 5–8 series, magenta at anthesis, inner bracts 2.7–3.0 cm long; middle bracts 3.0–3.5 cm wide. Corollas 2.0 cm long; pappus pink. Occurs in wet forest, mainly on the leeward side of the Ko‘olau Mountain Range, O‘ahu. One population was recently documented as extirpated from the windward Wai‘anae Range, O‘ahu.

DISCUSSION

Colonization of Hawai‘i

The evidence presented here does not support previous theories regarding the biogeography of *Hesperomannia* in Hawai‘i. There is no evidence that there were two distinct lines of colonization from Kaua‘i to Maui Nui, but contrarily each island group (Kaua‘i, O‘ahu,

and Maui Nui) is genetically distinct. Evidence suggests that following its arrival to the archipelago there was an apparent rapid colonization to each of the island groups (as evidenced by weak or lacking resolution among island clusters in the phylogeny) followed by isolated evolution occurring therein. The direction for this colonization among islands is not clear based on the sequence analysis. The progression rule model (Hennig 1966, Funk and Wagner 1995b) would suggest initial colonization occurred to the oldest of the islands, Kaua‘i, and then to subsequent islands as they emerged from the ocean and appropriate habitat became available. Although this cannot be discounted, the lack of resolution for dispersal among the islands based on sequence analysis suggests it is more likely that all islands groups were present at the time of colonization and that each was colonized nearly simultaneously. Interestingly, Carlquist (1957) examined morphological and anatomical features of these species and found that plants from Maui Nui and Oahu possess more primitive characteristics (leaf and peduncle trichomes present, median veins in corolla lobes present, and thick regions of subhypodermal fibers in the involucral bracts) as compared to the advanced traits in Kaua‘i’s *H. lydgatei* (leaves and peduncle glabrous, median veins of corolla lobes absent, and fibers in involucral bracts that never form a continuous band). This, too, suggests that all islands were present when the initial colonization took place and further postulates that dispersal among the islands was from Oahu and/or Maui Nui to Kauai.

Colonization among the islands from Maui Nui toward Kauai or rapid colonization across all islands as phylogenetic and anatomical analyses suggest necessitates reevaluation of when these events occurred. Previous studies indicated that the divergence of *Hesperomannia* from its most recent common ancestor occurred ca. 17 MYA (Kim et al. 1998, Keeley et al. 2007). However, geologic evidence indicates that the emergence of Maui Nui only occurred within the last 2.3 MY (Price and Elliot-Fisk 2004) implying that the colonization may have been more

recent than previously thought. Although the most recent common ancestors of *Hesperomannia* (*Gymnanthemum amigdalinum* and *Vernonia humbloti*) are from Africa, it is unlikely that the colonization from Africa to Hawai‘i occurred in a single step. Given the long time interval from their divergence (17 MY) and possibly a much more recent colonization (as recent as 2.3 MY), it is more probable that a stepping stone colonization to Hawai‘i occurred (most likely from Africa across Southeast Asia) with the intermediate species involved having gone extinct during the interim. This possibility seems all the more probable as both ancestral species have an east African distribution and *G. amigdalinum* extends into the Asian region of Yemen (Turrill et al. 1952).

Interisland Dispersal Models

The size and relative association of the islands was very different during the past 2.3 MY relative to the present day islands and may give insight into the colonization and subsequent dispersal of *Hesperomannia*. Kaua‘i, although taller in the past 3 MY, was approximately the same size (ca. 1400 km^2) as it is now (Carson and Clague 1995). However, O‘ahu and Maui Nui underwent dramatic alterations having been connected by two separate land bridges between 2.1 and 2.3 MYA (Carson and Clague 1995, Price and Elliot-Fisk 2004). The connection of O‘ahu with Penguin Bank (a now-submerged shield volcano west of Moloka‘i and once part of Maui Nui) was probably 500 m elevation at its highest point ca 2.2 MYA. The connection between O‘ahu and west Moloka‘i also existed and formed a broad plain probably only 200 m elevation at its maximum ca. 2.0 MYA. Although this landmass was quite large (estimated at ca. 7000 km^2), the bridges connecting Maui Nui to O‘ahu was brief, probably lasting only ca. 0.3 MY (Price and Elliott-Fisk 2004). From 2.2 to 1.8 MYA, east Moloka‘i continued to build to its maximum size (ca. 3000 m), the west Maui and Lana‘i shield volcanoes were developing, and the developing

Maui Nui had increased to over 5000 km^2 in size. With the eventual development of east Maui, Maui Nui reached a maximum size of over 14,000 km^2 ca. 1.2 MY ago (Price and Elliott-Fisk 2004).

Long distance dispersal must have accounted for the initial colonization of the ancestor of *Hesperomannia*. There has never been any evidence of *Hesperomannia* on East Maui, and as such it is likely that dispersal to Maui Nui occurred prior to its development (i.e., more than 1.5 MYA). Since colonization is likely to have occurred when all three islands were present, Maui Nui or Oʻahu Nui (Oʻahu and Molokaʻi when contiguous; Price and Elliott-Fisk 2004) would have been the largest landmasses for the ancestor of *Hesperomannia* to disperse to, and initial colonization is likely to have been on either of these islands. Once established, long distance dispersal must also be accounted for interisland dispersal (Oʻahu/Maui Nui to Kauaʻi or Kauaʻi to Oʻahu/Maui Nui) given that these islands are widely separated and have never been contiguous.

It is probable that vicariance, rather than long distance dispersal, accounts for the distribution of *Hesperomannia* species on Oʻahu and the islands of Maui Nui. Vicariance among these islands has been discussed before (Cowie and Holland 2006, Holland and Cowie 2006, Nelson 2006) although no plant examples have been promoted previously. The Koʻolau Mountains were linked with Molokaʻi (Carson and Clague 1995, Price and Elliott-Fisk 2004), and dispersal across the land bridge may have occurred. Subsequent loss of this land bridge would have served to genetically isolate these two lineages. Such a scenario would account for the morphological similarity of *H. swezeyi* (Koʻolau Mtn) to *H. arborescens* (Maui Nui). However, the Oʻahu-Molokaʻi land bridge was of low elevation (200 m), and likely did not provide suitable habitat for *Hespermannia* species that require a cooler and moister climate afforded at higher elevations. Further, *H. swezeyi* is most genetically similar to *H. oahuensis*

(Wai‘anae Mountains), and the O‘ahu species were both more similar to *H. lydgatei* (Kaua‘i) based on RAPD analysis. As such, a long-distance dispersal model is more probable to account for colonization from O‘ahu to Maui Nui, or vice-versa.

Much more likely examples of vicariance are afforded by the land bridge between Ko‘olau and Wai‘anae Mountains on O‘ahu and the land bridges between Moloka‘i, Lana‘i and west Maui. The connecting lands between the two O‘ahu volcanoes were much higher in the past (Price and Elliott-Fisk 2004), and this region was undoubtedly cooler and moister; estimates of island subsidence for O‘ahu are 1200 m or more (J. Price, pers. comm.), which would have altered the climate in this region tremendously. Even considering elevation of this region at the present level, glacial periods were far wetter in the mid-elevations of the islands (Gavenda 1992) and habitats on the separate mountain ranges would have been connected until transitions to interglacial periods caused them to become dry and separated once again. It is therefore likely that *Hesperomannia* populations were continuous from the Wai‘anae to Ko‘olau ranges at various times and became separated as erosion and island subsidence or climate change altered the ecology of the intervening lands. The presence of *H. swezeyi* at Palikea Gulch of the Wai‘anae Mountains may have been a remnant from a recent glacial episode that ecologically connected these ranges.

The minimum elevation of the land bridges among Maui Nui volcanoes is estimated to have been 1300 m during their early development (Carson and Clague 1995), and would have undoubtedly provided suitable habitat for dispersal among the separate volcanoes. As on O‘ahu, the bridging lands of Maui Nui disappeared as island erosion and subsidence occurred. Complete separation of each of the islands has occurred only within the last 0.4-0.6 MY (Price and Elliott-Fisk 2004). As this proceeded, Maui Nui first separated into two islands (Moloka‘i/Lana‘i and Maui/Kaho‘olowe) followed ultimately by the four islands as they are now recognized (Carson

and Clague 1995). It is important to note that the past circumscriptions of *Hesperomannia* species from Maui Nui included *H. arborescens* being restricted to Lanaʻi and Molokaʻi, and *H. arbuscula* restricted to west Maui (Wagner et al. 1990). Although we could make no genetic distinctions between these populations, there were subtle morphological differences among them that were recognized by past taxonomists (ie, their separate classification as *H. arborescens* and *H. arbuscula sensu* Wagner et al. 1990 and others) and are consistent with a vicariant model of differentiation.

Conservation Issues

All four species of *Hesperomannia* are presently rare and federally listed as endangered. The most abundant species is *H. lydgatei* with fewer than 200 individuals localized to one geographic area in South Kauaʻi. This is followed closely by *H. swezeyi* with approximately 150 individuals scattered in the Oʻahu Koʻolau Mountains, and *H. arborescens* on W. Maui and Molokaʻi (extirpated on Lanaʻi) with approximately 130 individuals among eight populations. Regeneration among populations of these three species is being observed. By far, the most critically rare species is *H. oahuensis*, where there are only 12 wild individuals in four populations, fewer than were sampled when this study was initially undertaken and with no *in situ* regeneration being observed. In light of the extreme rarity of all four species, the classification scheme presented here has already aided in conservation management decisions. For instance, great progress in the species conservation has already been made through hand pollination of *H. oahuensis* (Kawelo et al. 2011), and this research aided in the decision to cross pollinate among populations of the species as delimited herein. Furthermore, delineating species geographic boundaries is helping land managers to prioritize actions for the most critical populations.

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TABLE 1. Individuals of *Hesperomannia* sampled for RAPD and ITS analyses.

	Pop N[a]	N[a]	Herbarium Accession[b]	HPDL[c]	Correct Name[d]	Genbank Accession No.
Hesperomannia arbuscula						
O`ahu, Wai`anae Mts.						
Makaha	15	15	415770	2752-2764, 2952, 2975	*H. oahuensis*	JX444458
Palawai	9	7	none	3877-3883	*H. oahuensis*	JX444459
Waiʻanae Kai	11	11	514146	1960-1968, 2669, 3914	*H. oahuensis*	JX444457
W. Maui						
Honokōhau Valley	28	21	646652	2813-2833	*H. arborescens*	JX444452
ʻIao Valley	3	3	508235, 581758	3911-3913	*H. arborescens*	JX444454
Waiheʻe Valley	18	17	728623	3860-3876	*H. arborescens*	JX444453
Hesperomannia arborescens						
Molokaʻi						
Olokui Sea Cliffs	30	27	599373	3884-3910	*H. arborescens*	JX444455
O`ahu, Koolau Mts.						
Halawa Ridge	10	1	500948, 500949	2768	*H. swezeyi*	JX444462
Kaukonahua	200	25	501461	2901-2923, 3915, 3916	*H. swezeyi*	JX444464, JX444465
Kawaiʻiki	35	8	none	2924-2931	*H. swezeyi*	JX444463
Kawailoa Trail	1	1	none	2666	*H. swezeyi*	
Peahinaia	5	3	none	2976-2978	*H. swezeyi*	JX444460
Poamoho	3	3	413625	2665, 3919-3920	*H. swezeyi*	JX444461, JX444466
Oahu, Waianae Mts.						
Hesperomannia lydgatei						
Kauai						
Wahiawa / Kanaele Bog	61	61	500951	3591-3651	*H. lydgatei*	EF155777, JX444456
Outgroup Taxa Examined						
Baccharoides adoensis (Sch. Bip. ex Walp.) H. Rob (Africa-cultivated)						EF155745
Gymnanthemum amygdalinum Sch. Bip. ex Walp. (Africa)						AY504695
Linzia gerberiformis (Oliv. and Hiem.) H. Rob (Zimbabwe)						EF155752
Linzia melleri (Oliv. and Hiem.) H. Rob (Burundi)						EF155792
Linzia melleri (Oliv. and Hiem.) H. Rob (Malawi)						EF155790
Orbivestus cinarescens (Sch.Bip.) H. Rob (South Africa)						EF155794
Vernonia abyssinica Sch. Bip. ex Walp. (Ethiopia)						EF155805
Vernonia brachycalyx O. Hoffm. (Uganda)						EF155809
Vernonia humbloti Drake (Madagascar)						EF155819
Vernoniastrum nestor (S. Moore) H. Rob (Malawi)						EF155804

[a] Estimated population size (Pop N) and the number of individuals sampled (N) are given; population size estimates are from the date of collection and not its current status.

[b] Accessions representative of plant populations collected for this study, when available, and deposited at BISH. Specimen vouchers were not made during the course of this study; permits were granted only for DNA samples because of health and/or size of the population. Some sampled individuals from these populations have died during the course of this study without further regeneration.

[c] Accession numbers in the Hawaiian Plant DNA Library (Morden et al. 1996; Randall and Morden 1999).

[d] Species name that should be applied to plants in these populations based on results of this study. Outgroup Taxa from Keeley et al. (2007).

TABLE 2

Primer, nucleotide sequence, number of scored markers per primer and size range of scored markers (kb) used for genetic variability of *Hesperomannia*.

Primer	*Primer Sequence*	*# Scored Markers*	*Range of Scored Markers (kb)*
OPA-07	GAAACGGGTG	12	420-1500
OPA-09	GGGTAACGCC	16	400-1960
OPA-10	GTGATCGCAG	33	400-1200
OPA-12	TCGGCGATAG	28	600-2500
OPB-05	TGCGCCCTTC	23	650-2500
OPB-10	CTGCTGGGAC	24	490-2200
OPB-11	CTAGACCCGT	15	550-1900
OPB-18	CCACAGCAGT	35	350-2200
OPC-02	GTGAGGCGTC	16	420-2200
	Average # markers	**22**	

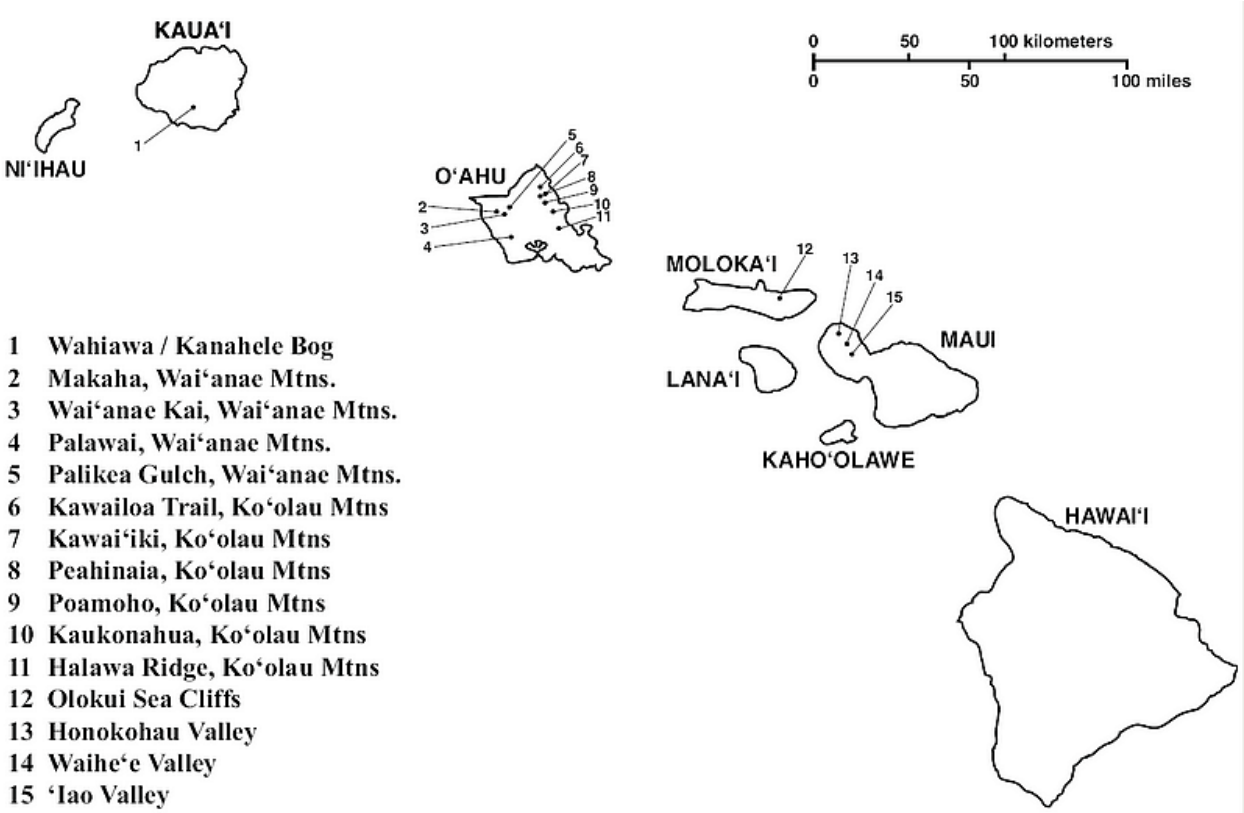

FIGURE 1. Map of the Hawaiian Islands with locations of populations sampled. See Table 1 for additional details.

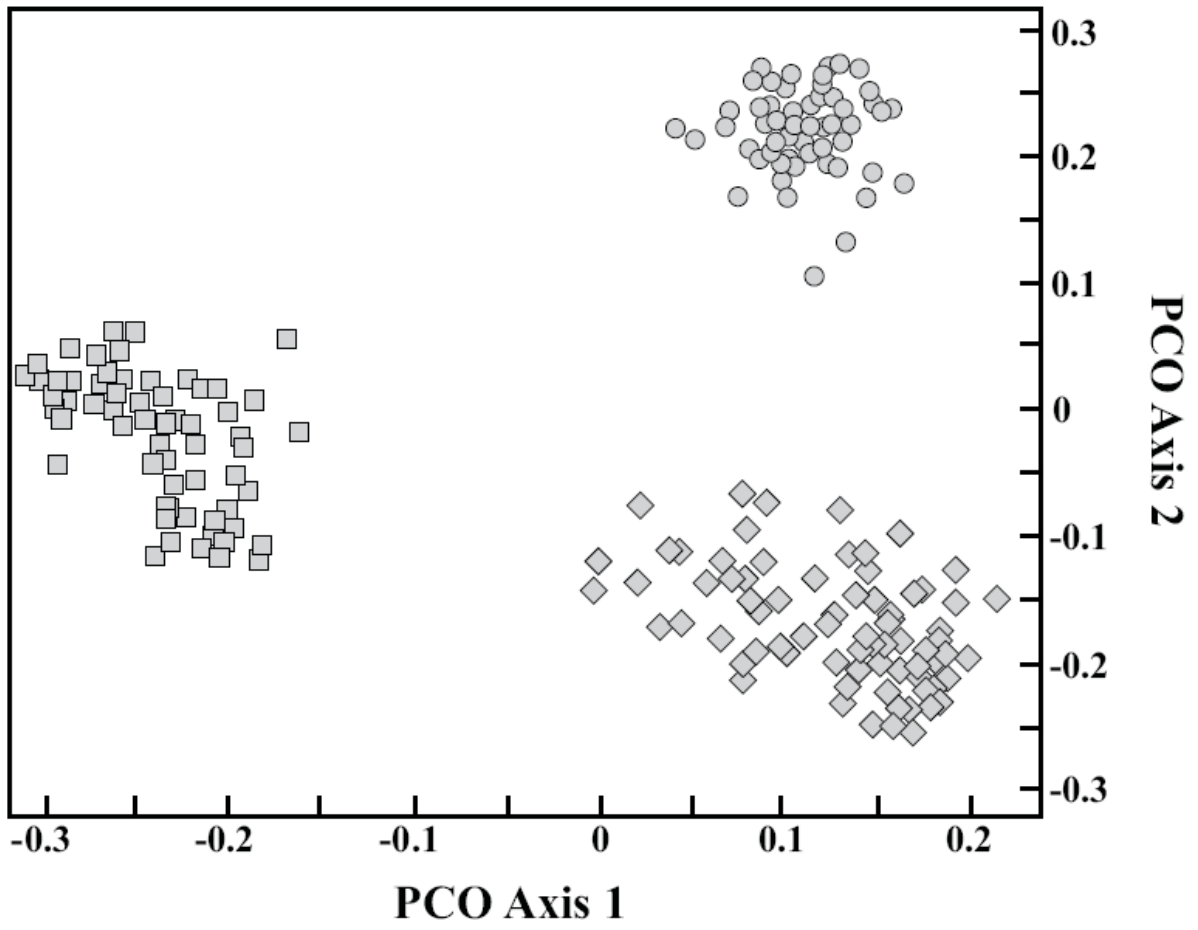

FIGURE 2. Principal coordinates analysis of all individual of *Hesperomannia* from populations on each of the three island groups based on RAPD data. The first (horizontal) axis represents 15% of the total variation and the second (vertical) axes represents 13% of the variation. Squares: Maui Nui individuals; circles: Kaua‘i individuals; diamonds: O‘ahu individuals.

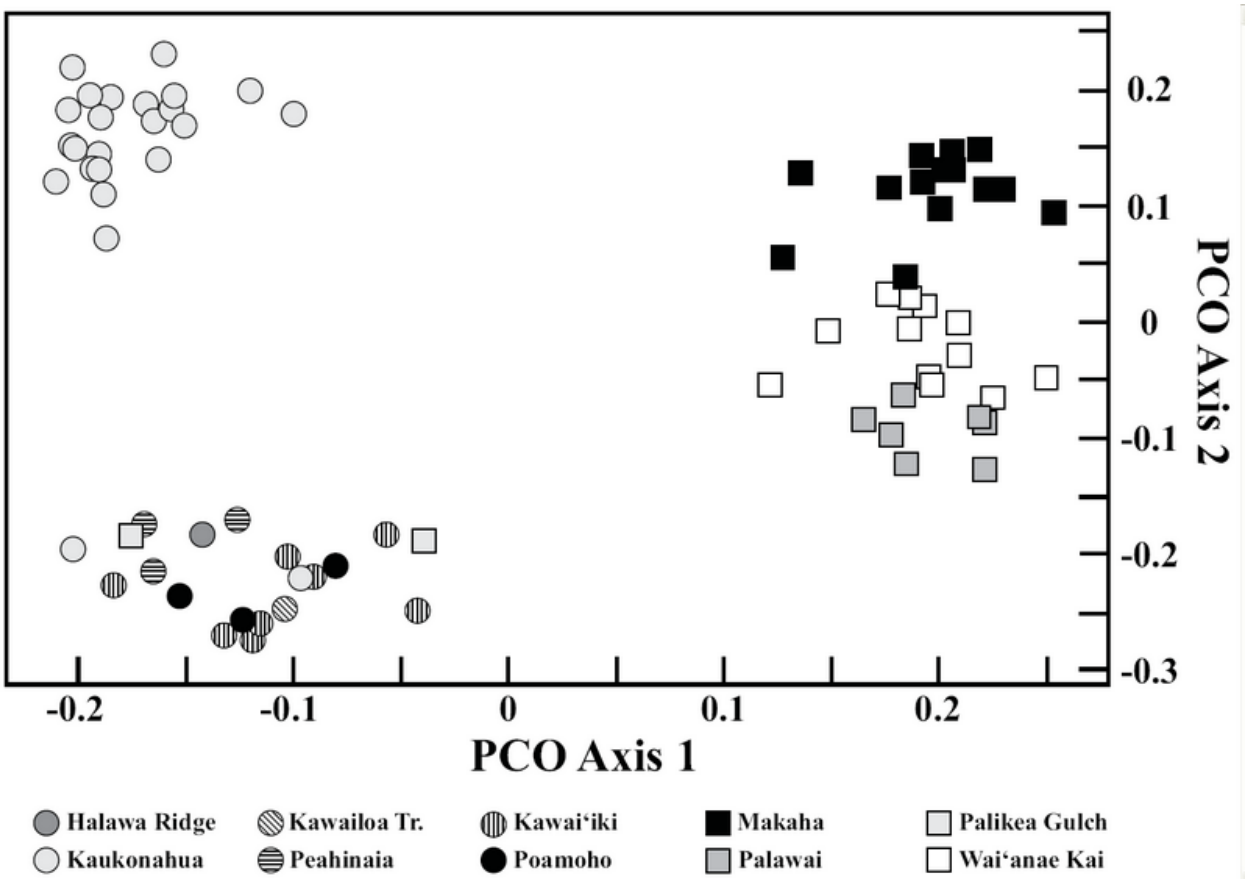

FIGURE 3. Principal coordinates analysis of O'ahu individuals of *Hesperomannia* from populations in the Ko'olau (circles) and Wai'anae Mountains (squares) based on RAPD data. The first (horizontal) axis accounts 15.3% of the total variation and the second (vertical) axes accounts for 12.6% of the variation.

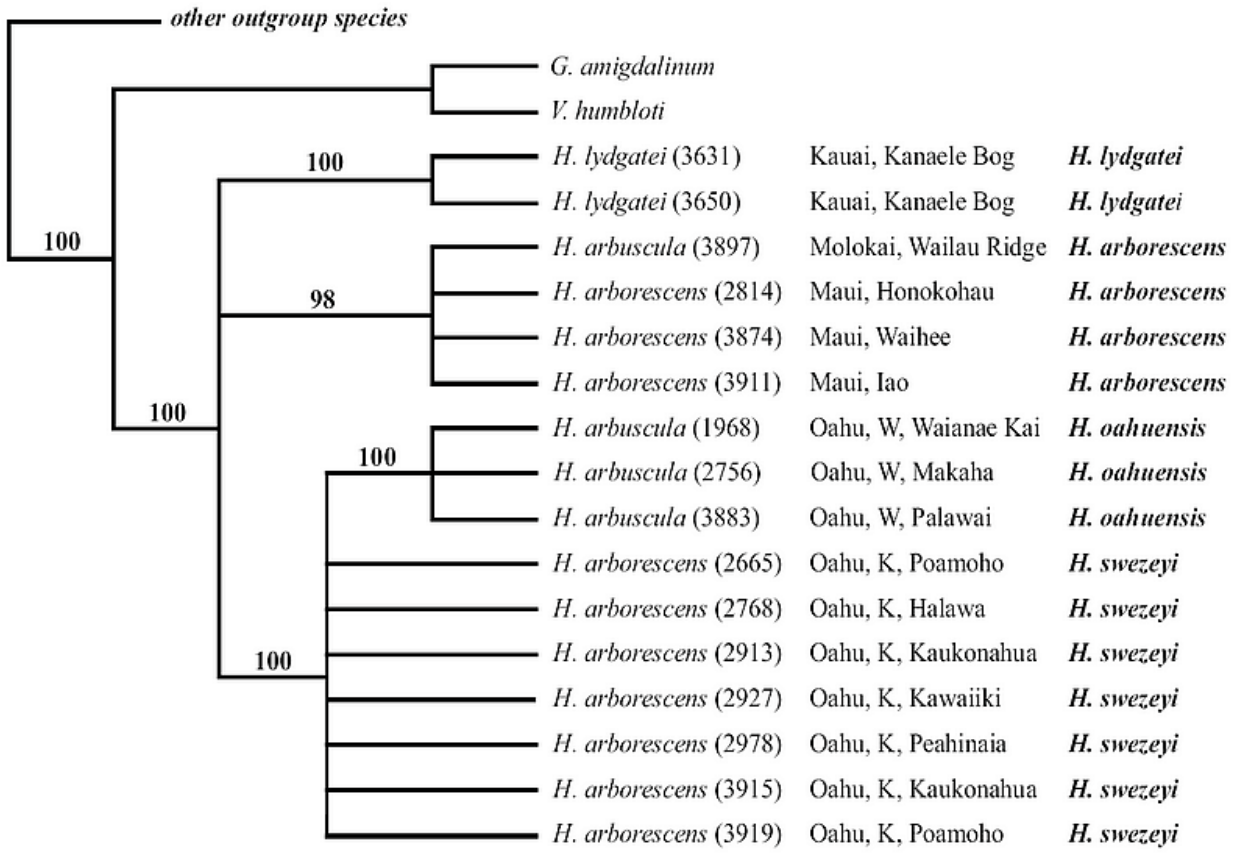

FIGURE 4. Phylogenetic analysis of *Hesperomannia* indicated by majority rule consensus tree of Bayesian analysis. Species names as known prior to this study, HPDL accession (in parentheses), and location of collection are adjacent to tree branches (K=Ko‘olau Mountains; W=Wai‘anae Mountains). Correct name to be used based on this study on right and in bold.

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APPENDIX 1

Specimens examined for morphological variation among species of *Hesperomannia*. Taxon, collector, collection number, herbarium, and herbarium accession number if available are provided.

***Hesperomannia arborescens* A. Gray:** *D. Forbes 322.2* (BISH-75449), *J. Lau 3231* (BISH-581759), *W. Hillebrand s. n.* (BISH-75453), *Hillebrand & Lydgate s. n.* (BISH-75454), *S. Meidell & H. Oppenheimer 126* (BISH-646562), *S. Meidell & H. Oppenheimer 141* (BISH-646579), *S. Montgomery s. n.* (BISH-413626), *S. Montgomery s. n.* (BISH-641412), *G. Munro 104* (BISH-75449), *G. Munro 492* (BISH-75457), *G. Munro 684* (BISH-75450), *G. Munro 1925* (BISH-75455), *H. Oppenheimer H90612* (BISH-728623), *S. Perlman* 10341 (BISH-599373), *K. Wood 6106* (BISH-650488).

***Hesperomannia lydgatei* Forbes:** *S. Carlquist s. n.* (HAW, HLA-7453), *C. H. Lamoureux 706* (HAW), *C. H. Lamoureux, T. Kato, F. Lamoureux 1513* (HAW), *S. Perlman 477* (BISH-427562, BISH-427484), *S. Perlman s. n.* (BISH-617601), *S. Perlman 12448* (BISH-622016), *R. Rice s. n.* (HAW), *H. U. Stauffer & R. Dehler 5912* (HAW-05632), *K. Wood & S. Perlman 12488* (BISH-612206).

***Hesperomannia oahuensis* (Hillebrand) Degener:** *S. Carlquist 1720* (BISH-24146), *S. Carlquist 1910* (BISH-24287), *G. Carr, J. Obata, & D. Palmer 985* (HAW), *D. Forbes 1591* (BISH-641413), *E. Funk 71* (HAW), *G. Gillett 1725* (HLA-334), *A. Gosline 108* (HAW-05535), *D. Herbst 1132* (BISH-457455), *D. Herbst 1416* (BISH-457494), *C. H. Lamoureux 1472* (HAW-00736), *K. Nagata 170* (HLA-334), *K. Nagata 818* (HLA 1582), *J. Obata 77-310* (BISH-

415770), *J. Obata 28008* (BISH-456130), *G. Pearsall s. n.* (HAW-00735), *S. Perlman 5466* (BISH-514146), *Tate & Takeuchi 2* (BISH-510785), *B. Stone 3293* (BISH-19249), *B. Stone 3450* (BISH-77768), *P. Welton 749* (BISH-631788).

Hesperomannia swezeyi **Degener:** *H. Akiyama s. n.* (BISH-754988), *B. Bishop s. n.* (BISH-75496), *O. Degener 7447* (BISH-75492), *O. Degener 10007* (BISH-76575), *O. Degener 10079* (BISH-75469), *R. Fosberg 9419* (BISH-75487), *Judd 1244* (BISH-10726), *S. Miyake 97* (BISH-19251), *K. Nagata & L. T. Gill 1443* (HLA-3776), *K. Nagata & R. Nagata 1201* (HLA-3591), *J. Obata 1952* (BISH-75503), *J. Obata & S. Perlman s. n.* (BISH-634281), *S. Perlman 6197* (BISH-616998, BISH-616999), *B. Stone 2788* (BISH-19250), *H. St. John 11547* (BISH-490661), *A. Suehiro s. n.* (BISH-75471), *O. H. Swezey s. n.* (BISH-75523), *J. Toba s. n.* (HAW-00738), *Topping 3285* (BISH-75504), *G. Webster 1588* (BISH-75479).