Molecular Phylogenetics and Evolution
Vol. 22, No. 1, January, pp. 31–42, 2002
doi:10.1006/mpev.2001.1019, available online at http://www.idealibrary.com on IDEAL®

Evolution of Insular Pacific *Pittosporum* (Pittosporaceae): Origin of the Hawaiian Radiation

C. E. C. Gemmill,*[,1,3] G. J. Allan,*[,2,3] W. L. Wagner,† and E. A. Zimmer*

*Laboratory of Molecular Systematics, National Museum of Natural History, Smithsonian Institution, Museum Support Center, MRC 534, 4210 Silver Hill Road, Suitland, Maryland 20746; and †Department of Botany, MRC 166, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0166

Received November 7, 2000; revised May 10, 2001; published online December 5, 2001

We investigated the origin of Hawaiian *Pittosporum* and their relationship to other South Pacific *Pittosporum* species using internal transcribed spacer sequences of nuclear ribosomal DNA. We performed both maximum-parsimony and maximum-likelihood analyses, which produced congruent results. Sequence divergence was 0.0% between Hawaiian members of *Pittosporum*. These taxa formed a strongly supported clade, suggesting a single colonization event followed by phyletic radiation. Sister to the Hawaiian clade were two South Pacific species, *P. yunckeri* from Tonga and *P. rhytidocarpum* from Fiji. This result presents convincing evidence for a South Pacific origin of Hawaiian *Pittosporum*. Our results also identify a monophyletic group comprising three species representing the Fijian Province and East Polynesia, two introductions onto New Caledonia, and at least one (but possibly two) introduction(s) onto New Zealand. Whether the New Zealand taxa form a monophyletic group is unclear from these data. Previous morphologically based hypotheses, however, suggest the presence of four different lineages occupying New Zealand. The nonmonophyly of the New Caledonian species was not surprising based on the extent of their morphological diversity. Although this latter result is not strongly supported, these species are morphologically complex and are currently the subject of taxonomic revision and molecular systematic analyses.

INTRODUCTION

Before the colonization of the Hawaiian Islands by the Polynesians 1600 years ago (Kirch, 1982), the Hawaiian flora evolved in isolation for at least 40 million years, producing one of the more distinctive biotas in the world. Of the approximately 1000 species of native Hawaiian flowering plants, 89% are found only on these islands (Wagner *et al.*, 1990; Sakai *et al.*, 1995), representing the highest degree of endemism for any known flora. The insular nature of the flora is due to various factors, including a limited number of original colonizers, radiation in a unique insular environment isolated from congeners, diverse geographic origins, and the unbalanced representation of certain groups relative to their nearest mainland congeners (Fosberg, 1948; Wagner *et al.*, 1990). The Hawaiian flora also is characterized by striking examples of phyletic radiations, which produced complexes of diverse, yet closely related species, most of which are single-island endemics. These features make the Hawaiian archipelago an excellent locality for the study of patterns and processes of organismal evolution (e.g., Simon, 1987).

Considering the importance of the Hawaiian flora, Fosberg (1948) noted that "Especially little has been written specifically on the relationships of Hawaiian genera and species to their relatives elsewhere." Since the time that this observation was made, a number of studies have addressed evolutionary relationships among Hawaiian relatives. The most celebrated of these are the works on the silversword alliance (Asteraceae; Baldwin, 1992; Baldwin *et al.*, 1990; Baldwin and Robichaux, 1995; Carr *et al.*, 1989; Witter, 1990), Hawaiian *Drosophila* (Drosophilidae; e.g., Carson and Kaneshiro, 1976), and the Hawaiian honeycreepers (Fringillidae; Johnson *et al.*, 1989; Tarr and Fleischer, 1995; Fleischer *et al.*, 1998). However, the origin and diversification of many taxa remain unclear. One contribution to our understanding of evolutionary patterns and processes within the Hawaiian Islands is the recent collaborative publication on contemporary cladistic research of Hawaiian organisms by Wagner and Funk (1995). This work emphasizes the value of the utilization of insular systems for the study of evolution

[1] Present address: Centre for Biodiversity and Ecology Research, Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand 2001.

[2] Present address: Department of Biology, Arcadia University, 450 South Easton Road, Glenside, PA 19038–3295.

[3] These authors contributed equally to this work.

1055-7903/01 $35.00

and is a practical approach to extend to the study of archipelagoes of the South Pacific.

The angiosperm genus *Pittosporum* is the only member of the Pittosporaceae (Asteridae) that extends beyond the Australian continent to the east and occupies archipelagoes in the South Pacific and the Hawaiian Islands (Fig. 1). In addition to Australia, the primary center of diversity, species of *Pittosporum* occur throughout tropical and warm temperate regions of New Zealand (which also has cool temperate and sub-Antarctic representation), Africa, Asia, and Malesia (Haas, 1977). The highest levels of endemism, however, occur on the larger islands of the Pacific (i.e., New Caledonia and New Zealand) and the Hawaiian Islands (Haas, 1977; Wagner *et al.*, 1990). Distributional patterns of *Pittosporum* throughout the remote islands of the Pacific are believed to be the result of long-distance dispersal of the black, resin-covered seeds via birds (Carlquist, 1974). Once established, species radiations are common within *Pittosporum*, as demonstrated by the large numbers of insular endemic taxa in the Hawaiian Islands (11 species; Wagner *et al.*, 1990), New Zealand (26 species; Allan, 1961), and New Caledonia (ca. 50 species; J.-M. Veillon, ORSTOM, pers. comm.). Of the 11 Hawaiian species, 7 are single-island endemics. These are found on the islands of Kauaʻi (3 spp.), Oʻahu (1 sp.), Molokaʻi (1 sp.), and Hawaiʻi (2 spp.). Other South Pacific islands that have endemic species include the Austral (also known as Tubuai; about 7 spp.), Fiji (3 spp.), Vanuatu (2 spp.), Samoa (1 sp.), Tonga (1 sp.), and Society (2 spp.) archipelagoes.

Hawaiian *Pittosporum*, or *hoʻawa*, form a morphologically complex assemblage with many polymorphic, overlapping characters (e.g., leaf size and shape and fruit shape, size, and extent of sculpturing) and exhibit the greatest degree of morphological variability among the South Pacific members of this genus, including sexual expression. Whereas most Hawaiian species are believed to have functionally unisexual flowers, at least one member, *P. confertiflorum*, has bisexual flowers also. Based on this and general morphological similarity, Haas (1977) hypothesized that the Hawaiian species evolved from a single long-distance immigration event from the South Pacific. Two endemic Fijian species (*P. rhytidocarpum* and *P. oligodontum*) with bisexual flowers and one endemic Tongan species (*P. yunckeri*) with unisexual flowers have been suggested as the progenitors of the Hawaiian complex and as most closely related to the Hawaiian species *P. confertiflorum* and *P. terminalioides*, respectively (Haas, 1977; Wagner *et al.*, 1990). Recent collections of mature capsules of *P. yunckeri* indicate an affinity with *P. terminalioides*, as both taxa have large, smooth, woody capsules (C. E. C. Gemmill, pers. obs.). The Hawaiian species are presumed to form a monophyletic lineage, although this hypothesis has not been rigorously tested.

Analyses of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) have proven particularly useful for uncovering the evolutionary and biogeographic history of a number of flowering plant lineages found in the Hawaiian Islands. Recent investigations have encompassed assessments of plant evolutionary and biogeographic relationships (Baldwin and Robichaux, 1995; Ganders *et al.*, 2000; Soltis and Soltis, 1998), geographic provenance and dispersal patterns (Wright *et al.*, 2000), age and rate diversification (Baldwin and Sanderson, 1998), and adaptive radiation (Baldwin, 1997). We examined the geographic origin and evolution of the insular Pacific members of *Pittosporum* using parsimony and maximum-likelihood analyses of nrDNA sequence variation. In particular, we investigated (a) whether the Hawaiian species are monophyletic, (b) how genetically divergent the Hawaiian members are from each other and from other Pacific *Pittosporum*, and (c) what the likely geographic origin of Hawaiian *Pittosporum* is.

MATERIALS AND METHODS

Plant Collection and DNA Isolation

Twenty-two ingroup taxa representing five South Pacific taxa, three New Zealand species, five New Caledonian species, and 9 of the 11 currently recognized Hawaiian taxa were included in this study (Table 1). The Hawaiian taxa represent the complete morphological and ecological range for Hawaiian *Pittosporum*. Two species, *P. undulatum* (Australia) and *P. balfouri* (Mauritius), were selected as putative outgroup species. These taxa were chosen based on preliminary phylogenetic analyses. For most taxa, total DNA was extracted from fresh leaf tissue. However, for some accessions, either herbarium or silica-dried material was used (see Table 1). DNA was isolated with a modified CTAB protocol (Doyle and Doyle, 1987) or with the Dneasy Mini Plant Kit (Qiagen).

Amplification and Sequencing of the ITS Region

The ITS region comprises two internal transcribed spacers, ITS1 and ITS2, and the 5.8S subunit of the nrDNA cistron (Baldwin, 1992; Baldwin *et al.*, 1995). The ITS region was amplified by polymerase chain reaction (PCR) with the 3′ universal eukaryote primer ITS-4 (White *et al.*, 1990) and the slightly modified "higher-plant" primer (ITS-5 HP, 5′-GGAAGGAGAAGTCGTAACAAGG-3′; Laboratory of Molecular Systematics (LMS), Smithsonian Institution). PCR was performed in a 100-μL total volume containing 25 mM $MgCl_2$, 2.5 mM each dNTP, 20 μM each primer, 10.0 μL of 10 μl *Taq* buffer, 5 μL dimethyl sulfoxide, 1 unit of *Taq* polymerase, and approximately 30 ng of

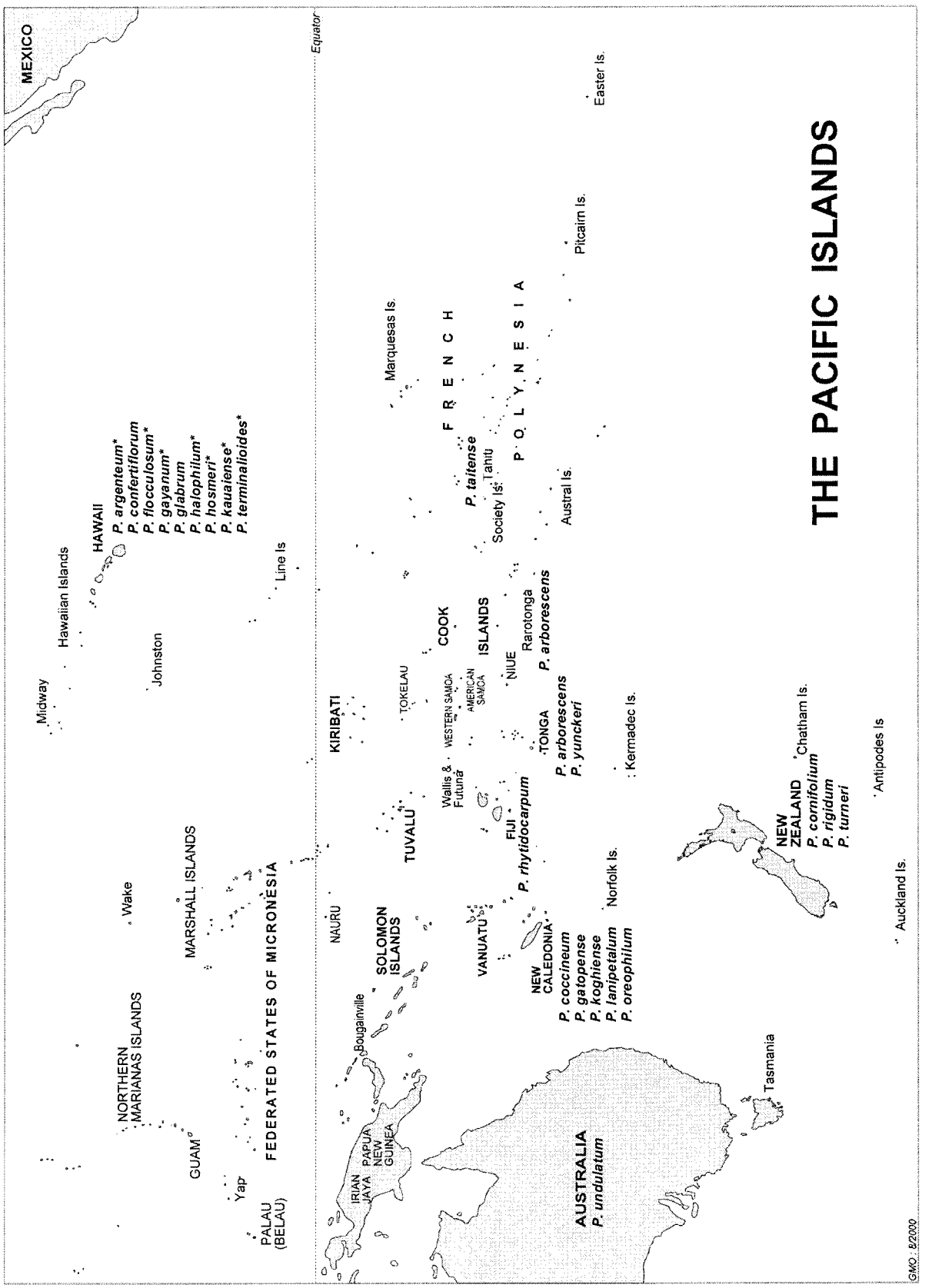

FIG. 1. Map of the Pacific Islands showing localities of the taxa included in this study. An asterisk indicates a Hawaiian single-island endemic. Note that *P. balfouri* from the island of Mauritius off the eastern coast of Africa is not shown.

TABLE 1

Taxa Sequenced for the ITS Region of Nuclear Ribosomal DNA along with Locality, Voucher, and GenBank Accession Nos.

Taxon	Locality	Voucher	GenBank Accession No.
Pittosporum arborescens Rich ex A. Gray	Kingdom of Tonga, Vava'u	CECG311*	AF302026
P. arborescens	Cook Islands, Rarotonga	CECG215*	AF302025
P. argentifolium Sherff	Hawaiian Islands, Moloka'i	CECG282+	AF302016
P. balfouri Cufod.	Mauritius	WFP91S3+	AF302015
P. coccineum Beauvis.	New Caledonia, Poya	JMV8146*	AF302033
P. confertiflorum A. Gray	Hawaiian Islands, O'ahu	CECG277+	AF302017
P. cornifolium A. Cunn.	New Zealand, cultivated	CECGp1–4+	AF302030
P. flocculosum (Hillebr.) Sherff	Hawaiian Islands, O'ahu	CECG229+	AF302018
P. gatopense Guillaumin	New Caledonia, Poya	JMV8129*	AF302034
P. gayanum Rock	Hawaiian Islands, Kaua'i	CECG255+	AF302019
P. glabrum Hook. & Am.	Hawaiian Islands, Kaua'i	CECG244+	AF302020
P. halophilum Rock	Hawaiian Islands, Moloka'i	NTBG950469+	AF302021
P. hosmeri Rock	Hawaiian Islands, Hawai'i	CECG239+	AF302022
P. kauaiensis Hillebr.	Hawaiian Islands, Kaua'i	NTBG950696+	AF302023
P. koghiense Guillaumin	New Caledonia, Mt. Dmuzac	CECG343*	AF302035
P. lanipetalum Tirel & Veillon	New Caledonia, Roche Ouaieme	JMV8170*	AF302036
P. oreophilum Guillaumin	New Caledonia, Mt. Koghi	CECG333*	AF302037
P. rhytidocarpum A. Gray	Fiji, Lomilagi Mountain	W2190202+	AF302029
P. rigidum Hook.	New Zealand, South Island	CECG298*	AF302031
P. taitense Putterl.	Society Islands, Bora Bora, Mt. Pahia	JYM612*	AF302027
P. terminalioides Planch ex A. Gray	Hawaiian Islands, Hawai'i	CECG240+	AF302024
P. turneri Petrie	New Zealand, North Island	CECG287*	AF302032
P. undulatum Guill.	Australia, cultivated	Bradford874*	AF302014
P. yunckeri A. C. Smith	Kingdom of Tonga, 'Eua	CECG303*	AF302028

Note. Taxonomy follows Wagner *et al.* (1990). Plants in cultivation at the National Tropical Botanical Garden (NTBG), Kaua'i, Hawaiian Islands, are from wild-collected seed. An asterisk (*) or plus sign (+) indicates either dried or fresh leaf material used for DNA extraction.

template DNA. The profile for PCR amplification included an initial denaturation cycle of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 48°C, 30 s at 72°C, and a final extension cycle of 7 min at 72°C. Samples were maintained at 4°C following amplification.

PCR products were purified from primers and dNTPs with either polyethylene glycol (PEG) or QIAquick PCR Purification kit (Qiagen). Cycle sequencing of these purified products was performed with the AmpliCycle Sequencing kit (Perkin–Elmer) and the following PCR conditions: 1 cycle of 4 min at 96°C and 25 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C. Samples were maintained at 4°C following cycle sequencing. Cycle sequencing products were purified with a series of 95% and then 75% ethanol washes. Direct automated sequencing of double-stranded PCR products was performed with Big Dye chemistry on an ABI automated sequencer, both at the LMS as described in Wen and Zimmer (1996) and at the University of Waikato's DNA Sequencing Unit. Two accessions (*P. rhytidocarpum* and *P. yunckeri*) were sequenced with FS chemistry following the manufacturer's protocol. Sequences were aligned by eye following initial alignments of gel trace files with Sequencher 3.1.1 software (Gene Codes, Inc.). GenBank accession numbers are given in Table 1, and the aligned sequences are available from the authors.

Data Analysis

Phylogenetic analyses were conducted with maximum-parsimony (MP) and maximum-likelihood (ML) methods. Parsimony analyses were performed via the heuristic search mode of PAUP* (Swofford, 1998) with DELTRAN optimization, TBR branch swapping option, and random addition of taxa. Gaps were treated as missing data. Character states (nucleotides) were treated as unordered and trees were rooted with *P. undulatum* and *P. balfouri*. Relative support of clades was assessed in a bootstrap analysis (Felsenstein, 1985) with a heuristic search mode (CLOSEST addition, TBR swapping, MULPARS, and DELTRAN options) and 100 replicates.

For molecular sequence data, recent theoretical work (e.g., Sullivan *et al.*, 1999) suggests that maximum-likelihood analyses also are powerful methods when used in combination with parsimony. Using a modification of the search strategy outlined by Sullivan *et al.* (1999), we generated a starting tree using maximum-parsimony and then four different substitution models, (1) Jukes–Cantor (Jukes and Cantor, 1969), (2) Kimura two-parameter (Kimura, 1980), (3) Hasegawa–

Kishino–Yano (Hasegawa *et al.*, 1985), and (4) a general time-reversible model (see REV of Yang, 1994), in combination with four models of among-site rate variation, (1) all sites with equal rates, (2) proportion of sites assumed to be variable with equal rate associated at variable sites (Hasegawa *et al.*, 1985; I), (3) rates of all sites assumed to follow a discrete approximation (Yang, 1994) of the gamma distribution (Γ), and (4) some sites assumed to be invariable with Γ-distributed rates at variable sites (Gu *et al.*, 1995). Maximum-likelihood heuristic searches were performed with the preferred model as determined by higher log likelihood values (GTR base substitution, with among-site heterogeneity and gamma rates), stepwise addition, and TBR swapping. A successive approximation approach (Farris, 1969; Swofford *et al.*, 1996) was employed to find the best trees (as identified by log likelihood values) until the same tree was found in all iterations.

RESULTS

ITS Sequence Variation

The lengths of ITS1 and ITS2 ranged from 171 to 207 bp (mean of 201) and from 217 to 225 bp (mean of 219), respectively. A majority of species showed a uniform length of 203 bp in the ITS1 region. However, two South Pacific taxa, *P. yunckeri* and *P. rhytidocarpum*, had notably shorter lengths (171 bp). Most species exhibited equal lengths in the less-variable ITS2 region (219 bp). The 5.8S region was of uniform length, and identical in sequence, for all taxa (168 bp). GC content ranged from 56 to 63% with an average of 59% in ITS1 and from 57 to 67% with an average of 60% in ITS2. A χ^2 test showed that nucleotide composition in ITS1 and ITS2 across all taxa, and between the two spacers, was not significantly different ($P > 0.9$).

The percentages of potentially informative sites were 24.8% and 18.2% for ITS1 and ITS2, respectively. The numbers of variable and constant nucleotide positions for ITS1 and ITS2 were 32 and 125, and 44 and 145, respectively. Manual alignment of the sequences required 45 gaps in ITS1 and 15 in ITS2. The number of single-base indels for ITS1 was 5, whereas that of ITS2 was 3. There were three indels of 5 or more bases in the spacer regions: two indels (32 bases for both *P. yunckeri* and *P. rhytidocarpum*) in ITS1 and one indel (7 bases for all taxa except *P. undulatum*) in ITS2. There were no indels in the 5.8S region. Pairwise sequence divergence (uncorrected for multiple hits) across all taxa ranged from 20.1% in ITS1 to 18.0% in ITS2 with an average of 10.2% across the two spacers. Pairwise sequence divergence among all Hawaiian taxa was 0.0% (Table 2). Taxa exhibiting the least amount of sequence divergence from the Hawaiian group were *P. rhytidocarpum* (1.5%) and *P. yunckeri* (1.7%).

Phylogenetic Relationships

The parsimony analysis with gaps treated as missing data yielded two most parsimonious trees of 283 steps, a consistency index (CI) of 0.71, a retention index (RI) of 0.80, and a rescaled consistency (RC) index of 0.70. By comparison, the maximum-likelihood tree was 287 steps in length, with CI, RI, and RC equal to 0.70, 0.85, and 0.59, respectively. The strict consensus of the two MP trees is shown in Fig. 2A. The two MP trees differ in their resolution of the two *P. arborescens* specimens (one each from Tonga and the Cook Islands) and *P. taitense*: either the Cook Island *P. arborescens* and *P. taitense* were each other's closest relatives, with *P. arborescens* from Tonga sister to both, or the relationships among the three samples were unresolved. In the ML tree (Fig. 2B), relationships among these three samples are similarly unresolved. In rooted analyses, *P. balfouri* was sister to a large clade containing all other representatives of *Pittosporum*. In both MP and ML unrooted analyses (preliminary analyses not shown), *P. undulatum* and *P. balfouri* were resolved as sisters to the remaining species.

The ingroup comprises several major clades. Both MP and ML analyses robustly support (100%) the placement of *P. rhytidocarpum* and *P. yunckeri* as the most closely related lineage to all Hawaiian taxa. Members of the Hawaiian group, in turn, form a strongly supported (99%) monophyletic group. However, relationships within the Hawaiian clade are unresolved. The New Caledonian species *P. gatopense* is sister to *P. rhytidocarpum* and *P. yunckeri*, but its placement is only weakly supported (62%). Both MP and ML analyses weakly support the monophyly of the remaining New Caledonian taxa. An unresolved clade composed of two accessions of *P. arborescens* (one from East Polynesia and one from the Fijian Province) and *P. taitense* (from East Polynesia) forms a strongly supported (100%) monophyletic group. Another clade comprising two New Zealand species (*P. rigidum* and *P. turneri*) is also strongly supported (100%) as monophyletic. However, it is unclear whether the New Zealand taxa form a monophyletic group; the position of *P. rigidum* and *P. turneri* relative to *P. cornifolium* is unresolved.

DISCUSSION

Origin and ITS Evolution of Hawaiian Pittosporum

Both MP and ML analyses strongly support (98 and 99%, respectively) the monophyly of Hawaiian *Pittosporum* (Figs. 2A and 2B). In addition, seven synapomorphic nucleotide positions (five in ITS1 and two in ITS2) identify the Hawaiian taxa as a distinct group (Fig. 3). Primary morphological features that support the monophyly of the Hawaiian taxa are the increased seed and fruit sizes and the relative woodiness of the

TABLE 2

Pairwise Sequence Divergences (Uncorrected for Multiple Hits) for *Pittosporum* ITS Data

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	*P. undulatum*																							
2	*P. balfouri*	0.089																						
3	*P. argenteum*	0.145	0.170																					
4	*P. confertiflorum*	0.145	0.170	**0**																				
5	*P. flocculosum*	0.145	0.170	**0**	**0**																			
6	*P. gayanum*	0.145	0.170	**0**	**0**	**0**																		
7	*P. glabrum*	0.145	0.170	**0**	**0**	**0**	**0**																	
8	*P. halophilum*	0.145	0.170	**0**	**0**	**0**	**0**	**0**																
9	*P. hosmeri*	0.145	0.170	**0**	**0**	**0**	**0**	**0**	**0**															
10	*P. kauaiensis*	0.146	0.170	**0**	**0**	**0**	**0**	**0**	**0**	**0**														
11	*P. terminalioides*	0.145	0.170	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													
12	*P. arborescens (C)*	0.101	0.132	0.134	0.134	0.134	0.134	0.134	0.134	0.134	0.134	0.134												
13	*P. arborescens* (T)	0.086	0.117	0.124	0.124	0.124	0.124	0.124	0.124	0.124	0.124	0.124	**0.017**											
14	*P. taitense*	0.096	0.122	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128	0.128	**0.026**	**0.009**										
15	*P. yunckeri*	0.147	0.166	**0.017**	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.124	0.113	0.118									
16	*P. rhytidocarpum*	0.145	0.164	**0.015**	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.121	0.111	0.116	**0.008**								
17	*P. cornifolium*	0.131	0.158	0.174	0.174	0.174	0.174	0.174	0.174	0.174	0.174	0.174	0.148	0.135	0.145	0.181	0.179							
18	*P. rigidum*	0.064	0.110	0.124	0.124	0.124	0.124	0.124	0.124	0.124	0.124	0.124	0.108	0.098	0.102	0.121	0.119	**0.111**						
19	*P. turneri*	0.062	0.107	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.105	0.095	0.100	0.124	0.122	**0.109**	**0.002**					
20	*P. coccineum*	0.088	0.129	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.141	0.126	0.136	0.149	0.147	0.135	0.086	0.083				
21	*P. gatopense*	0.093	0.124	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.141	0.126	0.133	0.121	0.119	0.140	0.088	0.090	**0.112**			
22	*P. koghiense*	0.069	0.110	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.127	0.112	0.116	0.121	0.119	0.130	0.071	0.069	**0.078**	**0.109**		
23	*P. lanipetalum*	0.064	0.110	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.112	0.098	0.107	0.117	0.114	0.114	0.062	0.059	**0.071**	**0.098**	**0.033**	
24	*P. oreophilum*	0.120	0.157	0.158	0.158	0.158	0.158	0.158	0.158	0.158	0.158	0.158	0.156	0.143	0.146	0.158	0.156	0.155	0.112	0.112	**0.127**	**0.150**	**0.088**	**0.079**

Note. Comparisons discussed in the text are highlighted in boldface. Brackets indicate within-region comparisons. Localities for *P. arborescens*: C, Cook Islands; T, Tonga.

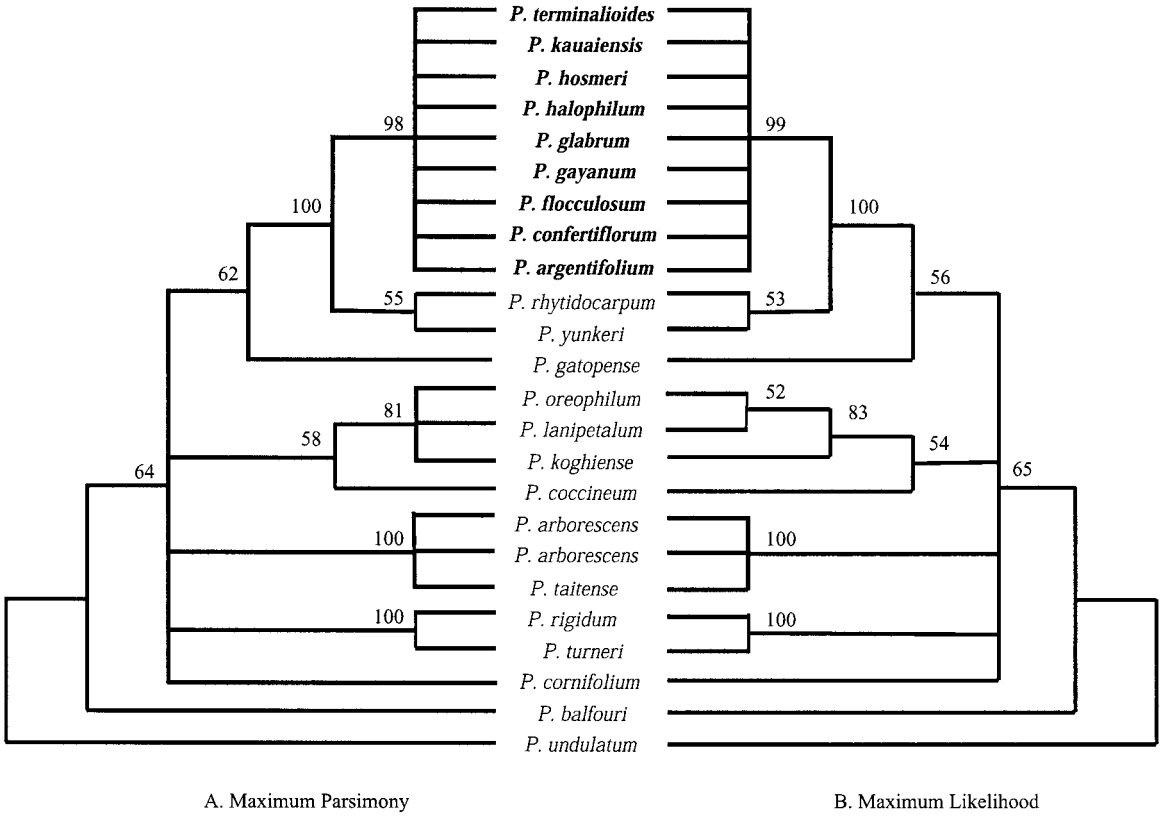

FIG. 2. (A) Maximum-parsimony strict consensus of two trees based on ITS sequences of 24 *Pittosporum* taxa. Numbers indicate bootstrap values based on 100 replicates. (B) Maximum-likelihood tree of ITS sequences for 24 *Pittosporum* taxa. Branches show bootstrap values from 100 replicates. Hawaiian *Pittosporum* are in boldface.

capsules in all Hawaiian species, as compared to other non-Hawaiian *Pittosporum* (Carlquist, 1974). Indeed, these reproductive features serve as the primary basis for distinguishing among species of Hawaiian *Pittosporum*, although differences in leaf texture and leaf indumentum are also useful (Wagner *et al.*, 1990). However, the level of morphological divergence among species is modest compared to other well-known endemic species groups (e.g., the Hawaiian silversword alliance). This moderate level of morphological divergence in Hawaiian *Pittosporum* closely matches the low levels of molecular divergence reported here.

The low levels of sequence divergence in the ITS region did not allow resolution of relationships within the Hawaiian clade. We believe that this lack of resolution may be indicative of a relatively recent origin for Hawaiian *Pittosporum* (i.e., a hard polytomy). Alternatively, the lack of resolution may be due to a slow rate of evolution in the ITS region. Other studies employing ITS, however, do not corroborate this latter hypothesis. Indeed, most molecular phylogenetic studies involving insular Pacific taxa (e.g., P. S. Soltis *et al.* (unpubl.), *Schiedea–Alsinidendron*; Baldwin and Robichaux, 1995; Baldwin *et al.*, 1998; silversword alliance, Wright *et al.*, 2000, *Metrosideros*) find levels of ITS evolution that are similar to those reported here. Consequently, we suggest that the lack of resolution among the Hawaiian taxa is indicative of a relatively recent phyletic radiation in the Hawaiian archipelago. This view is further supported by the fact that the ITS region appears to be sufficient for resolving relationships among members of other insular Pacific clades identified in this study (e.g., the New Caledonian clade), which is consistent with these clades occupying geologically older island systems (Kroenke, 1996).

ITS Sequence Divergence

Members of Hawaiian *Pittosporum* are the least genetically divergent from two other South Pacific taxa, *P. rhytidocarpum* and *P. yunckeri* (see Table 2), which show sequence divergence of 1.5 and 1.7%, respectively. *P. rhytidocarpum* and *P. yunckeri*, in turn, are highly genetically similar, with only 0.8% sequence divergence from each other. The next divergent taxon to the Hawaiian clade is *P. gatopense*, which exhibits seven times the sequence divergence observed in *P. rhytidocarpum* and *P. yunckeri* (12.2%). As cited above, these results are comparable to those observed in other studies involving Hawaiian sister groups (e.g., Baldwin and Sanderson, 1998).

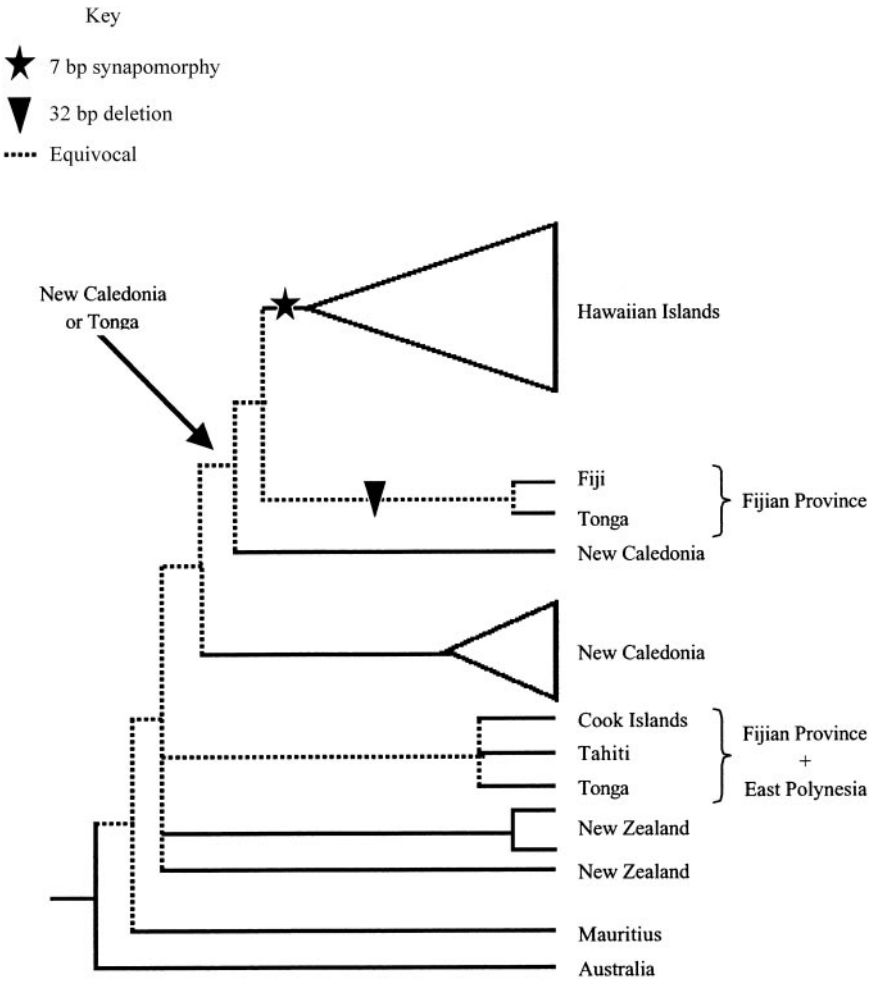

FIG. 3. Area cladogram showing the biogeographic relationships of regions inhabited by *Pittosporum*. South Pacific Fijian and Tongan areas are sister to the Hawaiian Islands. Note that two characters have been mapped onto the tree to show their phylogenetic distribution.

Members of the New Caledonian clade differ from each other on average by 8.4% and from the Hawaiian taxa by 13.7%. Within the mixed Fijian–East Polynesia clade, sequences diverge by 1.7% overall and by 12.9% compared to the Hawaiian taxa. The two New Zealand sister taxa diverge only by 0.2%, but differ by 12.5% in their sequences from the Hawaiian taxa.

One viable alternative to use of the ITS region for the resolution of relationships among closely related taxa may involve the use of the external transcribed spacer (ETS) region of nrDNA. This region has been successfully employed to resolve relationships among closely related species of the Hawaiian silversword alliance (Asteraceae; Baldwin and Markos, 1998) and appears to show useful levels of sequence divergence in Hawaiian Alsinoideae (Caryophyllaceae; Nepokroeff *et al.*, 2001). We are currently investigating the utility of the ETS region for the resolution of relationships among Hawaiian and South Pacific *Pittosporum*.

Geographic Origin of the Hawaiian Lineage

ITS analyses with both MP and ML methods reveal that Hawaiian *Pittosporum* are most closely related to Fijian *P. rhytidocarpum* and Tongan *P. yunckeri*. This finding suggests that the most likely landmass of origin for the progenitor of the Hawaiian lineage is the Fijian Province (including both Fiji and Tonga). This is supported by the monophyly of the Hawaiian taxa coupled with high bootstrap values (98 and 99%, respectively), which identify the Tongan and Fijian species as the sister group to the Hawaiian clade. The Fijian–Hawaiian biogeographic relationship is consistent with a morphological cladistic analysis of Australian *Pittosporum* (Cayzer *et al.*, 2000), which identifies a sister-taxon relationship between another Fijian species, *P. brackenridgei*, and Hawaiian *P. confertiflorum* (sole Hawaiian representative in this study).

An alternative explanation for the geographic origin of the Hawaiian lineage is that the Hawaiian members,

along with *P. rhytidocarpum* and *P. yunckeri,* are together derived from a single common ancestor. This alternative hypothesis, however, does not alter our conclusion that the Hawaiian lineage is of South Pacific origin and is the result of a single introduction event. This conclusion is consistent with Fosberg's (1948) inference of a single colonization event of Indo-Pacific affinity based on morphology. This idea was elaborated on by Haas (1977) who specifically identified *P. yunckeri* and *P. rhytidocarpum* as the likely progenitors of the Hawaiian lineage and placed them together into the *P. rhytidocarpum* group. Using data gathered from capsule and flower morphology, she hypothesized a single introduction via long-distance dispersal from the South Pacific to the Hawaiian Islands. Haas (1977) and Wagner *et al.* (1990) also suggested that the Hawaiian endemic *P. confertiflorum* was closest, morphologically, to the putative ancestor of Hawaiian *Pittosporum*. Alternatively, Wagner *et al.* (1990) suggested that the morphology of *P. terminalioides* may be more like that of the common ancestor. Given the lack of sufficient sequence divergence among members of the Hawaiian clade, however, we can neither affirm nor reject these hypotheses.

An interesting outcome of our findings is that in unrooted MP and ML analyses the Australian species, *P. undulatum,* and then the Mauritian species, *P. balfouri,* are successive sisters to the remaining species in the ITS phylogeny. That we designate *P. undulatum* as a member of the outgroup, however, precludes identification of Australia as the definitive center of origin for *Pittosporum*. Nevertheless, the idea that Australia is a center of species diversity for *Pittosporum* is not new. Cooper (1956), for example, recognized nine genera of Pittosporaceae (of which *Pittosporum* is the largest and the only one found outside Australia), which include a total of 48 species endemic to Australia. Thus, identification of Australia as a center of origin is a viable hypothesis, but one that requires further testing. We anticipate conducting direct tests of this hypothesis by including additional Australian taxa and other members representing the full geographic distribution of *Pittosporum* (e.g., Africa [1 sp.], Indo-China [approx. 11 spp.], and India [5 spp.]).

Despite the fact that we cannot specifically identify a center of origin for *Pittosporum,* separate introductions (presumably from Australia) to remote Pacific regions (in addition to the Hawaiian introduction) are likely to have occurred. Given the lack of resolution among the New Caledonian, New Zealand, Fijian Province, and East Polynesian taxa, however, it is impossible to specify the number of introductions. One possible scenario involves a single introduction from Australia, possibly to New Zealand, followed by dispersal to the other South Pacific islands. It should be noted, however, that this model is only one among several possibilities, given the unresolved relationships of the South Pacific taxa. Nevertheless, the wide Pacific distribution of *Pittosporum* is suggestive of a multiple introduction pattern. Consider, for example, that in addition to the geographic distribution cited here, *Pittosporum* species are also found on New Hebrides (*P. aneityense*), Fiji (*P. oligodontum* and *P. brackenridgei*), Samoa (*P. samoense*), the Society Islands (*P. orohenense*), and the Caroline Islands (*P. ferrugineum*), with the latter species being the most widespread of any *Pittosporum* (Haas, 1977). This broad distribution was also noted by Carlquist (1974), who suggested a long-distance dispersal model (via birds) to explain the colonization of remote Pacific islands by *Pittosporum* species.

Relationships among non-Hawaiian Taxa

The monophyly of the clade containing the two *P. arborescens* samples and *P. taitense* reflects relationships based on morphological variation observed among these taxa. Haas (1977) placed *P. arborescens* and *P. taitense* within the *P. arborescens* group. These taxa share many morphological features but can be distinguished by the entire leaf margin, more complex inflorescence structure, and larger fruits with more seeds in *P. arborescens*. It is interesting to note that the *P. arborescens* sample from Tonga has an ITS sequence more similar to that of *P. taitense* from Tahiti (0.9% sequence divergence) than it does to the other *P. arborescens* sample from the Cook Islands (1.7% sequence divergence) and that this sequence similarity is of the same magnitude as that between the Hawaiian taxa and *P. yunckeri* from Tonga. Because the specimen from the Cook Islands was once considered a separate species (*P. rarotongense*), species delimitations within this group should be reassessed carefully within the context of a complete revision of the insular species of the *P. arborescens* group.

Our analyses do not identify the New Caledonian taxa as a monophyletic group. However, the placement of New Caledonian *P. gatopense* as sister to the Hawaiian clade (plus its New Caledonian sister group) is not strongly supported, and the lowest pairwise sequence divergence estimate for *P. gatopense* is with *P. undulatum* (9.3%). This split also is not supported by any obvious morphological characteristics. Many of the New Caledonian species are taxonomically problematic due to insufficient sampling and morphological complexities presumably resulting from heteroblasty (i.e., two or more leaf forms on the same plant). The revision of the New Caledonian taxa currently underway (J.-M. Veillon, ORSTOM, pers. comm.) will provide an improved comparative basis for further molecular analyses of these taxa.

These data do not clearly resolve the issue of monophyly of the New Zealand taxa. The two sister taxa, *P. rigidum* and *P. turneri,* are divaricating shrubs (intertangled branches that spread at wide angles), a feature unique to the New Zealand species, whereas *P. corni-*

folium exhibits a regular growth form and is generally epiphytic. *P. turneri* is unusual and presents a more complex life history in having a divaricating juvenile form and a nondivaricating adult form (Cooper, 1956), which may be the result of hybridization between divaricating and nondivaricating species (B. D. Clarkson, pers. comm.). Previous authors (e.g., Allan, 1961; Cooper, 1956), based on morphological characters such as capsule number, presence of a papery endocarp, divaricating habit, and putative recent colonization events, have suggested that as many as four evolutionary lineages are represented in New Zealand. Despite these morphological observations, it remains unclear whether the New Zealand taxa are monophyletic on the basis of the current data set.

Comparison with Other Insular Pacific Taxa

A relevant biogeographic comparison to *Pittosporum* is found among the insular Pacific members of *Metrosideros* (Wright *et al.*, 2000). Using an ITS-based phylogeny, Wright *et al.* (2000) proposed four separate introductions into the Pacific, with a majority of taxa dispersed from New Zealand. They also suggested that colonization of remote insular regions (e.g., Polynesia) may be attributed to recent changes in wind patterns during the Pleistocene; unlike *Pittosporum*, *Metrosideros* has small, wind-dispersed seeds. The largest clade in their consensus tree includes 11 species dispersed over several Pacific islands (e.g., New Zealand, Society, Cook, Kermadec, and Hawaiian Islands). However, with the exception of 1 New Zealand species, taxa on these islands differ by only a single base substitution, resulting in an unresolved Pacific clade. This stands in striking contrast to the ITS phylogeny of *Pittosporum*, which reveals a strongly supported monophyletic Hawaiian lineage and allows the identification of its South Pacific sister group.

CONCLUSIONS

Our results support earlier morphologically based hypotheses (e.g., Fosberg, 1948; Haas, 1977; Wagner *et al.*, 1990) on the origin and derivation of Hawaiian *Pittosporum*. Both the MP and the ML analyses strongly support the monophyly of the Hawaiian taxa and identify two South Pacific species of the Fijian Province, *P. rhytidocarpum* and *P. yunckeri*, as the sister group (those also identified by Haas (1977) as likely ancestors of the Hawaiian taxa), thus supporting a South Pacific origin and a single colonization preceding diversification. These taxa, in turn, may represent pioneer species that migrated from New Caledonia. Of the areas sampled with multiple species (Hawaiian Islands, New Zealand, New Caledonia, and Tonga), it is only in the Hawaiian Islands that we have strong evidence for a single introduction, which may be a consequence of the remote locality of these islands. It will be of interest to see whether this pattern endures as we complete our sampling of all insular *Pittosporum* (e.g., *P. oligodonium* from Fiji and *P. orohenense* from Tahiti). We also suggest that the monophyly of the Hawaiian species, coupled with low levels of sequence divergence, may be indicative of a rapid speciation event following a single colonization from either Fiji or Tonga. Unfortunately, the ITS data do not allow adequate resolution for the reconstruction of relationships among members of the Hawaiian clade. These data further suggest (but do not confirm) multiple South Pacific island introductions, most likely from Australia. The tendency for *Pittosporum* to be dispersed by birds, coupled with its broad insular distribution, provides additional support for a multiple-introduction hypothesis. As discussed above, our sequence divergence results corroborate other studies of insular plant taxa based on ITS sequence variation. Current and future work will focus on the development of high-resolution markers such as the externally transcribed spacer of nrDNA and amplified fragment length polymorphisms (AFLP), the enhancement of our sampling base, and the completion of morphological cladistic analyses. For example, we are particularly interested in comparing phyletic patterns across the island systems of the Hawaiian Islands, New Caledonia, and New Zealand. Ultimately, these phylogenetic hypotheses should provide the necessary framework for future studies focusing on the evolution of morphological characters and breeding systems in *Pittosporum*.

ACKNOWLEDGMENTS

We are indebted to numerous people and organizations for field, laboratory, and herbarium assistance and permits, especially C. Imada, Bishop Museum; J. Bradford, Missouri Botanical Garden; G. McCormack, Cook Islands Heritage Program; Honolulu Botanic Gardens; Kingdom of Tonga; S. Wagstaff and D. Glenny, Landcare Research; S. Meidell, H. Oppenheimer, and R. Bartlett, Maui Pineapple Inc.; J.-Y. Meyer, T. Flynn, D. Lorence, S. Periman, and K. Wood, National Tropical Botanical Garden; J.-M. Veillon, ORSTOM; Le Service de l'Environnement, La Direction des Resources Naturelles, Nouvelle-Calédonie; B. Garnett, L. Perry, and B. Stormont, State of Hawaii DOFAW; J. Lau, The Nature Conservancy of Hawaii; C. Beard, B. D. Clarkson, W. Silvester, University of Waikato; and D. Orr, Waimea Falls Park and Arboretum. We thank T. A. Ranker, B. D. Clarkson, and two anonymous reviewers for their thoughtful comments on the manuscript. This research was funded by the Smithsonian Institution Scholarly Studies Program and the University of Waikato School of Science and Technology.

REFERENCES

Allan, H. H. (1961). "Flora of New Zealand," Vol. I, P. D. Hasselberg, Government Printer, Wellington, New Zealand.

Baldwin, B. G. (1992). Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Mol. Phylogenet. Evol.* **1:** 3–16.

Baldwin, B. G. (1997). Adaptive radiation of the Hawaiian silversword alliance: Congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. *In* "Molecular Evolution and Adaptive Radiation" (T. J. Givnish and K. J. Sytsma, Eds.), pp. 103–128. Cambridge Univ. Press, Cambridge, UK.

Baldwin, B. G., Crawford, D. J., Francisco-Ortega, J., Kim, S.-C., Sang, T., and Stuessy, T. F. (1998). Molecular phylogenetic insights on the origin and evolution of oceanic island plants. *In* "Molecular Systematics of Plants. II: DNA Sequencing" (D. E. Soltis, P. S. Soltis, and J. J. Doyle, Eds.), pp. 410–441. Kluwer Academic, Boston.

Baldwin, B. G., Kyhos, D. W., and Dvorak, J. (1990). Chloroplast DNA evolution and adaptive radiation in the Hawaiian silversword alliance (Asteraceae–Madiinae). *Ann. Mo. Bot. Gard.* **77:** 96–109.

Baldwin, B. G., and Markos, S. (1998). Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Mol. Phylogenet. Evol.* **10:** 449–463.

Baldwin, B. G., and Robichaux, R. H. (1995). Historical biogeography and ecology of the Hawaiian silversword alliance (Asteraceae): New molecular phylogenetic perspectives. *In* "Hawaiian Biogeography: Evolution on a Hot Spot Archipelago" (W. L. Wagner and V. A. Funk, Eds.), pp. 259–287. Random House (Smithsonian Inst. Press), New York.

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S., and Donoghue, M. J. (1995). The ITS regions of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Ann. Mo. Bot. Gard.* **82:** 247–277.

Baldwin, B. G., and Sanderson, M. J. (1998). Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proc. Natl. Acad. Sci. USA* **95:** 9402–9406.

Carlquist, S. (1974). "Island Biology," Univ. of Columbia Press, New York.

Carr, G. D., Robichaux, R., Witter, M. S., and Kyhos, D. W. (1989). Adaptive radiation of the Hawaiian silversword alliance (Compositae-Madiinae): A comparison with the Hawaiian picture-winged *Drosophila*. *In* "Genetics, Speciation, and the Founder Principle" (L. V. Giddings, K. Y. Kaneshiro, and W. W. Anderson, Eds.), pp. 79–97, Oxford Univ. Press, New York.

Carson, H. L., and Kaneshiro, K. Y. (1976). *Drosophila* of Hawaii: Systematics and ecological genetics. *Annu. Rev. Ecol. Syst.* **7:** 311–345.

Cayzer, L. W., Crisp, M. D, and Telford, J. R. H. (2000). Revision of *Pittosporum* (Pittosporaceae) in Australia. *Aust. Syst. Bot.* **13:** 845–902.

Cooper, R. C. (1956). The Australian and New Zealand species of *Pittosporum*. *Ann. Mo. Bot. Gard.* **43:** 87–187.

Doyle, J. J., and Doyle, J. L. (1987). A rapid DNA isolation procedure for small amounts of fresh leaf material. *Photochem. Bull.* **19:** 11–15.

Farris, J. S. (1969). A successive approximation approach to character weighting. *Syst. Zool.* **18:** 374–385.

Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* **125:** 1–15.

Fleischer, R. C., McIntosh, C. E., and Tarr, C. L. (1998). Evolution on a volcanic conveyor belt: Using phylogeographic reconstructions and K–Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Mol. Ecol.* **7:** 533–545.

Fosberg, F. R. (1948). Derivation of the flora of the Hawaiian Islands. *In* "Insects of Hawaii" (E. C. Zimmerman, Ed.), Vol. 1, pp. 107–109. Univ. of Hawaii Press, Honolulu.

Ganders, F. R., Berbee, M., and Pirseyedi, M. (2000). ITS base sequence phylogeny in *Bidens* (Asteraceae): Evidence for the continental relatives of Hawaiian and Marquesan *Bidens*. *Syst. Bot.* **25:** 122–133.

Gu, X., Fu, Y. X., and Li, W. H. (1995). Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotides sites. *Mol. Biol. Evol.* **12:** 546–557.

Haas, J. E. (1977). The pacific species of *Pittosporum* Banks ex Gaertn. (Pittosporaceae). *Allertonia* **1:** 73–167.

Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating the human–ape split by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22:** 160–174.

Johnson, N. K., Matern, J. A., and Ralph, C. J. (1989). Genetic evidence for the origin and relationships of Hawaiian Honeycreepers (Aves: Fringillidae). *Condor* **91:** 379–396.

Jukes, T. H., and Cantor, C. R. (1969). Evolution of protein molecules. *In* "Mammalian Protein Metabolism" (H. N. Munro, Ed.), pp. 21–132. Academic Press, New York.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16:** 111–120.

Kirch, P. (1982). The impact of the prehistoric Polynesians on the Hawaiian ecosystem. *Pac. Sci.* **36:** 1–14.

Kroenke, L. W. (1996). Plate tectonic development of the western and southwestern Pacific: Mesozoic to the present. *In* "The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes (A. Keast and S. E. Miller, Eds.) pp. 19–34. SPB Academic, Amsterdam.

Nepokroeff, M., Wagner, W. L., Weller, S. G., Soltis, P. S., Zimmer, E. A., Sakai, A. K., and Soltis, D. E. (2001). Diversification in the endemic Hawaiian subfam. Alsinoideae (Caryophyllaceae): Evidence from nrDNA ITS, ETS sequences and morphology. *Am. J. Bot.*, in press.

Sakai, A. K., Wagner, W. L., Ferguson, D. M., and Herbst, D. R. (1995). Biogeographical and ecological correlates of dioecy in the Hawaiian flora. *Ecology* **76:** 2530–2543.

Simon, C. (1987). Hawaiian evolutionary biology: An introduction. *Trends Ecol. Evol.* **2:** 175–178.

Soltis, P. S., and Soltis, D. E. (1998). Molecular evolution of 18S ribosomal DNA in angiosperms. *In* "Molecular Systematics of Plants II" (D. E. Soltis, P. S. Soltis, and J. J. Doyle, Eds.), pp. 188–210, Chapman & Hall, New York.

Sullivan, J., Swofford, D. L., and Naylor, G. J. P. (1999). The effect of taxon sampling on estimating rate-heterogeneity parameters of maximum-likelihood models. *Mol. Biol. Evol.* **16:** 1347–1356.

Swofford, D. L. (1998). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer, Sunderland, MA.

Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic inferences. *In* "Molecular Systematics" (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.), 2nd ed., Sinauer, Sunderland, MA.

Tarr, C. L., and Fleisher, R. C. (1995). Evolutionary relationships of the Hawaiian Honeycreepers (Aves, Drepanidinae). *In* "Hawaiian Biogeography: Evolution on a Hot Spot Archipelago" (W. L. Wagner and V.A. Funk, Eds.). Random House (Smithsonian Inst. Press), New York.

Wagner, W. L., and Funk, V. A. (Eds.). (1995). "Hawaiian Biogeography: Evolution on a Hot Spot Archipelago," Radom House (Smithsonian Inst. Press), New York.

Wagner, W. L., Herbst, D. R., and Sohmer, S. H. (1990). "Manual of the Flowering Plants of Hawaii," Univ. of Hawaii Press and Bishop Museum (Special Publication 83), Honolulu.

Wen, J., and Zimmer, E. A. (1996). Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): Inference from ITS sequences of nuclear ribosomal DNA. *Mol. Phylogenet. Evol* **6:** 167–177.

White, T. J., Burns, T., Lee, S., and Taylor, J. (1990). Amplification and direst sequencing of fungal ribosomal RNA genes for phylogenetics *In* "PCR Protocols: A Guide to Methods and Applications" (M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, Eds.), pp. 315–322, Academic Press, San Diego.

Witter, M. S. (1990). Evolution of the Madiinae: Evidence from enzyme electrophoresis. *Ann. Mo. Bot. Gard.* **77:** 110–117.

Wright, S. D., Yong, C. G., Dawson, J. W., Whittaker, D. J., and Gardner, R. C. (2000). Riding the ice age El Niño? Pacific biogeography and evolution of *Metrosideros* subg. *Metrosideros* (Myrtaceae) inferred from nuclear ribosomal DNA. *Proc. Natl. Acad. Sci. USA* **97:** 4118–4123.

Yang, Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *J. Mol. Evol.* **39:** 306–314.