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PHYLOGENETIC POSITION AND BIOGEOGRAPHY OF *HILLEBRANDIA SANDWICENSIS* (BEGONIACEAE): A RARE HAWAIIAN RELICT[1]

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The Begoniaceae consist of two genera, *Begonia*, with approximately 1400 species that are widely distributed in the tropics, and *Hillebrandia*, with one species that is endemic to the Hawaiian Islands and the only member of the family native to those islands. To help explain the history of *Hillebrandia* on the Hawaiian Archipelago, phylogenetic relationships of the Begoniaceae and the Cucurbitales were inferred using sequence data from 18S, *rbcL*, and ITS, and the minimal age of both *Begonia* and the Begoniaceae were indirectly estimated. The analyses strongly support the placement of *Hillebrandia* as the sister group to the rest of the Begoniaceae and indicate that the *Hillebrandia* lineage is at least 51–65 million years old, an age that predates the current Hawaiian Islands by about 20 million years. Evidence that *Hillebrandia sandwicensis* has survived on the Hawaiian Archipelago by island hopping from older, now denuded islands to younger, more mountainous islands is presented. Various scenarios for the origin of ancestor to *Hillebrandia* are considered. The geographic origin of source populations unfortunately remains obscure; however, we suggest a boreotropic or a Malesian–Pacific origin is most likely. *Hillebrandia* represents the first example in the well-studied Hawaiian flora of a relict genus.

Key words: *Begonia*; Begoniaceae; biogeography; divergence time; *Hillebrandia*; molecular phylogeny; paleoendemic; relict.

The Begoniaceae comprise a large, diverse clade of tropical and subtropical herbs, shrubs, and lianas. This family is morphologically well characterized, and family members are easily distinguished from related families by their mostly fleshy stems, stipulate, usually asymmetrical leaves, showy unisexual flowers with petal-like perianth segments and numerous centripetal stamens, and by their seeds that uniquely possess collar cells below an operculum. Botanists have traditionally found the Begoniaceae difficult to classify within larger taxonomic schemes because the species share obvious morphological features only with the Cucurbitaceae, Datiscaceae, and Tetramelaceae, a group of families considered to be morphologically isolated among flowering plants. Recent phylogenetic analyses of *rbcL*, 18S, and *atpB* sequence data, however, have provided new insights into the family's wider evolutionary relationships (Chase et al., 1993; Swensen et al., 1998; Soltis et al., 2000; Schwarzbach and Ricklefs, 2000; Wagstaff and Dawson, 2000). These studies indicate that the Begoniaceae are most immediately related to a morphologically diverse clade that includes the Cucurbitaceae, Datiscaceae, and Tetramelaceae as well as three other families that were not traditionally linked with the begonias—the Anisophylleaceae, Coriariaceae, and Corynocarpaceae. These seven families constitute the order Cucurbitales (sensu APG II, 2003), and recent investigations of wood anatomy in members of Cucurbitales have revealed some unusual features that are shared by all (Carlquist and Miller, 2001). The Cucurbitales is noteworthy because its constituent families differ considerably in appearance from one another (but see Brouillet [2001] for shared features and potential synapomorphies) and also because it includes families with large numbers of herbaceous species (Begoniaceae, Cucurbitaceae) as well as families with small numbers of predominantly woody species (Anisophylleaceae, Coriariaceae, Corynocarpaceae, and Tetramelaceae). Several genera in the order are either monotypic (*Combretocarpus*, *Hillebrandia*, *Octomeles*, *Poga*, and *Tetrameles*) or contain a small number of species (*Corynocarpus*–five species; *Datisca*–two species; *Polygonanthus*–two species). Recent molecular phylogenetic studies that have included members of the Begoniaceae (Swensen et al., 1998; Schwarzbach and Ricklefs, 2000; Wagstaff and Dawson, 2000) all suggest that the Begoniaceae form a well-supported clade that is sister to the Datiscaceae sensu stricto.

Three genera are currently recognized within Begoniaceae: *Begonia*, *Symbegonia*, and *Hillebrandia*. *Begonia* is by far the largest of these, with roughly 1400 species. Most of the species are monoecious perennials, but a few differ by being either dioecious or annual. *Begonia* species typically inhabit

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moist, shady locations in humid lowland or upland forests, with the greatest number being found in middle-elevation and cloud forest habitats. Many of the species are narrow endemics. The species of *Begonia* are divided among 63 sections (Doorenbos et al., 1998). Each section is restricted to a particular continent, with many sections and species occurring in Asia and America, relatively few in Africa, and none in Australia. The relationships among these sections are poorly understood, and a recent morphological study of the genus indicates that the circumscription of several sections is questionable (Doorenbos et al., 1998). Recent efforts have focused on reconstructing *Begonia* phylogeny using DNA sequence data (Swensen et al., 2001; Tebbitt et al., 2001; Forrest and Hollingsworth, 2003; Plana, 2003). The genus *Symbegonia* includes 12 species that are endemic to New Guinea. It is traditionally distinguished from the other genera of Begoniaceae by having female flowers with a connate perianth and male flowers with free sepals and petals. Recent molecular-based phylogenetic analyses suggest that *Symbegonia* is nested within *Begonia* (Swensen et al., 1998; Forrest and Hollingsworth, 2003). The genus *Hillebrandia* is monotypic and represented by *Hillebrandia sandwicensis*, an endemic of the Hawaiian Islands and the focus of this study.

Hillebrandia sandwicensis was first described by Oliver (1866) and named in honor of Dr. Wilhelm Hillebrand, a physician and botanist specializing in the Hawaiian flora. It is the only member of the Begoniaceae native to the Hawaiian archipelago. In appearance, *Hillebrandia* closely resembles *Begonia* but differs by its more numerous and more highly differentiated sepals and petals, its semi-inferior and incompletely closed ovary (inferior and completely closed in *Begonia*) (Gauthier, 1950, 1959; Charpentier et al., 1989), fruits that dehisce between the styles (loculicidal or rarely septicidal or indehiscent in *Begonia*) and by the pattern of ornamentation of its pollen (van den Berg, 1983). *Hillebrandia* flowers from February to June; after producing fruit, the aboveground parts die back to rhizomes, and the plants become dormant from late summer until January (MacCaughey, 1918; Lorence, 1987).

Hillebrandia sandwicensis is found on the islands of Kauai, Maui, and Molokai (Wagner et al., 1999) and was reported once from Mount Ka'ala on Oahu (Hillebrand, 1888) but is now thought to be extinct on that island. It is presently most abundant on Kauai and Maui (MacCaughey, 1918), but even on these islands it is becoming increasingly rare (Lorence, 1987). Throughout its range, the species is restricted to wet ravines in the montane rain forest zone at altitudes ranging from 900 to 1800 m (MacCaughey, 1918), a habitat similar to that of many *Begonia* and *Symbegonia* species. Curiously, historical and current records of *Hillebrandia* populations show the species to be rare and localized despite the wider occurrence of suitable habitat, especially on Hawaii, the most recently formed of the Hawaiian Islands.

The seeds of *Hillebrandia* are very small (~0.4 mm in length) with some surface sculpturing. Similar seeds are found in *Begonia* (called ''dust seeds'') and can be effectively distributed by wind over long distances. However, the enclosed rainforest habitat of *Hillebrandia* (and *Begonia*) is not conducive to long-distance dispersal by wind. This situation presumably led Carlquist (1974) to suggest that *Hillebrandia* seeds were most likely transported to the Hawaiian archipelago in mud adhering to the legs of birds, although anemochorous dispersal appears to be equally probable given the morphology of the seeds and a habitat little frequented by migratory birds. In *Begonia*, ITS-based phylogenies show clades that are strongly correlated with geographic origin, suggesting that long-distance colonization has occurred only rarely in the genus (Forrest and Hollingsworth, 2003). Thus, while the habitat of Begoniaceae may prevent frequent anemochorous long-distance disperal events, these events appear to have occurred frequently enough to distribute *Begonia* around the globe and *Hillebrandia* onto the Hawaiian archipelago.

Previous phylogenetic analyses using chloroplast *rbcL* and nuclear 18S DNA sequence data support earlier morphologically based theories (e.g., MacCaughey, 1918; Gauthier, 1950, 1959; Reitsma, 1983) that *Hillebrandia* is the first branching member of the Begoniaceae (Swensen et al., 1998). This is surprising given *Hillebrandia*'s endemism to the Hawaiian archipelago, a very remote island chain whose current islands average only 15 million years in age coupled with the extremely large size and wide distribution of the genus *Begonia*.

This study aims to test previous assertions that *Hillebrandia* is the sister group to *Begonia* rather than embedded within it, to determine whether it is an ancient or a recent arrival on the Hawaiian Islands, and to propose a possible route by which it may have reached these islands. Because there is no fossil evidence available to assess the age of *Hillebrandia* directly, the minimal age of the *Hillebrandia* lineage is estimated here using analyses of sequence data calibrated by fossil dates suggested for close relatives.

An improved understanding of the phylogenetic placement and biogeography of *Hillebrandia* will facilitate a better understanding of evolutionary processes within the Begoniaceae as a whole.

MATERIALS AND METHODS

DNA amplification and sequencing—The sources of plant material used in these analyses are listed in the Appendix (see Supplemental Data accompanying the online version of this article). Total genomic DNA was extracted from fresh leaf tissue using the 2× cetyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987) as modified by Rieseberg et al. (1992) or using the DNeasy Plant DNA Extraction Kit (Qiagen, Valencia, California, USA). Other sequences were obtained directly from Genbank (see Appendix).

Three gene regions were amplified for subsequent sequence analyses: *rbcL*, a highly conserved chloroplast gene encoding the large subunit of the protein ribulose bisphosphate carboxylase/oxygenase (rubisco); 18S rDNA, a highly conserved nuclear region encoding ribosomal RNA (rDNA); and ITS, the noncoding internal transcribed spacer regions adjacent to (and including) the 5.8S rDNA. Amplification primers for each gene region are listed in Table 1. The amplification parameters were specific to the gene of interest. For *rbcL*, an initial denaturation step at 95°C for 4 min was followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, followed by a final extension step at 72°C for 4 min. For 18S, the same parameters were used as for *rbcL*, except that 30 cycles were run and the extension step was lengthened by 30 s. For ITS, the same parameters were used as for *rbcL*, except that the annealing temperature was 60°C. All amplification reactions were 50 μL in volume. Agarose gel electrophoresis followed by ethidium bromide staining was used to verify the presence and size of amplification products by comparison to a 1-kb ladder molecular mass standard (Life Technologies, Grand Island, New York, USA). Successful amplifications were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol. The products were eluted in 40 μL distilled water and again verified by agarose gel electrophoresis.

Purified amplification products were sequenced in half-volume reactions (20 μL) using the ABI Prism Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, California, USA). The sequencing primers

TABLE 1. Primers used for amplification and sequencing of ITS, 18S, and *rbc*L. An asterisk denotes primers used in amplification; an apostrophe denotes primers used for sequencing, a double dagger denotes an amplification primer used specifically for Begoniaceae.

Gene	Primer name	Sequence (5 → 3′)	*N*	References
ITS	5P*	GGAAGGAGAAGTCGTAACAAGG	22	Eckenrode et al., 1985
	26S1Rev*′	CGCCTGACCTGGGGTCG	17	modification & reverse of N-nc25S1 (designed by Bult and Zimmer, 1993; published by Kuzoff et al., 1998)
	51NT′	AGGTGAACCTGCCGAAGGATCATTG	25	modification of ITS-5P (P. Fritsch, unpublished data)
	2EXT′	CACTACGTTCTTCATCGATGC	21	modification of ITS-2 (White et al., 1990)
	3EXT′	GCATCGATGAAGAACGTAGTG	21	modification of ITS-3 (White et al., 1990)
18S	25EF*	CTGGTTGATCCTGCCAG	17	Nickrent and Starr, 1994
	ITS-2*	GCTGCGTTCTTCATCGATGC	20	White et al., 1990
	309F′	TACCATCGAAAGTTGATAGGGCAG	24	Bult et al., 1992 (=C-18E)
	1322F′	TAACGAACGAGACCTCAGCCT	21	Nickrent and Starr, 1994
	1424F′	TCTAAGGGCATCACAGACCTGTTATTG	27	Bult et al., 1992 (=C-18J)
	554R′	AGGGCAAGTCTGGTGCCA	18	Bult et al., 1992 (=N-18G)
	1131R′	CAATTCCTTTAAGTTTCAGCC	21	Nickrent and Starr, 1994
	1433R′	ATCTAAGGGCATSACAGACC	20	Nickrent and Starr, 1994
rbcL	ORF 106-T*‡	CTCTCCGACTACGGATCCCATACTACCCCC	30	modification of ORF 106 (Cruzan et al., 1993; Hiratsuka et al., 1989)
	z-1375R*	AATTTGATCTCCTTCCATATTTCGCA	26	DNAX-Gerard Zurawski primer (unpublished data)
	z-1*′	ATGTCACCACAAACAGAAACTAAAGCAAGT	30	Zurawski (unpublished data)
	B-427′	GCTTATGTTAAAACTTTTCAAGGCCCTCC	29	*Begonia* specific modification of z-427 (Zurawski, unpublished data)
	z-895′	GCAGTTATTGATAGACAGAAAAATCATGGT	30	Zurawski (unpublished data)
	z-1020′	ACTTTAGGTTTTGTTGATTTATTGCGCGATGATT	34	Zurawski (unpublished data)
	z-346R′	AAATACGTTACCCACAATGGAAGTAAATAT	30	Zurawski (unpublished data)
	z-895R′	ACCATGATTCTTCTGCCTATCAATAACTGC	30	Zurawski (unpublished data)
	z-1204R′	CCCTAAGGGTGTCCTAAAGTTTCTCCACC	29	Zurawski (unpublished data)

used for each gene are listed in Table 1. Sequencing reactions were purified with Centri-sep spin columns (Princeton Separations, Adelphia, New Jersey, USA), and the cleaned products were analyzed on an ABI 373 automated sequencer. Sequence chromatograms were proofread using Sequencher (GeneCodes, Ann Arbor, Michigan, USA) software.

Sequence alignment and phylogenetic analysis—Two phylogenetic analyses were conducted: a taxonomically broad analysis of Cucurbitales using the *rbcL* and 18S sequences from 29 species and a more narrowly focused analysis of Begoniaceae using the *rbcL*, 18S, and ITS sequences from 31 species. Outgroups in both cases were chosen on the basis of previous analyses (Swensen, 1996; Schwarzbach and Ricklefs, 2000; Forrest and Hollingsworth, 2003). In the Cucurbitales analyses, the outgroup consisted of two species belonging to the order Fagales (*Myrica cerifera* [Myricaceae] and *Quercus rubra* [Fagaceae]). In the Begoniaceae analyses, two *Datisca* species were designated as the outgroup. The *Begonia* species included in the Begoniaceae analysis were selected to represent the morphological and geographical ranges of the genus. All sequences included in these analyses are listed in the Appendix.

Alignment of the *rbcL* sequences was produced manually whereas 18S and ITS sequences were aligned using the Clustal X program (a graphical user interface for Clustal W; Thompson et al., 1994), followed by manual editing. For ITS alignments, the gap opening penalty (GOP) and gap extension penalty (GEP) parameters were set to 10 and 5, respectively. For 18S alignments, the GOP and GEP parameters were 10 and 0.1 for pairwise alignments and 10 and 0.5 for multiple alignments.

Phylogenetic trees were estimated using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. The MP analyses were conducted using PAUP* (Swofford, 2001) via heuristic searches with tree bisection-reconnection (TBR) branch swapping and 1000 random-taxon-entry replications per search. Both combined and separate data sets were analyzed. A combinability test was not conducted for the combined data; recent studies have indicated that tests such as the ILD test (Farris et al., 1994), if it can be run to completion, may find congruence despite the fact that different topologies are produced by the different data sets (Dowton and Austin, 2002). Yoder et al. (2001) showed an inverse relationship between data set congruence and phylogenetic accuracy. Dolphin et al. (2000) also showed that phylogenetic noise can lead to significant incongruence. Bootstrap and decay analyses were conducted to assess branch support (Felsenstein, 1985; Bremer, 1988; Donoghue et al., 1992). For bootstrap analyses, 100 bootstrapped data sets were analyzed using the same approach as with the original parsimony analyses. We used AutoDecay (Eriksson, 2002) with PAUP* to calculate decay indices under parsimony. Constraint trees for each node were analyzed in PAUP* with converse constraints in effect. For each node, 100 random addition starting trees were swapped with TBR branch swapping.

For the maximum likelihood (ML) and Bayesian inference analyses, an evolutionary model was selected using MrModeltest 1.1b (J. Nylander, Uppsala University, personal communication), a simplified version of Modeltest 3.06 (Posada and Crandall, 1998) that specifically tests the 24 models common to PAUP* and MrBayes (Huelsenbeck and Ronquist, 2001). The hierarchical likelihood test in MrModeltest selected the most complex model available, the general time reversible model with gamma distribution of rates, and an estimated proportion of invariable sites (GTR + G + I). This model was selected for combined data sets as well as for the separate ones.

The ML trees were obtained in a two-step procedure. First, the parameters estimated by MrModeltest were used in PAUP*, which estimated a tree using 10 random addition sequence trees and TBR branch swapping. Parameters were then reestimated using the optimal tree found in step one, and this tree was swapped to completion.

The Bayesian inference analyses were performed using MrBayes 2.01 and the same model as in the ML analyses. In this case, all parameters were estimated during the analysis. Analyses started on random trees, and 1 000 000 generations of the Markov chain were run (mcmc) with four chains. A tree was sampled every 10 generations. For the Cucurbitales data set, 73 820 of the trees were used after the ''burnin'' of the chain was removed from con-

sideration (generations prior to 261 800). For the *Begonia* data set, 92 200 of the 100 000 trees sampled were subsequently used to calculate clade posterior probabilities using a 50% majority-rule consensus tree (all trees sampled before generation 78 000 were excluded; the ''burnin'' of the chain). Data sets and trees resulting from these analyses can be obtained from http://www.TreeBase.org.

Divergence time estimates—An estimate for the minimal age of *Hillebrandia* was calculated using two approaches: (1) a fixed-rate method and (2) two nonparametric methods that do not impose a fixed rate of nucleotide change for *rbcL*. All age estimates were obtained with the computer program r8s (Sanderson, 2002a).

For the fixed-rate age estimation, the Langley-Fitch method (LF; Langley and Fitch, 1974) was used to reconstruct divergence times using maximum likelihood under the assumption of a molecular clock. As part of this approach, a likelihood ratio test of rate constancy was conducted (Felsenstein, 1988). For this analysis, we used only the best ML tree obtained from the combined Cucurbitales data set. This tree was rooted using the outgroups, saved with estimated branch lengths, and the outgroups were pruned prior to analyses in r8s. We used the parameters num_restarts = 5 and num_time_guesses = 5. Age constraints were enforced for three nodes: (1) Coriariaceae at minimum age 55 million years (my) BP (Saporta, 1865), (2) Tetramelaceae at minimum age 55 my BP (Lankhanpal, 1970), and (3) Cucurbitaceae set to a range from maximum 65 to minimum 39 my BP (Cronquist, 1981; Muller, 1981).

For the second approach, age estimates were obtained using nonparametric rate smoothing (NPRS; Sanderson, 1997) and penalized likelihood (PL; Sanderson, 2002b). Neither rely on a fixed evolutionary rate. We used the same starting tree and parameters as with the LF method. A cross-validation analysis was conducted for the PL analysis yielding a smoothing value of 562 (log 2.75).

To assess error levels in age estimates, we used a bootstrapping approach (Sanderson and Doyle, 2001). One hundred bootstrap replicates of the data set were obtained using seqboot of the Phylip 3.5c package (Felsenstein, 1993) and imported into PAUP* for branch length estimation. The tree was fixed for all replicates; branch lengths and all model parameters were estimated for each bootstrap replicate using the GTR + G + I model as before. Cross validation analyses were conducted in r8s for each replicate for use in the bootstrap PL analyses. Bootstrap log files were scanned and intervals of confidence (95%) for the estimated nodal ages were calculated using software available from one of the authors (T. Eriksson).

RESULTS

Phylogenetic analyses—*Maximum parsimony*—Parsimony analysis of the Cucurbitales was conducted using a combined dataset of *rbcL* and 18S sequences. The parsimony analysis resulted in six equally parsimonious trees (Fig. 1; length = 990; consistency index [CI] = 0.4972 [excluding uninformative characters], retention index [RI] = 0.5028, rescaled consistency index [RC] = 0.4605). A total of 3272 nucleotide positions were analyzed (1428 from *rbcL* and 1844 from 18S). Of these characters, 2764 were constant, 243 were variable but parsimony uninformative, and 265 were parsimony informative. In the strict consensus tree (Fig. 1), the Begoniaceae form a well-supported clade with a bootstrap value of 100% and a decay index of 29. Within the Begoniaceae clade, *Hillebrandia* is first-branching and six species of *Begonia* plus one species of *Symbegonia* form a well-supported subclade (bootstrap 100%, decay index = 12). The Datiscaceae sensu stricto (s.s.) forms a sister clade to Begoniaceae. The order Cucurbitales and the families within are all strongly supported. A less strongly supported clade within the Cucurbitales is composed of Corariaceae and Cucurbitaceae (bootstrap 51%, decay index = 1). Anisophyllaceae is the sister group to the rest of the Cucurbitales clade.

The *rbcL* and 18S data were analyzed separately as described for the combined data analysis to test for the presence of any well-supported differences between them. Analysis of *rbcL* recovered three trees (not shown; length = 600; CI = 0.4815; RI = 0.7193; RC = 0.4340) and analysis of 18S data recovered 312 trees having much less resolution (eight nodes resolved) compared to *rbcL* (23 nodes) or to the combined analyses (22 nodes) (18S tree not shown; length = 381; CI = 0.5483; RI = 0.7697; RC = 0.5333). The only topological difference between the *rbcL* and 18S trees was the position of *Datisca* species. In the 18S analysis, *Datisca* appeared sister to the rest of the Cucurbitales, whereas with *rbcL*, *Datisca* appeared sister to Begoniaceae. In both cases, branch support for the placement of *Datisca* was low (<50% bootstrap and decay index = 2). There was no difference among the *rbcL*, 18S, or combined analyses with regard to the position of *Hillebrandia*. In all analyses, the Begoniaceae appear as a well-supported monophyletic clade in which *Hillebrandia* appears as the sister group of *Begonia* plus *Symbegonia*.

Parsimony analysis of the combined *rbcL*, 18S, and ITS sequence data for the Begoniaceae resulted in a single most parsimonious tree (Fig. 2; length = 2561; CI = 0.4611; RI = 0.4407; RC = 0.2428). A total of 4179 nucleotide positions were analyzed (1428 from *rbcL*, 1813 from 18S, and 939 from ITS). Of these characters, 3267 were constant, 355 were variable but parsimony uninformative, and 557 were parsimony informative. The Begoniaceae appear as a well-supported monophyletic group (bootstrap 100%; decay index = 124) with *Hillebrandia* sister to *Begonia* plus *Symbegonia*. Within Begoniaceae, species groups correspond to continental locations, with the African and Malagasy species forming a clade with low support (bootstrap <50%; decay index = 2) that is sister to the rest. Ten of the 11 American species sampled form a weakly supported clade (bootstrap <50%; decay index = 1). An additional American species, *B. herbacea* (Brazil), is sister to the clade joining the American and Asian clades. The 10 Asian species sampled form a clade with low support (bootstrap <50%, decay index = 2). This clade includes a representative of *Symbegonia*, the New Guinea endemic, a result that supports the transfer of the species within genus *Symbegonia* to *Begonia* as a new section (Forrest and Hollingsworth, 2003).

Separate analyses of *rbcL* and ITS data were also conducted for the Begoniaceae data set (analyses of 18S data alone for this data set could not be run to completion). The analysis of *rbcL* alone recovered 46 trees (not shown; length = 267; CI = 0.5864; RI = 0.6854; RC = 0.5134). Analysis of the ITS data alone recovered nine trees (not shown; length = 2042; CI = 0.4510; RI = 0.4166; RC = 0.2154). Comparison of the strict consensus trees from these separate analyses revealed three topological differences, none of which were well supported: (1) in *rbcL* trees, American *B. luxurians* and *B. ulmifolia* appear sister to *Hillebrandia*, whereas with ITS, these taxa are embedded within the American clade; (2) in *rbcL* trees, Asian *B. boisiana* appears apart from other Asian begonias, whereas in ITS trees, *B. boisiana* is embedded within the Asian clade; (3) in ITS trees, American *B. olbia* groups with other American begonias, whereas in *rbcL* trees, it appears as part of an unresolved clade containing Asian and African species. In general, the *rbcL* strict consensus (17 nodes) was less resolved than the ITS consensus (23 nodes), but in

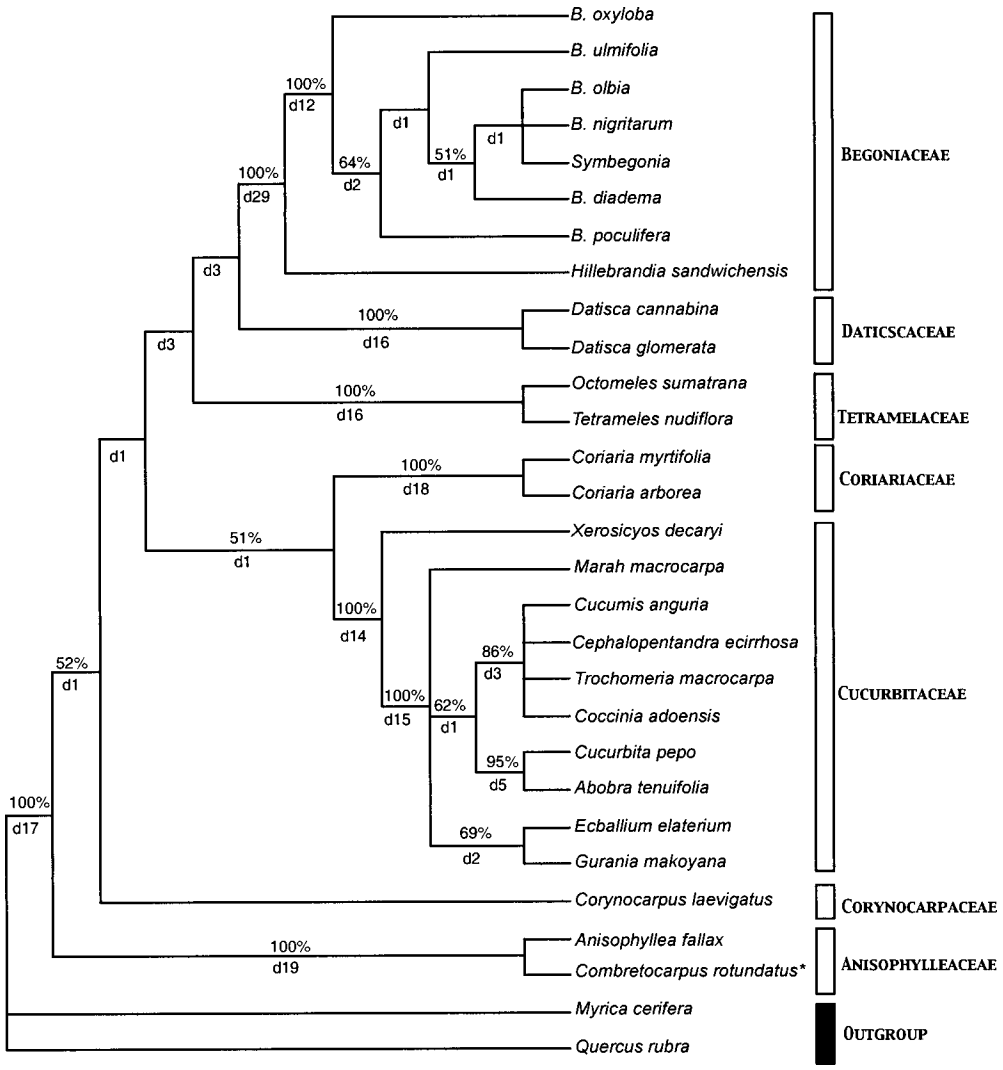

Fig. 1. Maximum parsimony tree for Cucurbitales based on analysis of combined data from chloroplast *rbcL* and nuclear 18S rDNA sequences. Strict consensus of six trees of 990 steps; consistency index (CI) = 0.4972 (excluding uninformative characters), retention index (RI) = 0.5028, rescaled consistency index (RC) = 0.4605. Family affiliations are shown to the right of taxon names. Percentages above the branches are bootstrap values; numbers preceded by "d" below the branches are decay values. An asterisk indicates a taxon for which only *rbcL* data was included in the analysis.

both cases, the sister group relationship of *Hillebrandia* to the rest of Begoniaceae was well supported.

Maximum likelihood and Bayesian inference—The best ML tree for the Cucurbitales *rbcL* + 18S combined dataset is shown in Fig. 3 (ln likelihood −10683.58853) with posterior probabilities indicated for each node. This tree was also sampled during the Bayesian analysis (in generation 322 170). The ML tree is not among the six most parsimonious trees found for the same data, but is five steps longer in terms of equally weighted parsimony. Its likelihood score is 0.9–1.2% better than those of the most parsimonious trees. Two topological differences exist between the ML tree (Fig. 3) and the MP consensus (Fig. 1). *Datisca* species are sister to Begoniaceae in the MP tree, but *Octomeles*/*Tetrameles* are sister to Begoniaceae in the ML tree. *Coriaria* appears with Cucurbitaceae in the MP tree, but with *Corynocarpus* in the ML tree. In both trees, however, the position of *Hillebrandia* relative to the rest of the Begoniaceae is the same.

The best ML tree found with the *Begonia rbcL* + 18S + ITS combined data is shown in Fig. 4 (ln likelihood −18685.63592) with posterior clade probabilities indicated for each node. The best tree sampled during the Bayesian inference analysis was found in generation 708 670 and had a ln likelihood of −18687.47432 when reestimated in PAUP*. When this latter tree was swapped on in PAUP*, the optimal tree was recovered. This likelihood tree is 14 steps longer than the MP tree (Fig. 2) in terms of equally weighted parsimony and has a likelihood score that is 0.066% better than the MP tree. Seven *Begonia* species (*poculifera*, *nigritarum*, *boisiana*, *fallax*, *fuchsioides*, *olbia*, *cinnabarina*) differ in their placement between the ML and MP trees, but these differences are confined to within their respective geographic clades (Asian, American, or African). The position of *Hillebrandia* relative to the rest of the Begoniaceae is the same in both ML and MP trees.

Divergence time estimates—Ages for the Begoniaceae crown group and for the *Hillebrandia* node were calculated

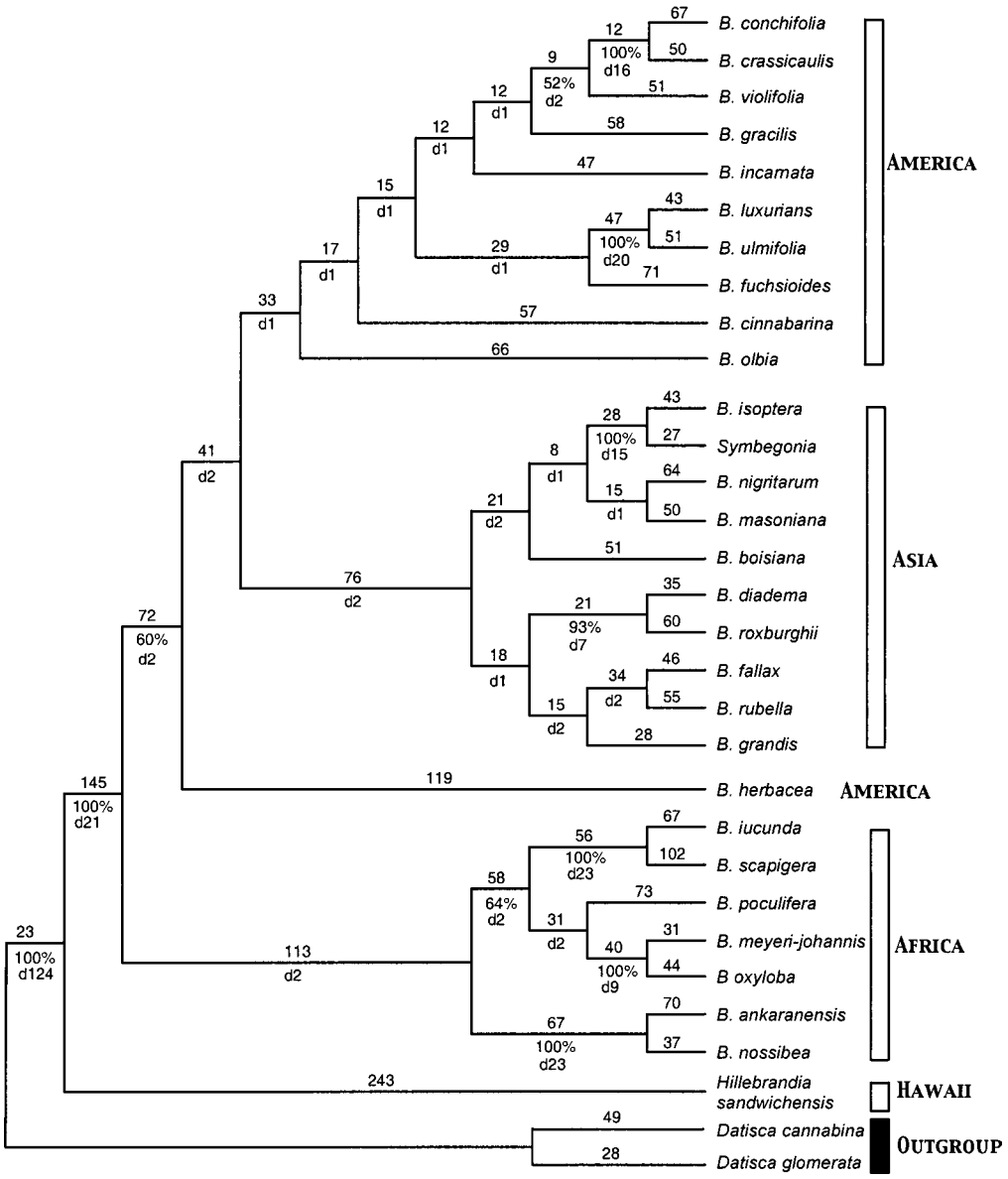

Fig. 2. Maximum parsimony tree for Begoniaceae based on analysis of combined data from *rbcL*, 18S rDNA, and ITS sequences. Single tree of 2561 steps; CI = 0.4611; RI = 0.4407; RC = 0.2428. Continental locations are indicated at right. Numbers above the branches are branch lengths (ACCTRAN optimization); percentages below branches are bootstrap values; numbers preceded by ''d'' are decay values. Bootstrap values below 50% are not listed.

using each of the three methods mentioned previously (Table 2). The likelihood ratio test for rate constancy conducted as part of the Langley-Fitch (LF) approach rejected the null hypothesis for rate constancy ($\chi^2 = 202.70$; df = 27).

The LF age estimations resulted in an age of 63.41 my BP (Paleocene) for the *Hillebrandia* node and 42.23 my BP for *Begonia* (including *Symbegonia*). For the non-fixed-rate estimations, the PL analysis gave corresponding estimates of 64.69 and 40.93 my BP, and the NPRS analysis gave estimates of 50.85 and 26 my BP. These dates estimate the minimum ages of the Begoniaceae crown group (~51–65 my BP; Eocene-Paleocene) and the *Begonia* crown group (~26–42 my BP; Oligocene-Eocene). In fact, *Hillebrandia*, or an ancestor to both *Hillebrandia* and *Begonia*, may have existed prior to these dates. Regardless, all estimates for the age of the node where the *Hillebrandia* lineage splits off from the *Begonia* lineage predate the origin of the oldest above-water island of the Hawaiian archipelago (Kure atoll; ~30 my BP) by at least 20 million years.

DISCUSSION

Phylogeny of Begoniaceae and Hillebrandia—The phylogenetic analysis of Cucurbitales provides a broad view of the relationship of Begoniaceae to other members of this order. Based on molecular analysis, Begoniaceae is a well-supported clade whose closest relatives belong to the small disjunct family Datiscaceae s.s. This association, originally proposed by Lindley (1846), has since been recognized in several classification schemes (Cronquist, 1981; Thorne, 1992; Takhtajan, 1997). Boesewinkel (1984) also supported this association on the basis of ovule and seed structure, and Brouillet (2001) supported it

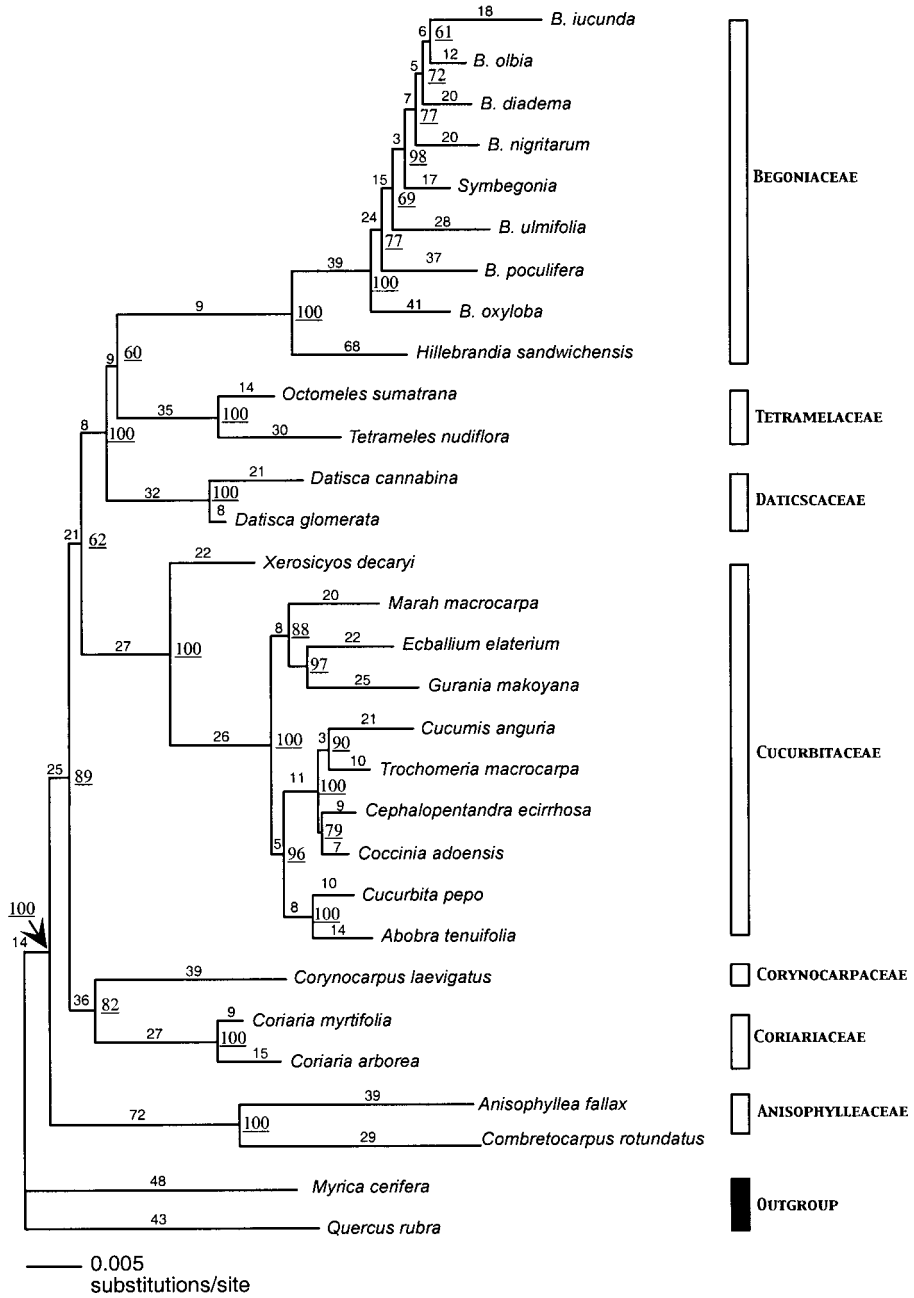

Fig. 3. Maximum likelihood tree for Cucurbitales based on combined data (ln likelihood −10683.58853) with posterior probabilities indicated at the right of each node and underlined. Branch lengths are shown above branches, optimized with ACCTRAN in PAUP*. This tree was also sampled during the Bayesian analysis (in generation 322 170).

on the basis of the herbacous habit, cymose inflorescences (instead of racemes of cymes), and bifid stylodia. The relationships of these two families and Tetramelaceae is further supported by the small, numerous, operculate seeds with honeycombed testa and spherical pollen. The inclusion of Begoniaceae within Cucurbitales is supported by recent molecular phylogenetic work (see citations in APG II, 2003), and a close relationship of the Begoniaceae with Cucurbitaceae and Datiscaceae was also recognized in serological work using seed extracts by Kolbe and John (1979). Begoniaceae and Datiscaeae, like the Cucurbitaceae, also produce cucurbitacins (Doskotch et al., 1969; Doskotch and Hufford, 1970; Kupchan et al., 1972).

The phylogenetic analyses presented here, as well as previous morphological comparisons of *Hillebrandia* and *Begonia* (Gauthier, 1950, 1959; van den Berg, 1983; Charpentier et al., 1989; Brouillet, 2001), provides substantial evidence to support *Hillebrandia* as the sister to the rest of Begoniaceae; this verification is a crucial step in understanding the biogeography of this species and its endemism to Hawaii.

Age estimates—The three methods utilized vary in their estimates of nodal ages. The NPRS estimates are the youngest, while the PL or the clock-based LF estimates are consistently older and quite close to each other. Sanderson (2002b) noted

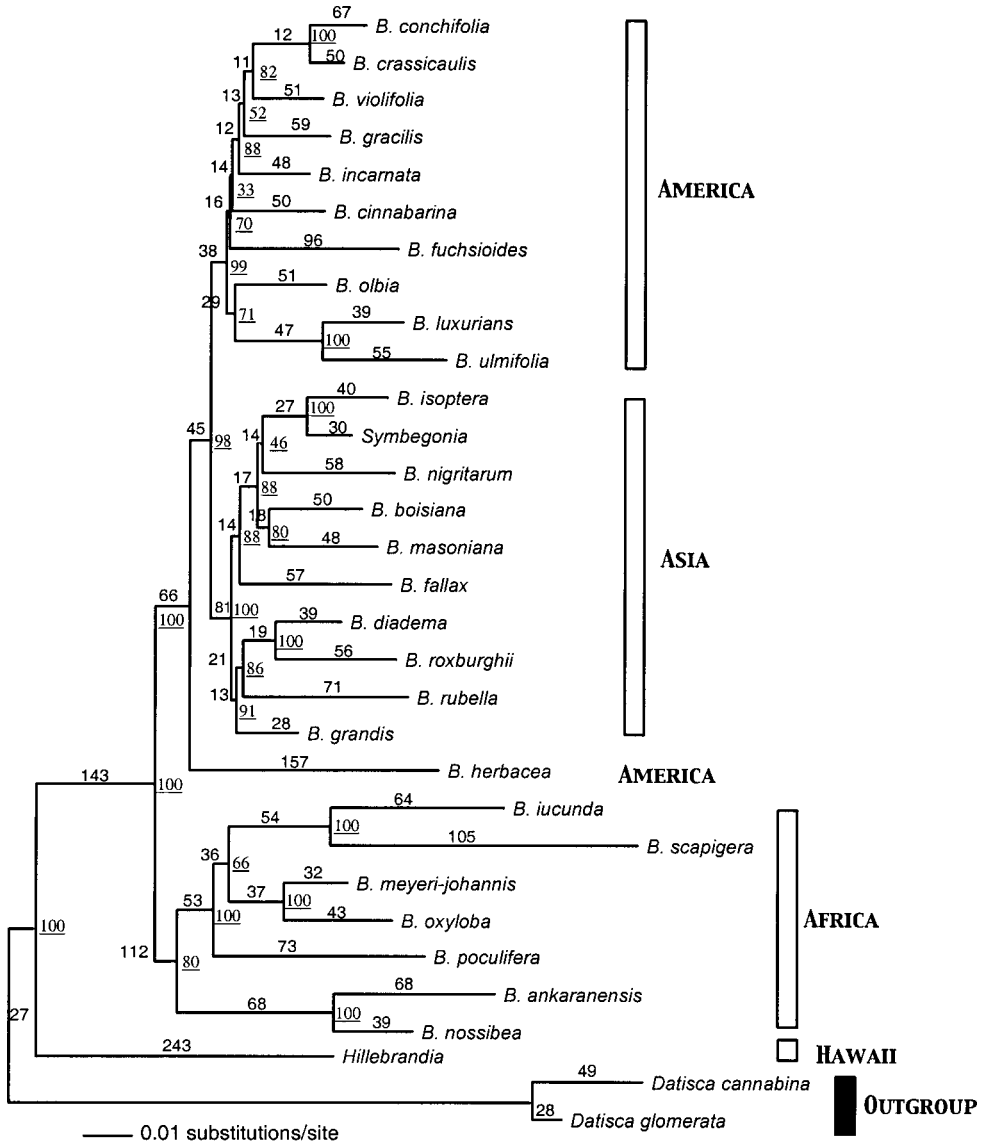

Fig. 4. Maximum likelihood tree for Begoniaceae based on combined data (ln likelihood −18685.63592). Posterior clade probabilities are shown to the right of each node and underlined; branch lengths, optimized using ACCTRAN in PAUP*, are shown above the branches.

TABLE 2. Estimated ages (million years before present, my BP) of the *Hillebrandia* node and the *Begonia* crown group using fixed-rated and non-fixed-rate methods with constraints.

Node/Method[a]	Estimated age (my BP)	Bootstrap estimate of age[b] (my BP)	Bootstrap estimate of SD (my BP)
Hillebrandia			
LF	63.41	64.472	6.986
NPRS	50.85	55.937	11.926
PL	64.69	72.164	15.440
Begonia			
LF	42.23	42.808	5.516
NPRS	26.00	28.614	6.980
PL	40.93	45.321	12.101

[a] LF, Langley-Fitch method (assumes a molecular clock); PL, penalized likelihood; NPRS, non-parametric rate smoothing (does not assume a molecular clock).

[b] Based on 100 bootstrap replications.

that NPRS tends to ''overfit'' the data, and this might account for the difference in estimated ages in our analyses. Another difference between NPRS and the other methods is the use of the LOG penalty function in the optimality criterion, which was necessary to be able to use NPRS for our data. Slight differences in settings between the methods might explain why the NPRS estimates are considerably lower. The PL method relies on striking a balance between a situation where any branch can have any rate and the strict clock situation where all branches have the same rate. This is accomplished through a ''cross validation'' procedure, which yields a low ''smoothing'' value for clearly non-clocklike data and a high value for clocklike data (Sanderson, 2002b). In our case, the smoothing value was intermediate and even though our data are not clocklike, the age estimates are almost identical to those reported by the LF method. So far, these methods are not very well known, and the effect of various parameter settings has not been investigated in detail. It is clear, however, that the

choice of estimation method (LF, PL, or NPRS) does not affect the overall conclusion from the age estimates that the *Hillebrandia* lineage is considerably more ancient than the Hawaiian islands. The age of *Hillebrandia* itself, however, cannot be known based on these estimates.

Biogeography of Hillebrandia—Phylogenetic placement and divergence time estimates both suggest that the split forming the *Hillebrandia* lineage is a relatively ancient event in the history of Begoniaceae and that *Hillebrandia* is the sister group to *Begonia*. Moreover, given that *Hillebrandia* is both morphologically and molecularly divergent from *Datisca*, the direct ancestor of *Begonia* and *Hillebrandia* appears to be extinct. While its endemism to the Hawaiian Islands might typically suggest that *Hillebrandia* is a more recent mainland derivative, our data provides no indication of a mainland predecessor or of recent colonization. Furthermore, in contrast to many Hawaiian endemics (e.g., *Munroidendron*, *Reynoldsia*, *Tetraplasandra* [Araliaceae] Plunkett et al., 1997; *Argyroxiphium*, *Dubautia*, *Wilkesia* [Asteraceae], Baldwin and Robichaux, 1995; *Bidens* [Asteraceae], Ganders et al., 2000; *Hesperomannia* [Asteraceae], Kim et al., 1998; *Clermontia* [Campanulaceae], Lammers, 1995; *Cyanea* [Campanulaceae], Givinish et al., 1995; *Vaccinium* [Ericaceae], Powell and Kron, 2002; *Geranium* [Geraniaceae], Pax et al., 1997; *Scaevola* [Goodeniaceae], Howarth et al., 2003; *Haplostachys*, *Phyllostegia*, *Stenogyne* [Lamiaceae], Lindqvist and Albert, 2002; *Gossypium* [Malvaceae], Dejoode and Wendel, 1992; *Metrosideros* [Myrtaceae], Wright et al., 2001; *Pittosporum* [Pittosporaceae], Gemmill et al., 2001; *Rubus* [Rosaceae], Howarth et al., 1997; *Viola* [Violaceae], Ballard and Sytsma, 2000), *Hillebrandia* is sister to the rest of its family and older than the current Hawaiian Islands. As such, it appears to be the only known example of a relict plant genus within the Hawaiian flora. *Hillebrandia* is of particular interest because several oceanic island taxa previously considered to be relictual based on their morphology have proven to be derived (suggesting recent colonization) based on phylogenetic analysis of molecular data. Prominent examples include the Hawaiian *Hesperomannia* (Kim et al., 1998), the Juan Fernandez *Dendroseris* (Kim et al., 1996), and the endemic *Echium* species of Macronesia (Böhle et al., 1996). While *Hillebrandia* is highly unusual in being a Hawaiian relict, it has been suggested that oceanic islands make good refugia for relict taxa because the maritime climate provides environmental stability during prolonged periods of widespread drought or cold elsewhere (summarized by Moore, 1979). We suggest that *Hillebrandia*, in part, owes its survival as a relict endemic genus on the Hawaiian Islands because these islands may have experienced a relatively stable maritime climate.

If *Hillebrandia* is a relict species, with Hawaii serving as a refuge, what is the origin of the ancestor of *Hillebrandia*? The present distribution pattern of *Hillebrandia* on the Hawaiian Islands suggests that it has been island hopping from older to younger islands in the same manner as documented in other Hawaiian groups (e.g., *Cyanea*, Givnish et al., 1994; silversword alliance, Baldwin and Robichaux, 1995; *Drosophila*, Desalle, 1995). However, unlike the members of these groups, *Hillebrandia* has not undergone speciation in association with the colonization of new islands and exhibits no morphological discontinuities between populations even when such populations are on different islands (W. L. Clement, personal observations). In fact, preliminary genetic diversity studies indicate similar levels of diversity in populations on Kauai and Molokai, suggesting that the existing populations may be remnants of much larger populations (W. L. Clement, unpublished data).

Hillebrandia is found at 900–1800 m elevation on Kauai, Maui, and Molokai and was historically collected at similar elevations on Oahu, but it is absent from the largest and most recently formed island, Hawaii. Because Hawaii contains suitable montane rainforest habitat for *Hillebrandia*, its absence from this relatively young island (~700 000 years old) may be due to the fact that this island's montane habitat has not been available long enough to allow *Hillebrandia* to colonize. The oldest islands no longer provide suitable montane habitat for *Hillebrandia*. *Hillebrandia* may, however, have colonized its present range by island hopping from older, now denuded, members of the Hawaiian archipelago, which would have provided suitable habitat since approximately 30 my BP. Given the absence of *Hillebrandia* from Hawaii, the youngest of the Hawaiian Islands, these island-hopping events were most likely rare. One might wonder if *Hillebrandia* was able to island hop from as far as the Emperor Sea Mounts that lie northward beyond the Hawaiian archipelago; however, the geological history of these islands reveals at least one obstacle in this pathway. After the formation of the last volcano (Koko) in the Southern Emperor Sea Mount Chain approximately 48 my BP, no other islands above 300 m existed until the formation of Kure approximately 30 my BP (Carson and Clague, 1995). Thus, by the time Kure began forming, all other high islands had been submerged for approximately 18 my. Assuming relatively constant climate and vegetational zones on the islands during this time, the altitudinal constraints of *Hillebrandia* and the absence of high island habitats for a significant period of time would have prevented arrival by this route. On the other hand, climate fluctuations over the past 50 my may have allowed *Hillebrandia* to survive at lower elevations. While detailed climate information for the entire history of the Hawaiian islands is lacking, data based on the last one million years indicate recurrent fluctuations in sea level and temperature (Nullet et al., 1998). During periods of cooler temperatures and lower sea levels, vegetation common at higher elevations occupied lower but more narrow zones. For example, fossil pollen records dating from the last glacial maximum on Oahu (about 20 000 years ago) suggest that at that time temperatures were 3°–5°C cooler than present at any given altitude (Hotchkiss and Juvik, 1999). This temperature decline corresponds to similar cooling in Brazil (Stute et al., 1995), the eastern Pacific (Pisias and Mix, 1997; Pisias et al., 1997), and New Guinea (Hope and Tulip, 1994). Colonization of *Hillebrandia* (or its ancestor) somewhere along the Emperor Sea Mount Chain might suggest a boreal or boreotropical origin. Extant begonias are almost all tropical (*B. grandis* grows in temperate regions) and relatively little (2.6%) of the Hawaiian taxa are thought to have a boreal origin (Fosberg, 1948). A boreotropical origin (Wolfe, 1975) in Eurasia would be consistent with the presence of derived taxa in South America, and with the disjunct distribution of *Hillebrandia*'s sister genus *Datisca*, with species in southwestern North America and southwestern Asia (Fig. 5). This scenario, however, would not explain why the African and Malagasy species appear to be basal within the phylogeny while the Asian species are derived (Forrest and Hollingsworth, 2003; Plana, 2003), unless the African species represent the earliest relicts of extinct Eurasian ancestors. Under the boreotropical hypothesis, the ancestor of *Hillebrandia*

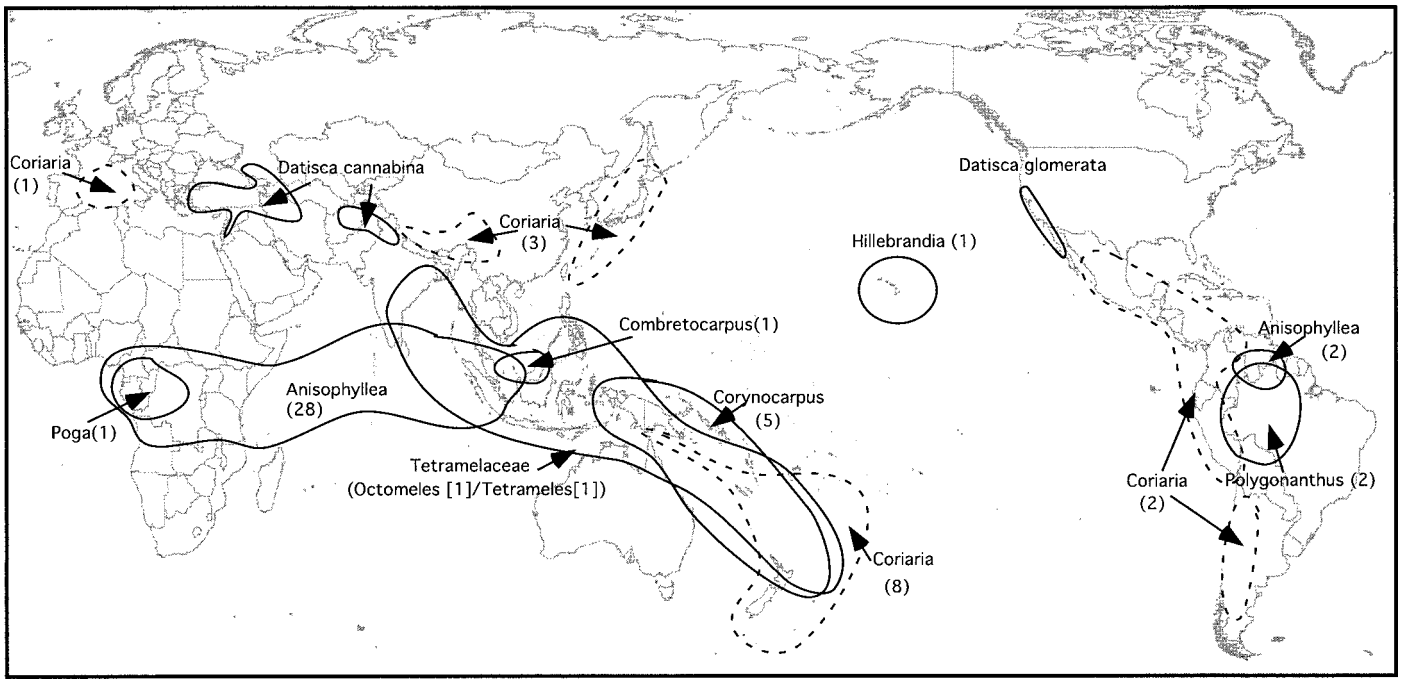

Fig. 5. Geographical distribution of Cucurbitales genera. Genus names are followed by numbers of species in parentheses. Anisophyllaceae includes *Anisophyllea* (30 spp.), *Combretocarpus* (1 sp.), *Poga* (1 sp.), and *Polygnanthus* (2 spp.); Tetramelaceae includes *Octomeles* (1 sp.) and *Tetrameles* (1 sp.); Datiscaceae includes *Datisca* (2 spp.); Coriariaceae includes *Coriaria* (15 spp.); Corynocarpus includes *Corynocarpus* (5 spp.). The pantropical *Begonia* (~1400 spp.) and Cucurbitacae (~825 spp.) are not included. Locality information and numbers of species follow Thompson and Gornall (1995), Juncosa and Tomlinson (1988), Davidson (1973), and A. Schwarzbach (Kent State University, personal communication).

may have dispersed across Asia toward the Pacific, then island hopped down the Emperor Sea Mount. The ancestor would had to have become extinct in Eurasia, possibly due to the cooling, drying climate at the end of the Eocene, followed by recolonization in Asia to produce the current distributions of the phylogenetically derived Asian begonias. American begonias would have been derived from Africa via trans-Atlantic dispersal or from Eurasia across the Bering land bridge or the lower latitude North Atlantic land bridge.

An alternative scenario is that *Hillebrandia* colonized the Hawaiian archipelago from the South Pacific-Malesia region. Several of the Cucurbitalean taxa have affinities to this area, although many genera contain widely disjunct species, making their geographic origins unclear (see Fig. 5). Datiscaceae s.s. is composed of the single genus *Datisca* with the American–Asian disjunction mentioned above; Tetrameleaceae comprises two monotypic genera distributed from India to southeast Asia and Malesia; *Corynocarpus*, the sole genus of Corynocarpaceae, is found in the southwest Pacific; *Coriaria*, the sole genus of Coriariaceae, is globally disjunct with species in the western Mediterranean, eastern Asia, Australasia, and Central and South America; Anisophyllaceae is composed of four genera and is found in Malesia, Africa, and South America; and Cucurbitaceae is cosmopolitan in distribution. A South Pacific–Malesia origin for *Hillebrandia* is consistent with Malagasy and African begonias occupying ancestral positions within the genus, and the American begonias appearing in derived positions, but requires the extinction of the direct ancestor of modern begonias in Malesia, followed by subsequent recolonization of this region by African begonias. Under this scenario, it is possible that the Malagasy and African *Begonia* were derived from western populations of a once-widespread proto-Begoniaceae, while the Hawaiian *Hillebrandia* was derived from eastern populations of such a taxon.

A third scenario is that *Hillebrandia* colonized the Hawaiian Archipelago from tropical America. Under this scenario, the early *Begonia* species would have had to colonize Madagascar and Africa from the Americas, then become extinct in the Americas, and then later recolonize this region from Africa, a scenario similar in complexity to both the boreotopical and South Pacific-Malesian origins, but with less support given that only two of the more basal genera of Cucurbitales have species in South America (Fig. 5).

While it is impossible to be certain of *Hillebrandia*'s origins, either a South Pacific–Malesian or a boreotropical origin appears plausible. A South Pacific–Malesian origin is consistent with current theory that the vast majority of the Hawaiian flora is of Malesian, Austral, or Pacific origin (Wagner et al., 1999). A boreotropic origin is not necessarily incompatible with a species radiation from Malesia, if the boreotropical proto-*Begonia* was pushed to southern Asia from more northern latitudes. The present-day boreotropic taxa have floristic affinities to Indomalaya (Wolfe, 1975).

Conclusions—Recent molecular phylogenies have led to a better understanding of the patterns of colonization of the Hawaiian Islands and the geographic sources of such colonists (e.g., DeJoode and Wendel, 1992; Baldwin and Robichaux, 1995; Givinish et al., 1995; Lammers, 1995; Howarth et al., 1997; 2003; Pax et al., 1997; Plunkett et al., 1997; Kim et al., 1998; Ballard and Sytsma, 2000; Ganders et al., 2000; Gemmill et al., 2001; Wright et al., 2001; Lindqvist and Albert, 2002; Powell and Kron, 2002). In all these cases, the Hawaiian Islands were colonized by derived members of their families.

The position of *Hillebrandia* as sister to, rather than derived from within, the rest of the Begoniaceae and its minimal age of 51–65 my BP (predating the current Hawaiian Islands) makes *Hillebrandia* the only known relict genus in the Hawaiian flora. We suggest that *Hillebrandia* has survived on the Hawaiian Archipelago while its source populations have become extinct because the Hawaiian Islands have provided a relatively stable climate and suitable montane habitat for at least 30 million years and during that time *Hillebrandia* has island hopped from older, now-denuded islands to younger more mountainous ones. However, *Hillebrandia* must at some time have colonized the Hawaiian Archipelago. The geographic origin of these source populations unfortunately remains obscure; however, we suggest a boreotropic or a Malesian–Pacific origin is most likely. Fossil material referable to the Begoniaceae would further improve upon these conclusions, in particular the age estimate and the exact origin of *Hillebrandia*.

LITERATURE CITED

APG [ANGIOSPERM PHYLOGENY GROUP] II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141: 399–436.

BALDWIN, B. G., AND R. H. ROBICHAUX. 1995. Historical biogeographical and ecology of the Hawaiian silversword alliance (Asteraceae): new molecular phylogenetic perspectives. *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: evolution on a hotspot archipelago, 257–287. Smithsonian Institution Press, Washington, D.C., USA.

BALLARD, J. H. E., AND K. J. SYTSMA. 2000. Evolution and biogeography of the woody Hawaiian violets (*Viola*, Violaceae): arctic origins, herbaceous ancestory and bird dispersal. *Evolution* 54: 1521–1532.

BOESEWINKEL, F. D. 1984. Ovule and seed structure in Datiscaceae. *Acta Botanica Neerlandica* 33: 419–429.

BÖHLE, U.-R., H. H. HILGER, AND W. F. MARTIN. 1996. Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). *Proceedings of the National Academy of Sciences, USA.* 93: 11740–11745.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

BROUILLET, L. 2001. Begoniaceae and the Cucurbitales: a morphological phylogenetic analysis. *In* Botany 2001: plants and people, abstracts, 94. Botanical Society of America, Albuquerque, New Mexico, USA.

BULT, C., M. KÄLLERSJÖ, AND Y. SUH. 1992. Amplification and sequencing of 16/18S rDNA from gel-purified total plant DNA. *Plant Molecular Biology Reporter* 10: 273–284.

BULT, C., AND E. A. ZIMMER. 1993. Nuclear ribosomal RNA sequences for inferring tribal relationships within Onagraceae. *Systematic Botany* 18: 48–63.

CARLQUIST, S. 1974. Island biology. Columbia University Press, New York, New York, USA.

CARLQUIST, S., AND R. B. MILLER. 2001. Wood anatomy of Corynocarpaceae is consistent with Cucurbitalean placement. *Systematic Botany* 26: 54–65.

CARSON, H. L., AND D. A. CLAGUE. 1995. Geology and biogeography of the Hawaiian Islands. *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: evolution on a hotspot archipelago, 14–29. Smithsonian Institution Press, Washington, D.C., USA.

CHARPENTIER, A., L. BROUILLET, AND D. BARABÉ. 1989. Organogénèse de la fleur pistillée du *Begonia dregei* et de l'*Hillebrandia sandwicensis* (Begoniaceae). *Canadian Journal of Botany* 67: 3625–3639.

CHASE, M. W., ET AL. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 528–580.

CRONQUIST, A. 1981. An integrated system of classification of the flowering plants. Columbia University Press, New York, New York, USA.

CRUZAN, M. B., M. L. ARNOLD, S. E. CARNEY, AND K. R. WOLLENBERG. 1993. CpDNA inheritance in interspecific crosses and evolutionary inference in Louisiana irises. *American Journal of Botany* 80: 344–350.

DAVIDSON, C. 1973. An anatomical and morphological study of Datiscaeae. *Aliso* 8: 49–110.

DEJOODE, D. R., AND J. F. WENDEL. 1992. Genetic diversity and origin of the Hawaiian Island cotton, *Gossypium tomentosum*. *American Journal of Botany* 79: 1311–1319.

DESALLE, R. 1995. Molecular approaches to biogeographic analysis of Hawaiian Drosophilidae. *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: evolution on a hotspot archipelago, 72–89. Smithsonian Institution Press, Washington, D.C., USA.

DOLPHIN, K., R. BELSHAW, C. D. L. ORME, AND D. L. J. QUICKE. 2000. Noise and incongruence: interpreting the results of the ILD test. *Molecular Phylogenetics and Evolution* 17: 401–406.

DONOGHUE, M. J., R. G. OLMSTEAD, J. F. SMITH, AND J. D. PALMER. 1992. Phylogenetic relationships of Dipsacales based on *rbcL* sequences. *Annals of the Missouri Botanical Garden* 79: 333–345.

DOORENBOS, J., M. S. M. SOSEF, AND J. J. F. E. DE WILDE. 1998. The sections of *Begonia* including descriptions, keys and species lists (Studies in Begoniaceae VI). *Wageningen Agricultural University Papers* 98–2: 266.

DOSKOTCH, R. W., AND C. D. HUFFORD. 1970. Hexanor–cucurbitacin D, a degraded cucurbitacin from *Begonia tuberhybrida* var. *alba*. *Canadian Journal of Chemistry* 48: 1787–1788.

DOSKOTCH, R. W., M. Y. MALIK, AND J. L. BEAL. 1969. Cucurbitacin B, the cytotoxic principle of *Begonia tuberhybrida* var. *alba*. *Lloydia* 32: 115–122.

DOWTON, M., AND A. D. AUSTIN. 2002. Increased congruence does not necessarily indicate increased phylogenetic accuracy—the behavior of the incongruence length difference test in mixed-model analyses. *Systematic Biology* 5: 19–31.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

ECKENRODE, V. K., J. ARNOLD, AND R. B. MEAGHER. 1985. Comparison of the nucleotide sequences of soybean 18S rRNA with the sequences of other small-subunit rRNAs. *Journal of Molecular Evolution* 21: 259–269.

ERIKSSON, T. 2002. AutoDecay, version 5.0.3. Distributed by the author, Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm, Sweden.

FARRIS, J. S., M. KALLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FELSENSTEIN, J. 1988. Phylogenies from molecular sequences: inference and reliability. *Annual Journal of Genetics* 22: 521–565.

FELSENSTEIN, J. 1993. PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, Washington, USA.

FORREST, L. L., AND P. M. HOLLINGSWORTH. 2003. A recircumscription of *Begonia* based on nuclear ribosomal sequences. *Plant Systematics and Evolution* 241: 193–211.

FOSBERG, F. R. 1948. Derivation of the flora of the Hawaiian Islands. *In* E. C. Zimmerman [ed.], Insects of Hawaii, vol. 1, 107–119. University of Hawaii Press, Honolulu, Hawaii, USA.

GANDERS, F. R., M. BERBEE, AND M. PIRSEYEDI. 2000. ITS base sequence phylogeny in *Bidens* (Asteraceae): evidence for the continental relatives of Hawaiian and Marquesan *Bidens*. *Systematic Botany* 25: 122–133.

GAUTHIER, R. 1950. The nature of the inferior ovary in the genus *Begonia*. *Contributions Institut de Botanique Universite de Montréal* 66: 5–91.

GAUTHIER, R. 1959. L'anatomie vasculaire et l'interprétation de la fleur pistillée de l'*Hillebrandia sandwicensis* Oliv. *Phytomorphology* 9: 72–87.

GEMMILL, C. E. C., G. J. ALLAN, W. L. WAGNER, AND E. A. ZIMMER. 2001. Evolution of insular Pacific *Pittosporum* (Pittosporaceae): origin of the Hawaiian radiation. *Molecular Phylogenetics and Evolution* 22: 31–42.

GIVNISH, T. J., K. J. SYTSMA, J. F. SMITH, AND W. J. HAHN. 1994. Thorn-like prickles and heterophylly in *Cyanea*: adaptations to extinct avian browsers in Hawaii? *Proceedings of the National Academy of Sciences, USA* 91: 2810–2814.

GIVNISH, T. J., K. J. SYTSMA, J. F. SMITH, AND W. J. HAHN. 1995. Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae, Lobelioideae). *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: evolution on a hotspot archipelago, 288–337. Smithsonian Institution Press, Washington, D.C., USA.

HILLEBRAND, W. 1888. Flora of the Hawaiian Islands: a description of their

phanerogams and vascular cryptogams; annotated and published after the author's death by W. F. Hillebrand. B. Westermann, New York, New York, USA.

HIRATSUKA, J., ET AL. 1989. The complete sequence of the rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. *Molecular and General Genetics* 217: 185–194.

HOPE, G., AND J. TULIP. 1994. A long vegetation history from lowland Irian Jaya, Indonesia. *Palaeogeography, Palaeoclimatology and Palaeoecology* 109: 385–398.

HOTCHKISS, S., AND J. O. JUVIK. 1999. A late Quaternary pollen record from Ka'au crater, O'ahu, Hawai'i. *Quaternary Research* 52: 115–128.

HOWARTH, D. G., D. E. GARDNER, AND C. W. MORDEN. 1997. Phylogeny of the *Rubus* subgenus *Idaeobatus* (Rosaceae) and its implications toward colonization of the Hawaiian Islands. *Systematic Botany* 22: 433–441.

HOWARTH, D. G., M. H. G. GUSTAFSSON, D. A. BAUM, AND T. J. MOTLEY. 2003. Phylogenetics of the genus *Scaevola* (Goodeniaceae): implications for dispersal patterns across the Pacific Basin and colonization of the Hawaiian Islands. *American Journal of Botany* 90: 915–923.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: a program for the Bayesian inference of phylogeny, version 2.01. Computer program distributed by the authors. University of California, San Diego, California, USA.

JUNCOSA, A. M., AND B. P. TOMLINSON. 1988. A historical and taxonomic synopsis of Rhizophoraceae and Anisophyllaceae. *Annals of the Missouri Botanical Garden* 75: 1278–1295.

KIM, H.-G., S. C. KEELEY, P. S. VROOM, AND R. K. JANSEN. 1998. Molecular evidence for an African origin of the Hawaiian endemic *Hesperomannia* (Asteraceae). *Proceedings of the National Academy of Sciences, USA* 95: 15 440–15 445.

KIM, S.-C., D. J. CRAWFORD, AND R. K. JANSEN. 1996. Phylogenetic relationships among the genera of the subtribe Sonchinae (Asteraceae): evidence from ITS sequences. *Systematic Botany* 21: 417–432.

KOLBE, P.-K., AND J. JOHN. 1979. Serologische Untersuchungen zur Systematik der Violales. *Botanische Jahrbücher für Systematik Pflanzengeschichte und Pflanzengeographie* 101: 3–15.

KUPCHAN, S. M., C. W. SIGEL, L. J. GUTTMAN, R. J. RESTIVO, AND R. F. BRYAN. 1972. Datiscoside, a novel antileukemic cucurbitacin glycoside from *Datisca glomerata*. *Journal of the American Chemical Society* 94: 1353–1354.

KUZOFF, R. K., J. A. SWEERE, D. E. SOLTIS, P. S. SOLTIS, AND E. A. ZIMMER. 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. *Molecular and Biological Evolution* 15: 251–263.

LAMMERS, T. G. 1995. Patterns of speciation and biogeography in *Clermontia* (Campanulaceae, Lobelioideae) *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: evolution on a hotspot archipelago, 338–362. Smithsonian Institution Press, Washington, D.C., USA.

LANGLEY, C. H., AND W. FITCH. 1974. An estimation of the constancy of the rate of molecular evolution. *Journal of Molecular Evolution* 3: 161–177.

LANKHANPAL, R. N. 1970. Tertiary floras of India and their bearing on the historical geology of the region. *Taxon* 19: 675–694.

LINDLEY, J. 1846. The vegetable kingdom: or, the structure, classification, and uses of plants, illustrated upon the natural system. Bradbury and Evans, London, UK.

LINDQVIST, C., AND V. A. ALBERT. 2002. Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). *American Journal of Botany* 89: 1709–1724.

LORENCE, D. H. 1987. The Hawaiian begonia. *Bulletin of the Pacific Tropical Botanic Garden* 17: 130–133.

MACCAUGHEY, V. 1918. An endemic begonia of Hawaii. *Botanical Gazette* 66: 272–275.

MOORE, D. M. 1979. Origins of temperate island floras. *In* D. Bramwell [ed.], Plants and islands, 75. Academic Press, London, UK.

MULLER, J. 1981. Fossil pollen records of extant angiosperms. *Botanical Review* 47: 1–142.

NICKRENT, D. L., AND E. M. STARR. 1994. High rates of nucleotide substitution in nuclear small-subunit (18S) rDNA from holoparasitic flowering plants. *Journal of Molecular Evolution* 39: 62–70.

NULLET, D., C. H. FLETCHER, S. HOTCHKISS, AND J. O. JUVIK. 1998. Paleoclimate and geography. *In* S. P. Juvik, J. O. Juvik, and P. R. Paradise [eds.], Atlas of Hawaii, 3rd ed., 64–66. University of Hawaii Press, Honolulu, Hawaii, USA.

OLIVER, F. W. 1866. On *Hillebrandia*, a new genus of Begoniaceae. *Transactions of the Linnean Society, London* 25: 361–363.

PAX, D. L., R. A. PRICE, AND H. J. MICHAELS. 1997. Phylogenetic position of the Hawaiian geraniums based on *rbcL* sequences. *American Journal of Botany* 84: 72–78.

PISIAS, N. G., AND A. C. MIX. 1997. Spatial and temporal oceanographic variability in the eastern equatorial Pacific during the late Pleistocene: evidence from *Radiolaria* microfossils. *Paleoceanography* 12: 381–393.

PISIAS, N. G., A. ROELOFS, AND M. WEBER. 1997. Radiolarian-based transfer functions for estimating mean surface ocean temperatures and seasonal range. *Paleoceanography* 12: 365–379.

PLANA, V. 2003. Phylogenetic relationships of the Afro-Malagasy members of the large genus *Begonia* inferred from *trnL* intron sequences. *Systematic Botany* 28: 693–704.

PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1997. Clarification of the relationship between Apiaceae and Araliaceae based on *matK* and *rbcL* sequence data. *American Journal of Botany* 84: 565–580.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

POWELL, E. A., AND K. A. KRON. 2002. Hawaiian blueberries and their relatives—A phylogenetic analysis of *Vaccinium* sections *Macropetalum*, *Myrtillus*, and *Hemimyrtillus* (Ericaceae). *Systematic Botany* 27: 768–779.

REITSMA, T. 1983. Placentation in Begonias from the African continent. In J. J. F. E. de Wilde [ed.], Studies in Begoniaceae I, vols. 83–9, 21–53. Agricultural University, Wageningen, The Netherlands.

RIESEBERG, L. H., M. A. HANSON, AND C. T. PHILBRICK. 1992. Androdioecy is derived from dioecy in Datiscaceae: evidence from restriction site mapping of PCR-amplified chloroplast DNA fragments. *Systematic Botany* 17: 324–336.

SANDERSON, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* 12: 1218–1231.

SANDERSON, M. J. 2002a. r8s. Analysis of rates (''r8s'') of evolution (and other stuff), version 1.5. Section of Evolution and Ecology, University of California, Davis, California, USA.

SANDERSON, M. J. 2002b. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101–109.

SANDERSON, M. J., AND J. A. DOYLE. 2001. Sources of error and confidence intervals in estimating the age of angiosperms from *rbcL* and 18S rDNA data. *American Journal of Botany* 88: 1499–1516.

SAPORTA, G. 1865. Études sur la végétation du sud-est de la France l'époque tertiaire. *Annales des Sciences Naturelles Botanique, series 5* 3: 5–264.

SCHWARZBACH, A. E., AND R. E. RICKLEFS. 2000. Systematic affinities of Rhizophoraceae and Anisophyllaceae, and intergeneric relationships within Rhizophoraceae based on chloroplast DNA, nuclear ribosomal DNA, and morphology. *American Journal of Botany* 87: 547–564.

SOLTIS, D. E., ET AL. 2000. Angiosperm phylogeny inferred from a combined data set of 18S rDNA, *rbcL*, and *atpB* sequences. *Botanical Journal of the Linnean Society* 133: 381–461.

STUTE, M., M. FORSTER, H. FRISCHKORN, A. SEREJO, J. F. CLARK, P. SCHLOSSER, W. S. BROECKER, AND G. BONANI. 1995. Cooling of tropical Brazil (5°C) during the last glacial maximum. *Science* 269: 379–383.

SWENSEN, S. M. 1996. The evolution of actinorhizal symbioses: evidence for multiple origins of the symbiotic association. *American Journal of Botany* 83: 1503–1512.

SWENSEN, S. M., W. L. CLEMENT, L. L. FORREST, AND M. C. TEBBITT. 2001. *Hillebrandia sandwicensis*: Evolutionary relationships and biogeography. *In* Botany 2001: plants and people, abstracts; 95. Botanical Society of America, Albuquerque, New Mexico, USA.

SWENSEN, S. M., J. N. LUTHI, AND L. H. RIESEBERG. 1998. Datiscaceae revisited: monophyly and the sequence of breeding system evolution. *Systematic Botany* 23: 157–169.

SWOFFORD, D. L. 2001. PAUP*: phylogenetic analysis using parsimony and other methods, version 4.0. Sinauer, Sunderland, Massachusetts, USA.

TAKHTAJAN, A. 1997. Diversity and classification of flowering plants. Columbia University Press, New York, New York, USA.

TEBBITT, M. C., L. L. FORREST, W. S. HOOVER, AND S. M. SWENSEN. 2001. Relationships of fleshy-fruited Asiatic *Begonia* (Begoniaceae) based on ITS sequence data and restriction site mapping of PCR amplified chloroplast and mitochondrial fragments. *In* Botany 2001: plants and people,

abstracts, 95. Botanical Society of America, Albuquerque, New Mexico, USA.

THOMPSON, J. D., D. G. HIGGINS, AND T. B. GIBSON. 1994. Clustal W: improving the sensitivity of progressive multiple alignment through sequence weighting, position specific gap penalties, and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.

THOMPSON, P. N., AND R. J. GORNALL. 1995. Breeding systems in *Coriaria* (Coriariaceae). *Botanical Journal of the Linnean Society* 117: 293–304.

THORNE, R. F. 1992. Classification and geography of flowering plants. *Botanical Review* 58: 225–348.

VAN DEN BERG, R. G. 1983. Pollen characteristics of the genera of the Begoniaceae. *Mededeling Landbouwhogeschool Wageningen* 83: 55–66.

WAGNER, W. L., D. R. HERBST, AND S. H. SOHMER. 1999. Manual of the flowering plants of Hawaii, revised ed. Bishop Museum Special Publication 97. University of Hawaii Press, Honolulu, Hawaii, USA.

WAGSTAFF, S. J., AND M. I. DAWSON. 2000. Classification, origin, and patterns of diversification of *Corynocarpus* (Corynocarpaceae) inferred from DNA sequences. *Systematic Botany* 25: 134–149.

WHITE, T. J., T. BRUNS, S. LEF, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White [eds.], PCR protocols: a guide to methods and applications, 315–322. Academic Press, New York, New York, USA.

WOLFE, J. A. 1975. Some aspects of plant geography of the northern hemisphere during the late cretaceous and tertiary. *Annals of the Missouri Botanical Garden* 62: 264–279.

WRIGHT, S. D., C. G. YONG, S. R. WICHMAN, J. W. DAWSON, AND R. C. GARDNER. 2001. A trans–equatorial dispersal pathway for *Metrosideros* (Myrtaceae) inferred from nrDNA (ITS + ETS). *Journal of Biogeography* 28: 769–774.

YODER, A. D., J. A. IRWIN, AND B. A. PAYSEUR. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* 50: 408–424.