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Evolution on the Hawaiian Hotspot: Biogeography and Divergence Time Estimation of Kadua (Rubiaceae)

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EVOLUTION ON THE HAWAIIAN HOTSPOT: BIOGEOGRAPHY AND DIVERGENCE TIME ESTIMATION OF KADUA (RUBIACEAE)

by

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ABSTRACT

EVOLUTION ON THE HAWAIIAN HOTSPOT: BIOGEOGRAPHY AND DIVERGENCE TIME ESTIMATION OF KADUA (RUBIACEAE)

Kenneth L. Parker
Old Dominion University, 2012
Director: Dr. Timothy J. Motley

Recent studies on the colonization time of Hawaiian plant and animal lineages have suggested certain lineages arrived to the archipelago before the formation of the extant islands. *Kadua*, a recently resurrected genus, is mostly endemic to the Hawaiian Archipelago, with many member taxa autochthonous to individual islands. This work investigates the colonization time of *Kadua* to the Hawaiian Islands. Sequence analyses of the quickly evolving nuclear regions ITS, ETS and 5s-NTS regions for 20 Hawaiian and 7 French Polynesian species of *Kadua* were used to create dated phylogeny calibrated with island ages. The phylogeny suggests a single colonization of Hawaii around nine million years ago, with a subsequent dispersal from Hawaii to French Polynesia. *Kadua* appears to have colonized Hawaii after Gardner Island formed, around fifteen million years ago. Gardner Island was the largest of the Hawaiian Islands before the extant islands began forming with Kaua`i, around five million years ago.

ACKNOWLEDGMENTS

I extend my thanks to the people who contributed to the success of this Thesis. My advisor, Dr. Timothy J. Motley provided support and guidance throughout my research and while editing this manuscript, and deserves special recognition. My committee gave insight and revisions on my research and writing. My parents provided support throughout graduate school. Also, fellow lab members assisted with lab procedures and computer analysis.

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INTRODUCTION

Kadua

Kadua(Rubiaceae) is a recently resurrected genus, circumscribing theformer species of *Hedyotis*occurring in Hawaii and French Polynesia(Terrell *et al.*, 2005). Twenty-one of the 28 total member taxa are endemic to the Hawaiian Archipelago; eight are single-island endemics. The remaining seven taxa are scattered throughout the Pacific islands of French Polynesia. Six of the seven French Polynesianspecies are single island endemics (*K. romanzoffiensis* is a widespread strand species).

Species of *Kadua* exhibit a variety of life forms, most commonly semi-woody perennials, but also woody shrubs and trees. In the tribe Spermacoceae and the genus *Hedyotis,*a large mostly Asian genus from which Kadua was segregated, the most common and ancestral species are herbaceous annuals;secondary woodiness is a derived characteristic(Lens *et al.*, 2009). Taxonomically, *Kadua* is divided into two subgenera, each with distinct fruit types. Subgenus *Gouldia* has berry-like fruits and contains four distinct species divided into two sections. Subgenus *Kadua* has capsular fruits and contains the remaining 24 taxa (see Fig 1), divided into six sections (Terrell *et al.*, 2005). Two French Polynesian sections, *Austrogouldia* and *Oceanica*, are included in subgenus *Kadua*. The fruit types (berry vs. capsule) each imply a different dispersal strategy. Birds carrying fruits between islands may reduce barriers to gene flow that are otherwise present for capsular fruited species, which simply drop their ripened seeds to the ground.

Hawaii

Hawaii is an archipelago situated on a hotspot in the middle of the Pacific Ocean. The volcanoes have been well-studied geologically, withK-Ar age estimates and historical elevational maximums(Clague, 1996). The 129 Hawaiian volcanoes are divided into two distinct ridges, the Emperor Seamounts (85 – 43.5 Ma) and the Hawaiian Ridge (42.5 Ma – present). Only the youngest volcanoes breach sea level, and only those lying atop the Hawaiian hotspot are still active. Historically, the Emperor Seamounts subsided and eroded below sea level before the volcanoes of the Hawaiian Ridge broke sea level, effectively wiping out the majority of terrestrial lineages in the archipelago.

Most Hawaiian lineages are restricted to the extant high islands, Kaua'i (5.1 Ma), O'ahu (3.7 Ma), Moloka'i (1.9 Ma), Maui (1.3 Ma), and Hawai'i (0.43 Ma) (Carson & Clague, 1995). Older volcanoes have been reduced to atolls or small pinnacles of rock, and are home to far fewer terrestrial taxa. As islands age, the species residing there must either disperse or risk extinction. Until the formation of Kauai (max 2600m above sea level), few islands grew much higher than 1000m above sea level before eroding, providing a very small target for colonization and an isolated location (Macarther & Wilson, 1967). Gardner Island (15 Ma), however, grew to over 2600m above sea level, making it the largest island, and most likely site of colonization in the Hawaiian chain before the formation of Kauai, around 5.1 million years ago.

Due to its geographic isolation, allopatric speciation has led to a rich endemic angiosperm flora in Hawaii, (Price & Clague, 2002). Furthermore, many Hawaiian taxa are restricted to single islands. These single-island endemics are assumed to be autochthonous, and therefore useful for calibrating nodes of phylogenies when performing divergence time estimation.

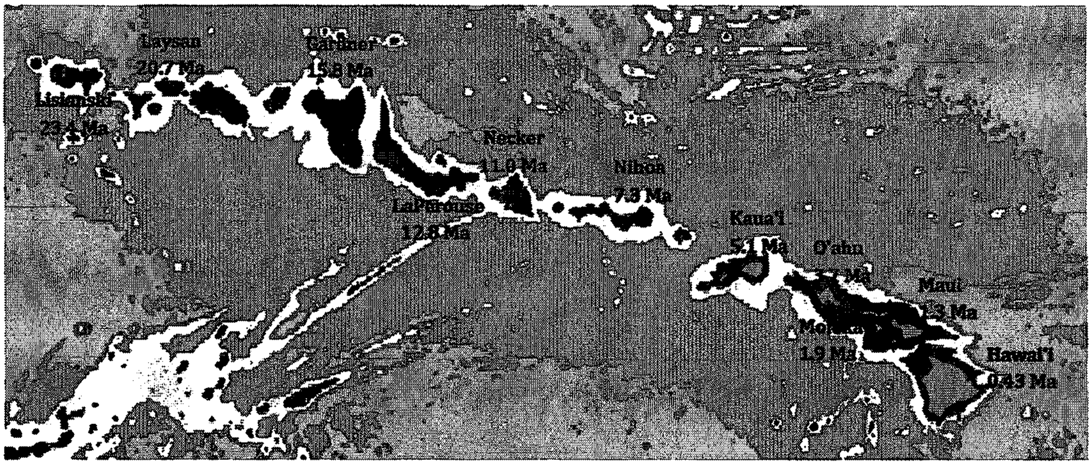

Figure 1. Map of Hawaiian Ridge. NOAA 1-minute GIS raster data layer. Current islands shown in gray. Black indicates ocean depth of 2 km, white indicates 4 km. Extant and submerged islands labeled with estimated age.

Time of Colonization

The time tree of the Rubiaceae has been determined to the tribal level byBremer & Eriksson(2009). The tribe Spermacocceae (containing *Kadua*) diverged around 28 Ma; therefore the age of *Kadua* should be younger than this date. Additionally, the phylogeny of the Spermacocceae has been investigated using chloroplast and nuclear markers (Kårehed *et al.*, 2008; Groeninckx *et al.*, 2009), but only includes a subset of the taxa within *Kadua*.

To estimate the age of the relatively young *Kadua* clade, we used methods put forth by Fleischer *et al.*(1998), which use the known K-Ar ages of the Hawaiian Islands to calibrate nodes of a phylogenetic tree where single-island endemic taxa diverge. This method has been used by others in the divergence time estimation of groups such as Hawaiian silverswords (Baldwin & Sanderson, 1998), lobeliads (Givnish *et al.*, 2009), and *Drosophila*(Russo *et al.*, 1995).

Most of the colonization events to the Hawaiian Archipelago appear to have occurred within the last 5 million years (Price & Clague, 2002). A few angiosperm lineages, however, have been present on the Hawaiian Island chain since before the emergence Kauai, such as *Lobelia*, *Rhus*, *Hesperomannia*, *Diellia*, and possibly *Hillebrandia*, although this is disputed(Kim *et al.*, 1998; Clement *et al.*, 2004; Schneider *et al.*, 2005; Givnish *et al.*, 2009; Harbaugh *et al.*, 2009). These recent findings suggest that Hawaiian biota may be much older than the extant islands; they provide some insight into the natural history of the archipelago and how often dispersal to the islands occurs.

Island colonization occurs in two steps, dispersal and establishment.Niche preemption limits colonization in most island systems, making it difficult for new arrivals to become established. Hawaii's extreme isolationmakes dispersal the limiting factor for the archipelago(Silvertown, 2004; Silvertown *et al.*, 2005).

The objectives of this study are to: 1) Create a phylogeny of *Kadua*; 2) Discuss the biogeographic patterns of the genus; and 3) Estimate when *Kadua* colonized the Hawaiian Islands.

MATERIALS AND METHODS

Taxon Sampling and Sequencing

In total we obtained 45 ITS sequences, 39 5S-NTS sequences, and 36 ETS sequences, representing 24 describedtaxa within *Kadua*, two undescribed taxa, as well as an outgroup(*Hedyotis foetida).*Outgroup selection was based on the large phylogenies of Spemacoceae(Kårehed *et al.*, 2008; Groeninckx *et al.*, 2009). The regions were easily amplified and aligned for most taxa, but sequences from some regionscould not be obtained from some samples, and additional material was unavailable for subsequent extractions. DNA was confirmed present in the sample using PCR (polymerase chain reaction), where the strongest bands produced when stained with Ethidium bromide were of equal length. Those regions that could not be obtained were entered as missing data in the matrix. For detailed information on taxa sampled, regions sequenced, and voucher specimens, see Table 1. All samples were collected under compliance of the state or governmental regulations issued to the individual collectors or tissue samples were obtained from herbarium material with permission from individual herbaria.

DNA was extracted from silica-gel dried material or herbarium specimens using the CTAB method (Doyle & Doyle, 1987).Polymerase Chain Reactions and DNA purification for ITS and 5S-NTS regions were performed as described by (Kårehed *et al.*, 2008). PCR for ETS was performed with 25-µl reactions, including 12.5 µl Taq mixture, 1.25 µl DMSO, 1 µl 3' primer (10 µM), 1 µl 5' primer (10 µM), 8 µl water, and 1 µl template DNA. Amplifications began with an initial denaturation phase at 94°C for 5 minutes, followed by 35 cycles of 30 s at 94°C, 30 s at 47°C, and 2 min at 72°C, with a final extension phase at 72°C for seven minutes. Primers used for both PCR and sequence reactions were for ITS, the forward primer P17 and the reverse primer 26S-82R (Popp & Oxelman, 2001) or P25(Oxelman, 1996). The 5S-NTS region was amplified using the primers PI and PII (Cox *et al.*, 1992). The primer pair ETS-Erit-F (Negron-Ortiz & Watson, 2002) and 18S-IGS(Baldwin & Markos, 1998) were used for ETS.

Table 1. Taxon sampling of *Kadua* in this study.

Name	Voucher[1]	Herbarium	Location
Subg. Gouldia			
Sect. Gouldia			
K. affinis DC	TM1733	NY	HI
	TM1751	NY	O
	VA1313	NY	M
	TM1759	NY	O
	TM1786	NY	O
	TM1766	NY	HI
K. fosbergii (W.L. Wagner & D.R. Herbst) W. L. Wagner & Lorence	TM1677	NY	O
	TM1757	NY	O
K. axillaris (Wawra) W. L. Wagner & Lorence	TM1724	NY	M

Table 1. Continued

Name	Voucher[1]	Museum	Location
Sect. Phyllozygia			
K. tryblium (D. R. Herbst & W. L. Wagner) W. L. Wagner & Lorence	CM474	BISH	K
Subg. Kadua			
Sect. Oceanica			
K. romanzoffiensis Cham & Schltdl	MHS2579	BISH	TU
Sect. Austrogouldia			
K. nukuhivensis (J. Florence & Lorence) W. L. Wagner & Lorence	KW6337	PTBG	MQ
K. tahuatensis (Lorence & J. Florence) W. L. Wagner & Lorence	SP15945	PTBG	MQ
K. rapensis subsp. *rapensis* F. Br.	TM2827	NY	RA
	SP17953	PTBG	RA
	SP18029	PTBG	RA
K. rapensis subsp. *taverna* F. Br.	SP17938	PTBG	RA
	SP18010	PTBG	RA
	SP17951	PTBG	RA
Sect. Protokadua			
K. coriacea (J. E. Smith) W. L. Wagner & Lorence	TM1702	NY	HI
	TM1703	NY	HI
	TM1704	NY	HI
Sect. Gouldiopsis			
K. centranthoides Hook. & Arn.	TM1739	NY	HI
	TM1737	NY	HI
	TM1979	NY	MO
K. foggiana (Fosberg) W. L. Wagner & Lorence	DL5324	BISH	K
K. knudsenii Hillebr.	SLMsn	BISH	K
K. formosa Hillebr.	TL5577	BISH	M
Sect. Kadua			
K. acuminata Cham. & Schltdl.	TM1781	NY	O
	TM1783	NY	O
	TM1694	NY	O
K. fluviatilis C. N. Forbes	NTBG 960163	PTBG	K
	TM1747	NY	O

Table 1. Continued

Name	Voucher[1]	Museum	Location
Sect. Wiegmannia			
K. cookiana Cham & Schltdl.	NTBG950509	PTBG	K
K. cordata Cham & schltdl.	TM1708	BISH	O
	NTBG8	PTBG[2]	K
K. cordata subsp. *remyi*	KN2524	NY	L
K. cordata subsp.*waimeae* (Wawra) W.L. Wagner & Lorence	TF1584	BISH	K
K. degeneri subsp. *degeneri* (Fosberg) W.L. Wagner & Lorence	KW5062	PTBG	O
	JL2015	BISH	O
K. degeneri subsp. *coprosmifolia*(Fosberg) W.L. Wagner & Lorence	LPsn	BISH	O
K. elatior (H. Mann) W.L. Wagner & Lorence	WLW6350	BISH	K
K. flynnii (W. L. Wagner & Lorence) W.L. Wagner & Lorence	SP15631	PTBG	K
K. littoralis Hillebr.	TM1778	NY	HI
	TM1780	NY	HI
	SPsn	PTBG	Mo
K. parvula A. Gray	NTBG950256	PTBG	O
K. laxiflora H. Mann	SP6647	PTBG	Mo
K. st.-johnii (B. C. Stone & Lane) W. L. Wagner & Lorence	KW5724	PTBG	K
K. haupuensis Lorence & W. L. Wagner	KW7492	PTBG	K

The names of included species are listed within established sections. Voucher number[1] and herbarium abbreviations, and island or archipelago of origin are listed. Abbreviations include: HI= Island of Hawaii, M=Maui, MO=Moloka'i, L=Lanai, O=O'ahu, K = Kaua'i, TU = Tupai Atoll, RA = Rapa, MQ=Marquesas.

[1] Abbreviations in voucher number refer to collector ID: KW = Kenneth Wood, TM = Timothy Motley, SP = Steve Perlman CM = Clifford Morden, JL = Joel Lau, LN = Leilani Pyle, NTBG = National Tropical Botanical Garden living collection, WLW = Warren Wagner, KN = Ken Nagata, TF = Timothy Flynn, VA Victor Albert, DL = David Lorence, MHS = Marie Sachet, SLM = Steven Montgomery.

[2] Living collection.

Phylogenetic Analysis

Sequences were aligned using MAFFT version 6 (Katoh & Toh, 2008), and then subsequently checked and aligned by eye, using Geneious (Drummond *et al.*, 2009)to visualize the sequences. To select models for the data matrix, jModeltest (Guindon & Gascuel, 2003) was used. Three substitution schemes were considered, with (+F) uneven base pair frequencies, invariable sites (+I), and four categories under the gamma distribution allowed. Akaike Information Criterion (AIC) was used to determine the best-fit model to the data set: GTR+I+G for ETS, ITS, and 5S-NTS. Bayesian analysis was performed using MrBayes v3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The algorithm was run for 100,000 generations, sampling every 1,000 generations, set to end when split frequencies fell below 0.01. Burn-in of the first 2,500 trees was performed. Each of the three regions was run separately to compare results, then combined in the final analysis.

The phylogeny obtained from the Bayesian analysis was used to determine taxon sets for the divergence time analysis. The dated phylogeny was estimated using BEAST 1.4.8 (Drummond & Rambaut, 2007). General Time Reverse was chosen as a substitution model, and an uncorrelated lognormal relaxed molecular clock model was used to estimate rate of divergence. Crown nodes of certain clades (represented by taxonomic sections) were then calibrated using the ages of volcanoes home to single-island endemics diverging early in the clade. For instance, the crown node of subgenus *Gouldia* is calibrated to 5.1±0.2 Ma, the age of Kauai, because the monotypic section *Phyllozygia* (*K. tryblium*) is endemic to Kauai and sister to section *Gouldia*. Additionally, the crown node of section *Wiegmannia* was calibrated at 3.7±0.1, the age of Oahu. Nodes were calibrated using a normal prior. To see the list of taxa included in each clade, please refer to Table 1. The algorithm was run for 1,000,000,000 generations, sampling every 100,000 trees. Log likelihoods of the resulting tree files were examined using Tracer v 1.5 (Rambaut & Drummond, 2007). The tree files were combined using TreeAnnotator, part of the BEAST software package, with a burn-in of 2500 trees, and viewed in FigTree v1.3.1. (Morariu *et al.*, 2008).

RESULTS

All phylogenies of the independent molecular regions 5S-NTS (Figure 2a), ETS (Figure 2b), and ITS (Figure 2c) produced similar topologies with regard to clade composition and taxonomic sections, although with less support and resolution than when the data were combined. All three regions provide a clear division between the two subgenera. Incongruence within *Protokadua* exists among all three phylogenies, especially with reference to *K. coriacea*. The sections within subgenus *Kadua* are supported in all three phylogenies, but the 5S-NTS tree offers the best resolution of relationships between the sections.

Kadua is a strongly-supported monophyletic group, with 100% posterior probability for the genus, as confirmed by Kårehed *et al.*(2008) and Groeninckx *et al.*(2009). Seed-based morphological groupings by Terrell *et al.*(2005) coincide with the clades found here, with a few differences. The French Polynesian sections,*Oceanica* and *Austrogrouldia*,are included in the same clade as *Kadua coriacea*(sect. *Protokadua*); there is strong support for thisclade. *Kadua st.-johnii* is included within section *Gouldiopsis* with high support, despite its previous placement within section *Wiegmannia*. Fleshy fruits evolved independently twice within *Kadua*, first in subgenus *Gouldia* and again in*K. romanzoffiensis* (subg. *Kadua* sect. *Protokadua*).

In the combined analysis (Figure 3), several individuals collected on different islands of the same species do not form monophyletic groups. *Kadua cordata* appears in several places throughout section *Wiegmannia*, therefore the species may require further study and revision. In section *Gouldia*, *K. affinis* and *K. fosbergii* are both paraphyletic. In the subclade containing *K. axillaris*, the sister individuals of*K. affinis* and *K. fosbergii* were both sampled from Oʻahu.*K. axillaris* does not occur on Oʻahu, but on the younger islands of Maui and Hawaiʻi.

Using the methodology of Fleischer *et al.* (1998), we used island age to calibrate crown nodes of the total evidence phylogeny (Figure 4). Two nodes were used for calibration, the crown node of subgenus *Gouldia* is calibrated to 5.1±0.2 Ma, the age of Kauaʻi, because the monotypic section *Phyllozygia* (*K. triblium*) is endemic to the island this sentence is a bit long!and is assumed to have diverged from sister section *Gouldia* at approximately that time. Additionally, the crown node of section *Wiegmannia* was calibrated at 3.7±0.1 Ma, the age of Oʻahu. This is the approximate time the Oʻahu endemic, *K. parvula*, is assumed to have diverged from the rest of the section. According to the island-age calibrated phylogeny, *Kadua* diverged from its outgroup, *Hedyotis foetida* around 9.2 million years ago (Figure 4, Table 2). This divergence time is interpreted as the colonization date of the Hawaiian Islands. Subsequent divergence within *Kadua* occurred around eight million years ago, separating subgenera*Kadua* from *Gouldia*(Figure 4, Table 2). If these results are overlaid on a map of the islands we are able to hypothesize the point of colonization and diversification on the archipelago. Although not shown in the figures, when this analysis is performed only using a single calibration point (Oʻahu), the divergence of section *Phyllozygia* from *Gouldia* occurs around 5.1 million years ago. Additionally, using a single calibration point results in a colonization time similar to that produced by using both calibration points.

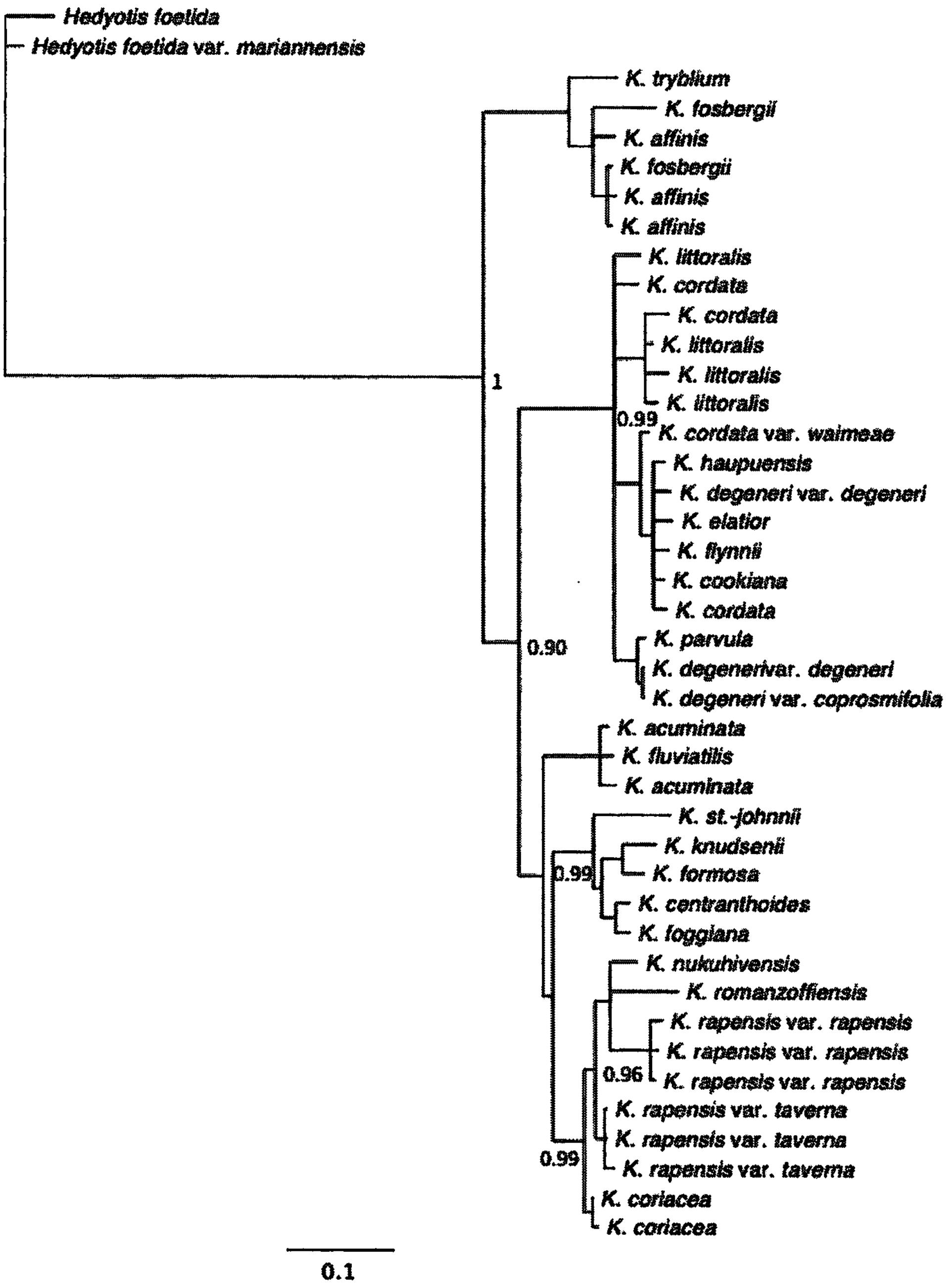

Figure 2A: 5S-NTS Phylogeny

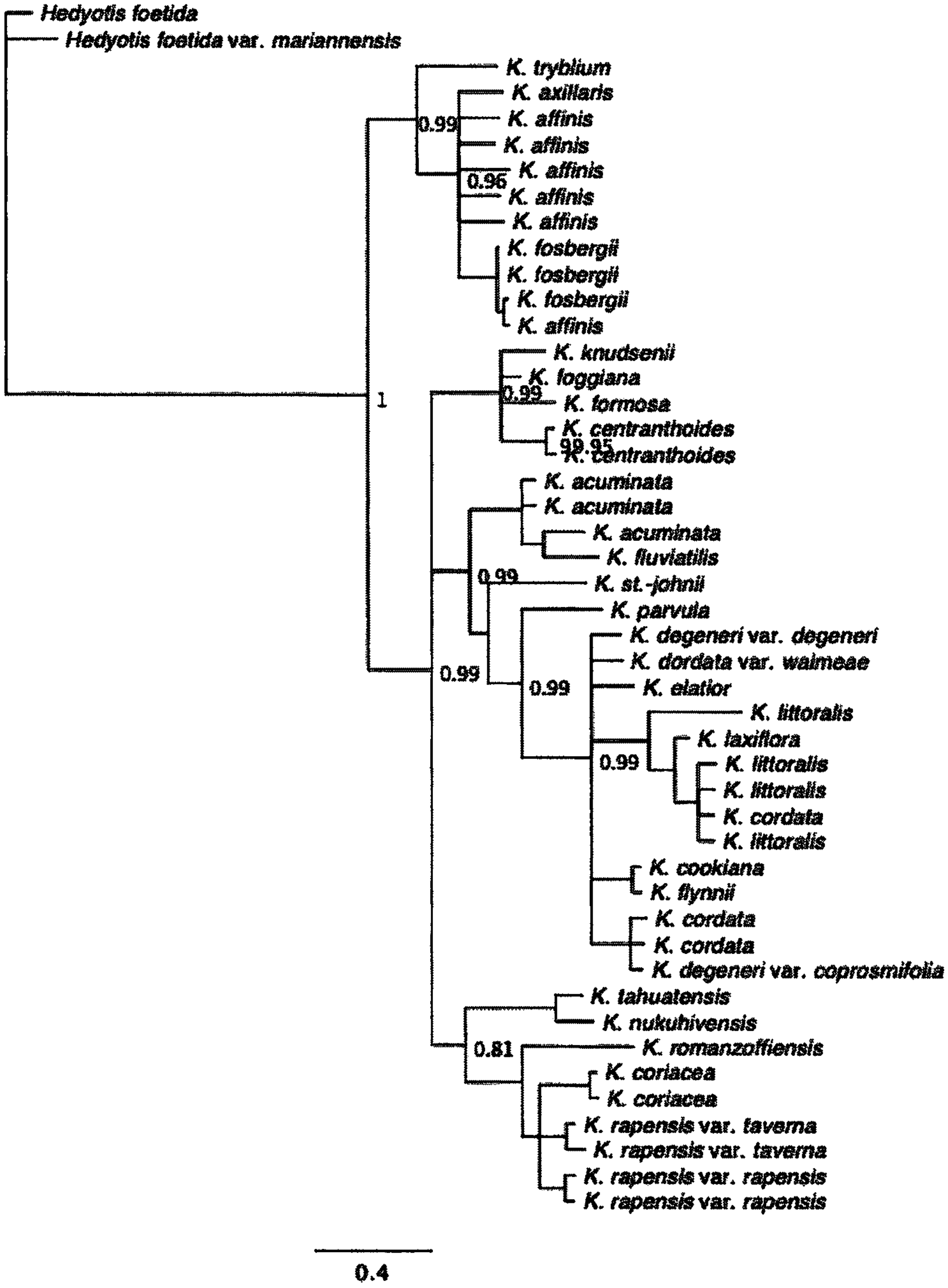

Figure 2B: ETS Phylogeny

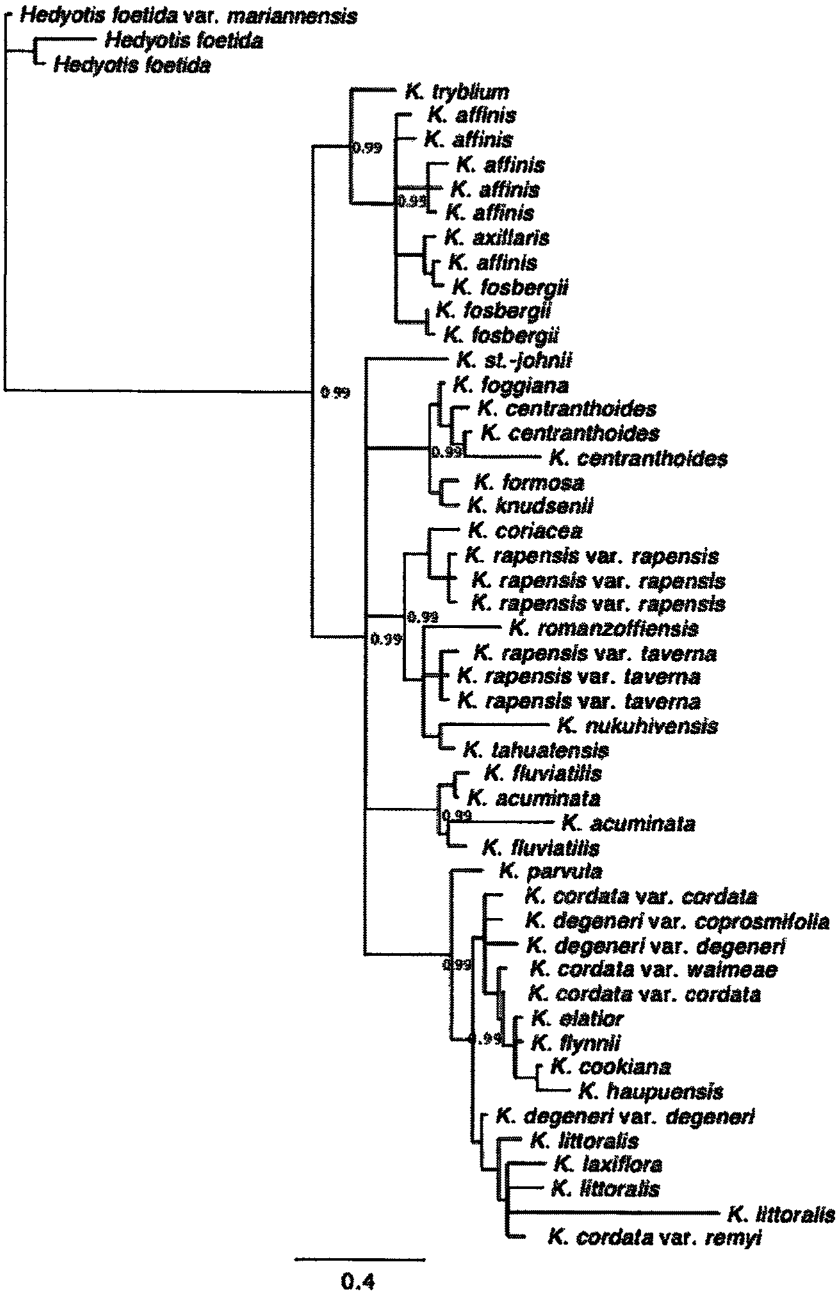

Figure 2C: ITS Phylogeny

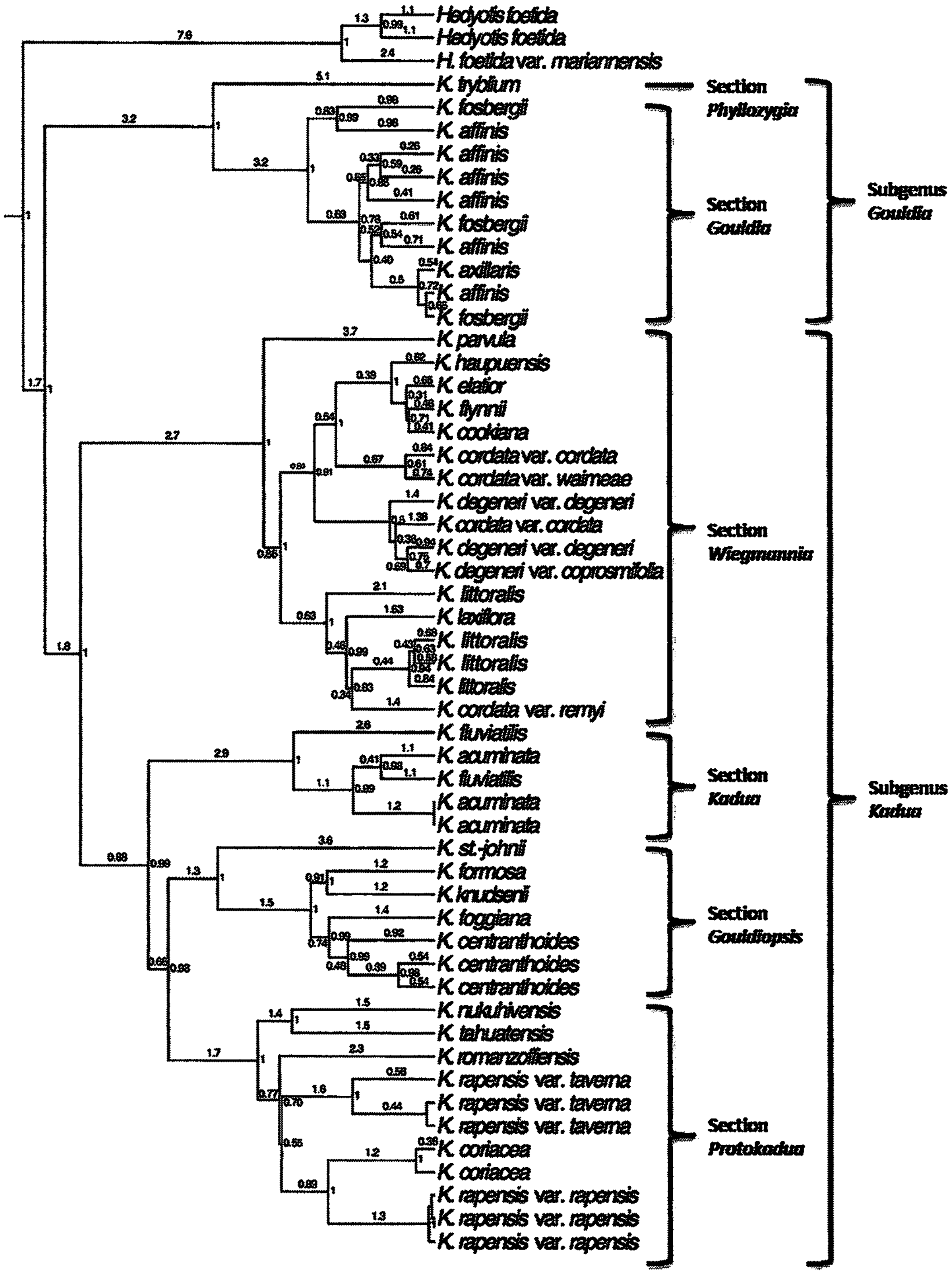

Figure 3. Combined ETS, ITS, and 5S-NTS phylogeny of *Kadua*. Nodes are labeled with posterior probability values. Branches labeled with branch lengths.

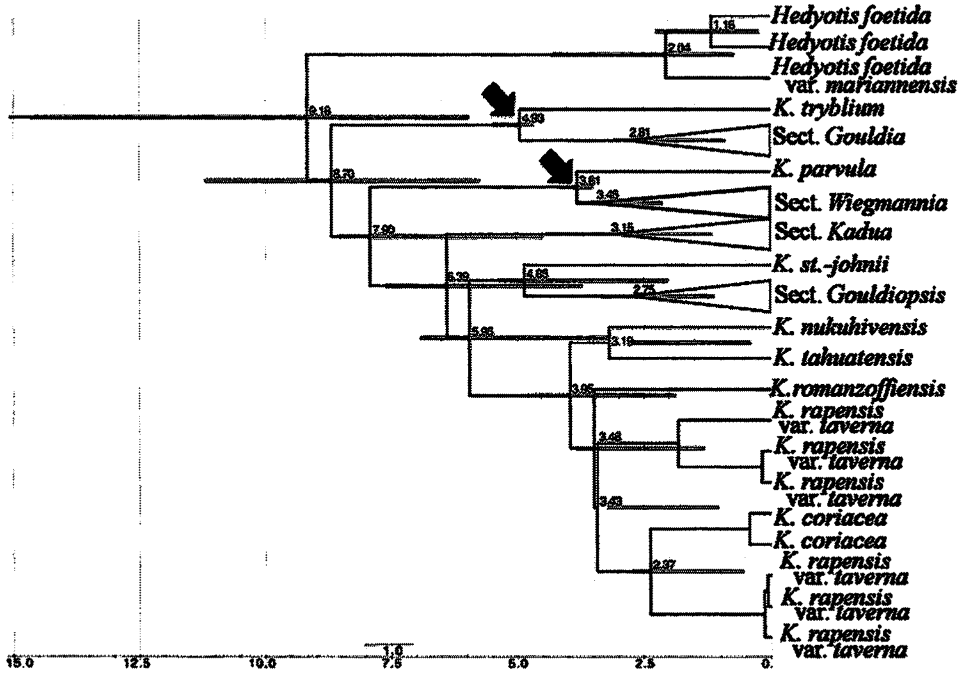

Figure 4. Dated phylogeny of *Kadua*. Divergence between *K. parvula* and the rest of *Wiegmannia* calibrated at 3.5 million years. Divergence between *K. tryblium* and sect. *Gouldia* calibrated at 5.1 million years. Arrows indicate calibration points.

Table 2. Ages of the major clades of *Kadua*. Lineage age refers to age of branch, crown node refers to date of divergence. 95% Highest Posterior Density of trees sampled in divergence time estimation analysis.

Clade	Lineage Age	95% HPD of Lineage Age	Crown Node Age	95% HPD of Node Age
Kadua	9.2	5.9-14.6	8.7	5.7-11.1
Subgenus *Gouldia*	8.7	5.7-11.1	4.9	4.7-5.5
Section *Phyllozygia*	4.9	4.7-5.5		
Section *Gouldia*	4.9	4.7-5.5	2.8	0.9-3.1
Subgenus *Kadua*	8.7	5.7-11.1	7.9	4.5-8.5
Section *Kadua*	6.4	3.7-7.6	3.1	1.1-4.4
Section *Protokadua*	5.9	3.1-6.9	4	1.9-4.8
Section *Wiegmannia*	7.9	4.5-8.5	3.8	3.5-3.9
Section *Gouldiopsis*	5.9	3.1-6.9	4.9	2.0-5.4

DISCUSSION

Colonization

Kadua has resulted from a single colonization of the Hawaiian Islands, with Gardner Island as the probable point of immigration. Gardner Island,formedaround 15.8 million years ago, was taller and larger than other Hawaiian Islands prior to the formation of Kauai, at approximately 5.1 million years ago; until then, Gardner was the only island to rise more than 2000 meters above sea level in the Hawaiian Islands. Because of its size,lineages arriving to the islands prior to five million years ago would have most likely colonized Gardner Island. The age of *Kadua* estimated here (9.2mya) makes it one of the oldest Hawaiian lineages to have been dated. This age for *Kadua* is well within the estimated age, 28.5 million years, for the entire Spermacoceae tribe (Bremer & Eriksson, 2009). In Hawaii, the only other organisms with proposed older colonizations include the fern genus *Diellia* (23mya)(Schneider *et al.*, 2005)and *Lobelia* (13.6mya) (Givnish *et al.*, 2009).

Diversification and Emigrations

The estimated 9.2 million year colonization age for *Kadua* provided time for the genus to diversify into more than twodozen species and emigrate from the island chain twice. The placement of non-Hawaiian *Kadua* within section *Protokadua* suggests an emigration event around six million years ago to French Polynesia, followed by a subsequent dispersal back to Hawaii by *Kadua coriacea* within the last 2.5 million years.The disparate placement between the two established varieties of *K. rapensis* suggests that these two lineages should be treated as separate species. Additionally, sections *Austrogouldia* and *Oceanica*, established by Terrel *et al.*(2005), need to be revised and should be combined with *K. coriacea* in section *Protokadua*.The phylogenetic placement of *Kaduast.-johnii* within *Goudiopsis* is inconsistent with its current inclusion in *Wiegmannia*.

The imbalance of species number between subgenera *Gouldia* and *Kadua* is likely attributable to the differences in fruit types. Seeds of fleshy fruits are carried more easily between islands and among similar habitats and mountain ranges by avian vectors. Alternatively, capsular fruits are dispersed far more randomly and require major climatic events to disperse long distances. Thus the berry-fruited species of*Gouldia*, dispersed by birds and restricted to high elevation rain forests, havefewer barriers to gene flow than do the capsular-fruited species, reliant on winds for dispersal that occur in a variety of habitats. The emigrations within *Protokadua* suggest that capsular fruits may be more successful at long-distance dispersal and establishment outside the archipelago. *Kadua romanzoffiensis*, the only berry-fruited member in subgenus Kadua, which was placed with section *Protokadua*is an example that indicates that the evolutionary switch between berry and capsular fruit is not difficult and has occurredtwice in *Kadua*.

Despite the 9.2 million year colonization age,much of the extant diversity within *Kadua* was generated during the formation of the modern islands. The first divergence, around eight million years ago, divided the genus into two subgenera. This divergence is likely associated with the switch from capsular to berry fruits in subgenus *Gouldia*. Subgenus *Kadua* appears to have not begun diversifying until around 5 million years ago, after the formation of Kauai. Apparent lack of diversity in the five million years following colonization is attributable to extinctions occurring as older islands have subsided. Taxa present on the older islands without the ability to disperse to younger islands were lost to erosion and subsidence of land masses(Price & Clague, 2002).

CONCLUSION

The divergence between *Kadua* and its sister clade, represented here by*Hedyotis foetida*, is estimated at around 9.2 million years ago. This indicates a colonization of Hawaii before the formation of the modern islands, making *Kadua* one of the older plant lineages in the islands today. The presence of *Kadua* and other plant lineages in Hawaii prior to the formation of the modern islands suggests a stepping stone dispersal pattern, whereby a lineage avoids extinction on a subsiding island by the ability to migrate successfully to a younger island. Methods to test dispersal patterns by Sanmartin *et al.*(2008) on islands could be employed to test this stepping stone hypothesis, but phylogenetic data from several other groups of organisms would be required. Likely other lineages, without dated phylogenies, also colonized the Hawaiian Islands before the modern islands formed. Resolution among taxa within some clades is poorly resolved in the phylogeny, such as section *Gouldia* and parts of *Wiegmannia*; this may be explained by lack of barriers to gene flow, resulting in inter-specific gene transfer. A population-level study may be needed to provide insight into the evolutionary history of these clades.

Calibrating dated phylogenies using the ages of islands with endemic species provides a useful alternative when no fossils are available. Phylogenies calibrated in this way are producing a new picture of the flora and fauna of the Hawaiian Islands. Lineages in Hawaii are forced to continually colonize younger islands, or face extinction as older islands subside. This constant pressure for dispersal results in emigration from the Hawaiian Archipelago. The emigration of *Kadua* from Hawaii to French Polynesia is unique, as Hawaii has historically been considered a dead-end for dispersal (Carlquist, 1974). Other lineages that have managed to emigrate from Hawaii, such as *Melicope*(Harbaugh *et al.*, 2009) and *Santalum*(Harbaugh & Baldwin, 2007), are thought to be bird-dispersed (Carlquist, 1967). The emigration of *Kadua* occurring with a capsular-fruited clade stands out, being associated with wind- rather than bird-dispersal. Additionally, the re-colonization of the Hawaiian Archipelago by *Kadua coriacea*may be the first example of a Hawaiian group returning to the islands after emigration.

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