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A Comparative Study in Ancestral Range Reconstruction Methods: Retracing the Uncertain Histories of Insular Lineages

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Abstract.— Island systems have long been useful models for understanding lineage diversification in a geographic context, especially pertaining to the importance of dispersal in the origin of new clades. Here we use a well-resolved phylogeny of the flowering plant genus *Cyrtandra* (Gesneriaceae) from the Pacific Islands to compare four methods of inferring ancestral geographic ranges in islands: two developed for character-state reconstruction that allow only single-island ranges and do not explicitly associate speciation with range evolution (Fitch parsimony [FP; parsimony-based] and stochastic mapping [SM; likelihood-based]) and two methods developed specifically for ancestral range reconstruction, in which widespread ranges (spanning islands) are integral to inferences about speciation scenarios (dispersal-vicariance analysis [DIVA; parsimony-based] and dispersal-extinction-cladogenesis [DEC; likelihood-based]). The methods yield conflicting results, which we interpret in light of their respective assumptions. FP exhibits the least power to unequivocally reconstruct ranges, likely due to a combination of having flat (uninformative) transition costs and not using branch length information. SM reconstructions generally agree with a prior hypothesis about dispersal-driven speciation across the Pacific, despite the conceptual mismatch between its character-based model and this mode of range evolution. In contrast with narrow extant ranges for species of *Cyrtandra*, DIVA reconstructs broad ancestral ranges at many nodes. DIVA results also conflict with geological information on island ages; we attribute these conflicts to the parsimony criterion not considering branch lengths or time, as well as vicariance being the sole means of divergence for widespread ancestors. DEC analyses incorporated geological information on island ages and allowed prior hypotheses about range size and dispersal rates to be evaluated in a likelihood framework and gave more nuanced inferences about range evolution and the geography of speciation than other methods tested. However, ancestral ranges at several nodes could not be conclusively resolved, due possibly to uncertainty in the phylogeny or the relative complexity of the underlying model. Of the methods tested, SM and DEC both converge on plausible hypotheses for area range histories in *Cyrtandra*, due in part to the consideration of branch lengths and/or timing of events. We suggest that DEC model-based methods for ancestral range inference could be improved by adopting a Bayesian SM approach, in which stochastic sampling of complete geographic histories could be integrated over alternative phylogenetic topologies. Likelihood-based estimates of ancestral ranges for *Cyrtandra* suggest a major dispersal route into the Pacific through the islands of Fiji and Samoa, motivating future biogeographic investigation of this poorly known region. [Ancestral range reconstruction; Bayesian inference; *Cyrtandra*; dispersal-mediated allopatry; Gesneriaceae; likelihood methods; stochastic mapping. dispersal; vicariance.]

Islands have long been useful models for understanding organismal interactions and histories (Wallace, 1902; Carlquist, 1974; Grant, 1998; Emerson, 2002) and insular studies have contributed greatly to our understanding of how and why lineages evolve (Lomolino, 2000). Emergent from this is a widely accepted view that oceanic island lineages are commonly established via chance dispersal events, following which founder effects (sensu Mayr, 1963) and genetic isolation from their source populations (Carlquist, 1981) drive rapid allopatric speciation (Price and Wagner, 2004; Cowie and Holland, 2006). We refer hereafter to this mode of island speciation as *dispersal-mediated allopatry*, in contrast to the notion of dispersal merely causing range expansion, without influencing the probability of speciation. In recent years, a wealth of insular studies have been conducted that either implicitly or explicitly assume dispersal-mediated allopatry in explaining insular lineage diversification patterns (e.g., Cronk et al., 2005; Harbaugh and Baldwin, 2007).

Modes of geographic divergence other than dispersal-mediated allopatry, such as vicariance, may be important in some cases. For example, islands in the Maui Nui complex of Hawai'i (Maui, Moloka'i, Lana'i, and Kahoolawae) were intermittently connected and disconnected during key periods over the last 2.2 million years (Price and Elliot-Fisk, 2004); biogeographic patterns here have been attributed to past vicariance events (Cowie and Holland, 2006). However, the general importance of vicariance in Maui Nui and other insular systems remains a subject of debate (Nelson, 2006). Moreover, other scenarios of geographic divergence, e.g., in which widespread ranges persist through speciation events (Ree et al., 2005) or sympatric speciation events (Savolainen et al., 2006; but see also Stussey, 2006), are also conceivable. Acknowledging these alternatives raises the question of how phylogenetic data are best used to infer whether dispersal-mediated allopatry is indeed the predominant mode of insular lineage divergence.

Inferring historical biogeographic patterns requires methods for reconstructing ancestral ranges on phylogenetic trees. A variety of such methods have been used or developed for this purpose (Crisci, 2001). Here,

we categorize these along two distinct lines: first, in how geographic ranges are conceptualized, and second, in the optimality criterion used for choosing between alternative hypotheses. Conceptually, *character state reconstruction* methods employ discrete-state transition models and treat areas as characters or character states. Alternatively, *explicit ancestral range reconstruction* methods treat the geographic range of a species as expanding or contracting according to biogeographic and evolutionary processes (dispersal, local extinction, and lineage divergence), with widespread ranges being necessary intermediate steps in the evolution of disjunctions. Ancestral ranges can be reconstructed using either of these two kinds of methods using parsimony or likelihood.

To date, methods for ancestral range reconstruction encompassing these categories have not been rigorously compared in an empirical system. In this study, we compare four methods: two originally developed for character-state reconstruction, namely Fitch parsimony (FP; parsimony-based; Fitch, 1971) and stochastic mapping (SM; likelihood-based; Nielsen, 2002), and two developed specifically for ancestral range reconstruction, namely dispersal-vicariance analysis (DIVA; parsimony-based; Ronquist, 1997) and dispersal-extinction-cladogenesis (DEC; likelihood-based; Ree and Smith, 2008a). These four methods, summarized in Table 1, were selected specifically to compare inferences under coding schemes that differ in allowing widespread ranges, and under contrasting optimality criteria. Our motivation is to test the performance of these methods in reconstructing ancestral range evolution in a well-resolved phylogeny of the angiosperm genus *Cyrtandra* J.R. & G.Forster (Gesneriaceae), using a prior hypothesis of dispersal-mediated allopatry as a common benchmark. We use the empirical system as a means of illuminating the benefits and limitations arising from each method's underlying assumptions.

Background Information

The genus *Cyrtandra* is the largest in the Gesneriaceae family (>500 species; Burtt, 2001; Cronk et al., 2005) and is one of the most widely dispersed plant genera in southeast Asia and the Pacific. *Cyrtandra* likely evolved in the Indo-Malayan region (Burtt, 2001) and, today, is found throughout the Pacific including northeast to Hawai'i and due east to the Marquesas Islands. Bird ectozoochory has been hypothesized as a means for dispersal in *Cyrtandra* (W. L. Wagner, personal communication). Although endozoochory is another possibility, it is unlikely the seeds would survive passage through the gut of a bird. Chromosome counts have been made on 36 species of *Cyrtandra*, sampled from across the entire range of species, and are with rare exception $n = 17$ (Möller and Kiehn, 2004).

Species of *Cyrtandra* are morphologically diverse and include a variety of habit, fruit, and flower characteristics. However, species in the Pacific islands east of Papua New Guinea, approximately half (>250) of all species of *Cyrtandra* (Cronk et al., 2005), are remarkably similar in being white flowered with fleshy berries and a predominantly understory shrub/small tree habit. Pacific species of *Cyrtandra* are strongly supported as monophyletic based on morphology and molecular phylogenetic data (Gillett, 1973; Cronk et al., 2005). Different Pacific species of *Cyrtandra*, regionally isolated from one another, inhabit ecologically similar perennially wet upland tropical forests throughout the Pacific Islands. These species are almost always narrowly distributed endemics occupying no more than a single archipelago, a single island, or even a single valley. According to Price and Wagner (2004), genera such as *Cyrtandra* containing a large proportion of endemic species and characterized by a high degree of ecological specialization and moderate dispersability have speciated via dispersal-mediated allopatry. Dispersal-mediated allopatry has been suggested for *Cyrtandra* by several researchers (e.g., Gillett, 1973; Burtt, 2001; Cronk et al., 2005) and has been inferred in other insular lineages with similar life histories (Price and Wagner, 2004). No formal analyses have been published on diversification patterns for *Cyrtandra* to date, however. Although recent studies (Atkins et al., 2001; Cronk et al., 2005) included hypotheses on range inheritance scenarios in *Cyrtandra*, they were not explicitly tested.

MATERIALS AND METHODS

Taxon Sampling

Approximately 20% of all species of Pacific *Cyrtandra* are represented here and includes 61 sampled individuals with two outgroup species (both species of *Aeschynanthus* L.; Table 2; Fig. 1). *Aeschynanthus* was

TABLE 1. Summary table comparing the four ancestral range reconstruction methods applied in the current study.

Method	Optimality criterion	Range concept	Implementation; authors
Fitch parsimony [FP]	Unordered parsimony	Single areas only.	MacClade 4.03; Maddison and Maddison, 2001
Stochastic mapping [SM]	Likelihood	Single areas only.	SIMMAP 1.0b2; Bollback, 2005
Dispersal vicariance analysis [DIVA]	Parsimony	Presence/absence in multiple areas.	DIVA 1.1a; Ronquist, 1997
Dispersal-extinction-cladogenesis [DEC]	Likelihood	Presence/absence in multiple areas.	Lagrange 2.0; Ree and Smith, 2008b

TABLE 2. Taxon sampling list for 61 individuals sampled in the current study including two outgroup species (*Aeschynanthus* sp.). ID numbers are J. R. Clark's DNA extraction numbers and are here used for reference. Please see also Figures 2 and 3. GenBank accession numbers are included for all taxa for each of the three genic regions analyzed in the current study. Species lacking a GenBank number under the *psbA-trnH* column, as indicated by an * in place of a number, were not sequenced in this study. *C.* = *Cyrtandra*; *A.* = *Aeschynanthus*. ITS = internal transcribed spacer regions 1 and 2, including the 5.8s subunit; ETS = external transcribed spacer region; *psbA-trnH* = chloroplast sequence data.

Species	ID no.	Collector and no.; voucher	Origin	ITS	ETS	*psbA-trnH*
C. tintinabula Rock	C0012	Perlman 17676; PTBG	Hawai'i, Hawai'i	EU919930	EU919869	*
C. wagneri Lorence and Perlman	C0013	Perlman 17673; PTBG	Hawai'i, Hawai'i	EU919931	EU919870	EU919991
*C.*sp.	C0016	Plunkett 1837; US	Fiji, Viti Levu	EU919932	EU919871	*
*C.*sp.	C0017	Plunkett 1838; US	Fiji, Viti Levu	EU919933	EU919872	EU919992
*C.*sp.	C0018	Plunkett 1843; US	Fiji, Viti Levu	EU919934	EU919873	EU919993
*C.*sp.	C0019	Plunkett 1875; US	Fiji, Viti Levu	EU919935	EU919874	EU919994
*C.*sp.	C0020	Plunkett 1898; US	Fiji, Viti Levu	EU919936	EU919875	EU919995
C. wainihaensis Léveillé	C0021	Clark 549; SEL	Hawai'i, Kaua'i	EU919937	EU919876	EU919996
C. wawrae C.B. Clarke	C0022	Clark 550; SEL	Hawai'i, Kaua'i	EU919938	EU919877	EU919997
C. longifolia (Wawra) Hillebrand ex C.B. Clarke	C0023	Clark 551; SEL	Hawai'i, Kaua'i	EU919939	EU919878	EU919998
C. kauaiensis Wawra	C0026	Clark 556A; SEL	Hawai'i, Kaua'i	EU919940	EU919879	EU919999
C. pulchella Rich ex A. Gray	C0029	Lorence 8525; PTBG	Samoa, Tau	EU919941	EU919880	EU920000
C. samoensis A. Gray	C0030	Lorence 8633; PTBG	Samoa, Ofu	EU919942	EU919881	EU920001
C. samoensis A. Gray	C0031	RP 71221; PTBG	Tonga	EU919943	EU919882	EU920002
C. ootensis var. *mollissima* Fosberg & Sachet	C0032	Wood 6563; PTBG	Marquesas, Tahuata	EU919944	EU919883	EU920003
C. kusaimontana Hosokawa	C0033	Flynn 5995; PTBG	Micronesia, Kosrae	EU919945	EU919884	EU920004
C. urvillei C.B. Clarke	C0034	Lorence 7838; PTBG	Micronesia, Kosrae	EU919946	EU919885	EU920005
C. kealiae Wawra	C0035	Clark 566; SEL	Hawai'i, Kaua'i	EU919947	EU919886	EU920006
C. laxiflora H. Mann	C0037	Clark 568; SEL	Hawai'i, O'ahu	EU919948	EU919887	EU920007
C. hawaiensis C.B. Clarke	C0038	Clark 569; SEL	Hawai'i, O'ahu	EU919949	EU919888	EU920008
C. propinqua C. Forbes	C0039	Clark 570; SEL	Hawai'i, O'ahu	EU919950	EU919889	EU920009
C. calpidicarpa (Rock) St. John & Storey	C0040	Clark 571; SEL	Hawai'i, O'ahu	EU919951	EU919890	EU920010
C. kaulantha St. John & Storey	C0041	Clark 572; SEL	Hawai'i, O'ahu	EU919952	EU919891	EU920011
C. sandwicensis (Léveillé) St. John & Storey	C0045	Clark 576; SEL	Hawai'i, O'ahu	EU919953	EU919892	EU920012
C. grandiflora Gaudichaud	C0046	Clark 577; SEL	Hawai'i, O'ahu	EU919954	EU919893	EU920013
C. cordifolia Gaudichaud	C0048	Clark 579; SEL	Hawai'i, O'ahu	EU919955	EU919894	EU920014
C. sp.	C0050	Clark 581; SEL	Hawai'i, O'ahu	EU919956	EU919895	EU920015
C. kealiae ssp. *urceolata* W.L. Wagner & Lorence	C0054	Perlman 18805; PTBG	Hawai'i, Kaua'i	EU919957	EU919896	EU920016
A. tricolor Hook.	C0055	MSBG 1974-1760-W; SEL	Indonesia	EU919958	EU919897	EU920017
A. longicaulis Wallich ex R. Brown	C0056	MSBG 1974-2207-W; SEL	Indonesia	EU919959	EU919898	EU920018
C. feaniana F. Brown	C0059	Price 200; US	Marquesas, Hiva Oa	EU919960	EU919899	EU920019
C. ootensis var. *ootensis* F. Brown	C0060	Wood 10047; PTBG	Marquesas, Hiva Oa	EU919961	EU919900	EU920020
C. ootensis var. *molissima* Fosberg & Sachet	C0061	Perlman 18399; PTBG	Marquesas, Fatu Hiva	EU919962	EU919901	EU920021
C. thibaultii Fosberg & Sachet	C0062	Meyer 2541; PTBG	Marquesas, Ua Pou	EU919963	EU919902	EU920022
C. ootensis var. *mollissima* Fosberg & Sachet	C0063	Wood 10266; PTBG	Marquesas, Tahuata	EU919964	EU919903	EU920023
C. jonesii (F. Brown) Gillett	C0064	Wood 10484; PTBG	Marquesas, Ua Huka	EU919965	EU919904	EU920024
C. nukukivensis Forest and Brown	C0065	Wood 10428; PTBG	Marquesas, Ua Pou	EU919966	EU919905	EU920025
C. cf. *richii* A. Gray	C0068	Clark 646; SEL	Samoa, U'polu	EU919967	EU919906	EU920026
C. pogonantha A. Gray	C0071	Clark 649; SEL	Samoa, U'polu	EU919968	EU919907	EU920027
C. richii A. Gray	C0072	Clark 650; SEL	Samoa, Savai'i	EU919969	EU919908	EU920028
C. compressa C.B. Clarke	C0074	Clark 652; SEL	Samoa, Savai'i	EU919970	EU919909	EU920029
C. aurantiicarpa Gillett	C0076	Clark 655; SEL	Samoa, Savai'i	EU919971	EU919910	EU920030
C. coccinea Blume	C0089	Hoover & Agus ARs 167 ; US	Indonesia, Java	EU919972	EU919911	*
C. sp.	C0092	Hoover & Agus ARs 173; US	Indonesia, Java	EU919973	EU919912	EU920031
C. sp.	C0093	Hoover & Agus ARs 175 ; US	Indonesia, Java	EU919974	EU919913	EU920032
C. sp.	C0094	D 536; US	Indonesia	EU919975	EU919914	EU920033
C. sp.	C0095	Wiriadinata, H. 12709; US	Indonesia, Java	EU919976	EU919915	EU920034
C. sp.	C0096	Wiriadinata, H. 12713; US	Indonesia	EU919977	EU919916	EU920035
C. picta Blume	C0097	Wiriadinata, H. 12715; US	Indonesia	EU919978	EU919917	EU920036
C. pendula Blume	C0098	Wiriadinata, H. 12716; US	Indonesia	EU919979	EU919918	EU920037
C. sulcata Blume	C0100	Hoover & Agus ARs 160; US	Indonesia, Java	EU919980	EU919919	*
C. spathulata St. John	C0102	Clark 664; SEL	Hawai'i, Maui	EU919981	EU919920	EU920038
C. grayana Hillebrand	C0103	Clark 666; SEL	Hawai'i, Maui	EU919982	EU919921	EU920039
C. munroi C. Forbes	C0104	Clark 675; SEL	Hawai'i, Maui	EU919983	EU919922	*
C. grayi C.B. Clarke	C0105	Clark 676; SEL	Hawai'i, Maui	EU919984	EU919923	EU920040
C. sp.	C0112	Plunkett 1980; US	Fiji, Viti Levu	EU919985	EU919924	EU920041
C. milnei Seem. ex A. Gray	C0113	Clark 687; SEL	Fiji, Viti Levu	EU919986	EU919925	*
C. anthropophagorum Seem. ex A. Gray	C0114	Clark 688; SEL	Fiji, Viti Levu	EU919987	EU919926	EU920042
C. leucantha A.C. Smith	C0116	Clark 693; SEL	Fiji, Viti Levu	EU919988	EU919927	*
C. occulta A.C. Smith	C0117	Clark 694; SEL	Fiji, Viti Levu	EU919989	EU919928	*
C. cf. *occulta* A.C. Smith	C0119	Clark 702; SEL	Fiji, Viti Levu	EU919990	EU919929	*

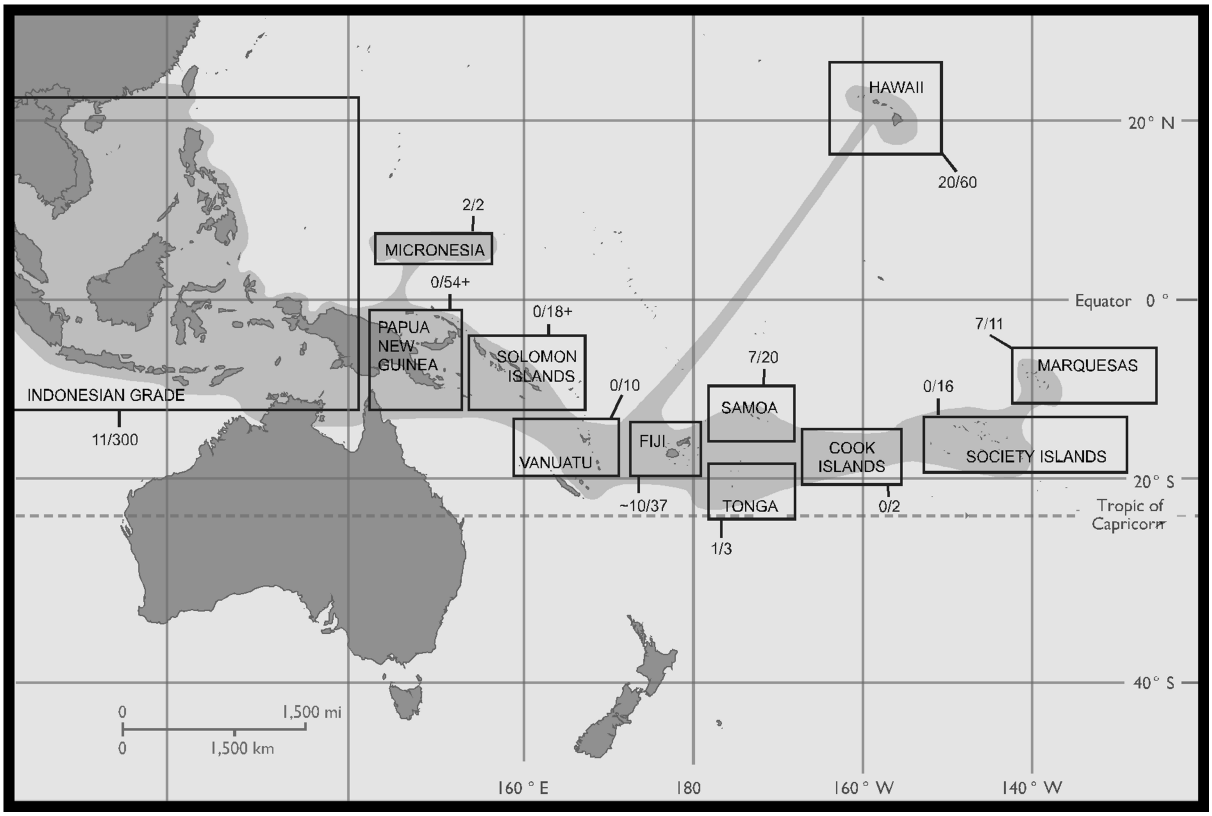

FIGURE 1. Southeast Asian and Pacific distribution of *Cyrtandra*. Numbers before the forward slash are approximate number of species sampled in this study; numbers after slash are conservative estimates for species numbers in the defined areas based on herbarium records (Skog and Boggan, 2007).

selected as the appropriate outgroup based on current understanding of phylogenetic relationships among paleotropic gesneriads (Mayer et al., 2003; Cronk et al., 2005). Specimens sampled are here used as representatives of distinct lineages/populations that are generally considered species by taxonomists (sensu Gillett, 1973; Wagner et al., 1990). Samples included represent lineages present on most high island systems in the Pacific and all attempts were made to include at least one specimen from principal lineages as defined by Gillett (1973) and Wagner el al. (1990; Table 2). Several putative species from Indonesia and Fiji were included that have not yet been identified to species, but initial analysis showed them to be genetically distinct. Our sampling, influenced by the availability of material, is appropriate for the questions we are asking based on current thinking on species sampling for phylogenetic dating (see Bremer et al., 2004, for a discussion on sampling issues in phylogenetic dating).

Phylogenetic Analysis.—Silica gel–dried leaf material was used for total genomic DNA extraction using the CTAB procedure of Doyle and Doyle (1987). All genic regions used were amplified with the polymerase chain reaction (PCR) using specific primers dependent upon the region amplified. PCR products were purified prior to sequencing using the Exonuclease enzymatic reaction (New England Biolabs). Direct sequencing of purified DNA PCR products was conducted using the Big Dye 3.1 terminator cycle-sequencing reaction (Applied Biosystems, Inc.). Purified cycle sequence products were cleaned using Edge Biosystems DTR gel purification system and analyzed on an Applied Biosystems Model 3730 Automated DNA Sequencer. For each individual, forward and reverse sequencing reactions were performed for sequence confirmation. Sequence chromatograms were proofed, edited, and contigs were assembled using Sequencher 4.5 (Gene Codes Corporation, Inc.). Edited contigs were then aligned using ClustalX (Thompson et al., 1997) with subsequent manual refinement.

ITS.—The internal transcribed spacer region, including ITS1, ITS2, and the 5.8S subunit, was amplified using ITS5 and ITS4 primers as described by Roalson et al. (2003). PCR reaction conditions: initial denaturizing at 95°C; 34 cycles of 1 min at 95°C, 1 min at 48°C, 1 min at 72°C; followed by a 7-min extension at 72°C.

ETS.—The 5′ end external transcribed spacer region was amplified using the primers 18S-ETS (Baldwin and Marcos, 1998) and ETS-B developed for *Mimulus* L. (Phrymaceae) by Beardsley and Olmstead (2002). PCR reaction conditions: initial denaturizing at 95°C; 34 cycles of 1 min at 95°C, 1 min at 50°C, 1 min at 72°C; followed by a 7-min extension at 72°C.

psbA-trnH.—The chloroplast *psbA-trnH* region was amplified using the primers psbAf and trnHr as

described in Smissen et al. (2004). PCR reaction conditions: initial denaturizing at 95°C; 30 cycles of 1 min at 95°C, 30 s at 52°C, 30 s at 72°C; followed by a 7-min extension at 72°C. Note: 9 of the 61 species analyzed were recalcitrant to sequencing for *psbA-trnH*. Preliminary analyses with and without these species did not alter topologies significantly (data not shown). The inclusion of these species provides additional biogeographic information relevant to the current study.

Aligned sequences were analyzed using maximum likelihood (ML) and Bayesian inference (BI) methods. ML analyses were performed using PAUP* 4.0b10 (Swofford, 2002). Heuristic searches were performed using TBR branch swapping with initial starting tree generated using neighbor-joining reconstruction. DNA evolution model parameters were selected using DT-ModSel (Minin et al., 2003). Bootstrap support indices were generated for each node using 1000 heuristic bootstrap replicates executed over 1000 random addition cycles with 100 trees saved per cycle (Hillis and Bull, 1993). BI analyses were performed using MrBayes v. 3.1 (Huelsenbeck and Ronquist, 2001). Four chains were run for 30,000,000 generations each and sampled every 10,000 generations. Model selection was conducted using DT-ModSel (Minin et al., 2003). Multiple independent BI analyses were run to test for convergence and mixing. Initially, ML analyses were run on individual gene trees and then compared with one another to assess compatibility of genic regions for combined analysis (data not shown). No well-supported branches (≥75% bootstrap support) among the various topologies were in conflict; therefore, the three genic regions were combined and analyzed. Results from these independent ML and BI analyses of combined datasets were used in the dating and ancestral range reconstruction methods.

Estimation of Phylogeny Divergence Times

We used the r8s program (v. 1.7.1; Sanderson, 2004) to estimate a chronogram for *Cyrtandra* based on our maximum likelihood tree from the combined analysis using semiparametric rate smoothing (SPRS) by penalized likelihood and the truncated Newton algorithm (Sanderson, 2002). Combined genic region data sets are commonly employed in phylogenetic dating studies (e.g., Dumont et al., 2005; Forest et al., 2005; Morgan et al., 2007). The r8s program requires that all nodes be resolved prior to analysis; therefore, unresolved branches in the ML tree were resolved in PAUP* to very short branch lengths (0.000001). Smoothing parameters were derived using cross-validation (data not shown).

Confidence intervals were calculated by creating 100 bootstrap replicate data matrices of the combined ITS-ETS-*psbA-trnH* gene matrix using the SEQBOOT program in Felsenstein's (2004) PHYLIP package. The replicate datasets were used to estimate branch lengths on the ML topology and resulting phylograms were then analyzed using the r8s Bootkit developed by Eriksson (2002). Standard deviations were generated for specified nodes as described in the documentation (Eriksson, 2002).

No macro-fossils for the Gesneriaceae are known, (Wiehler, 1983), so we used estimated ages of Pacific island groups as maximum age calibration points (sensu Roalson et al., 2008). This approach stems from the idea that a lineage that has diversified within an area and is endemic to that area most probably post-dates the origin of that area. For example, although it is highly probable that plant lineages inhabited the Hawaiian Islands throughout their history (>84 million years ago [Ma]), most lineages now present in Hawai'i are thought to have arrived since the formation of the now extant high islands (less than 5 Ma; Price and Clague, 2002; Price and Wagner, 2004). This prevailing hypothesis is based on numerous studies involving dating of Hawaiian lineages (e.g., Baldwin and Marcos, 1998) and on geologic evidence that suggests a "lull" in high islands presence in the chain of several million years prior to the formation of Nihoa and Kaua'i around 5.2 to 4.7 Ma. This lull was apparently a bottleneck in many Hawaiian lineages; subsequent colonization of Hawaiian Islands likely occurred from areas outside the chain.

Given this line of reasoning, we used known geologic ages of three major island systems to calibrate our phylogeny. The extant Hawaiian Islands have an estimated age of 5.1 Ma (Price and Clague, 2002), Marquesas (6 Ma; Florence and Lorence, 1997), and Fiji (40 Ma; Evenhuis and Bickel, 2005). The Fijian date was used as a conservative maximum age for the Indonesian grade/Pacific split given that Fiji is the oldest Pacific island system represented in our study.

Ancestral Range Analysis

Area coding.—Ancestral range patterns were inferred in all analyses by recognizing seven geographic areas: (1) a broadly defined "Indonesia" referring to the collective ranges of the more western species representing the grade of species within which the Pacific lineage is nested; (2) Fiji; (3) Hawai'i; (4) Samoa; (5) Tonga; (6) Micronesia; and (7) Marquesas.

Character state reconstruction methods.—In applying character state reconstruction methods to ancestral range reconstruction, we coded geographic ranges as discrete, multistate characters that do not allow for ranges spanning more than one area. Methods currently exist that allow for multiple character, binary-state coding integrated using a step-matrix approach (e.g., "presence coding" as described in Hardy and Linder, 2005). However, similar coding methods are applied in the explicit ancestral range reconstruction methods (see below); here, we chose not to implement such methods in favor of multistate coding that best approximates dispersal-mediated allopatry, a probable divergence scenario in insular lineage radiations (for similar application of multistate coding, see Kron and Luteyn, 2005; Harbaugh and Baldwin, 2007).

Fitch parsimony (FP; Fitch, 1971) is an algorithm for finding ancestral states that minimize the number of changes required to explain an observed distribution of character states at tip nodes on a phylogeny,

without reference to relative or absolute time encoded in the lengths of branches and with the directional costs of transitions between states weighted equally (Felsenstein, 2004). Because the algorithm is agnostic about time, it makes no assumptions about whether an inferred change from one area to another on a phylogeny occurs coincidentally with lineage divergence (i.e., at the internal node) or along the branch connecting the ancestor to its descendant. The former scenario is consistent with dispersal-mediated allopatry, whereas the latter implies dispersal and subsequent extinction in the source area. Analysis was performed using MacClade 4.03 (Maddison and Maddison, 2001) using the single most likely tree from the likelihood analysis.

Stochastic mapping (SM), a Bayesian approach to character state reconstruction (Nielsen, 2002; Huelsenbeck et al., 2003), may also be applied to ancestral ranges. Unlike FP, SM is based on a probabilistic model of transitions between states in continuous time and generates inferences of ancestral states by simulating evolutionary sequences on the phylogeny that yield the observed data at its tips. Here, transitions are explicitly modeled as occurring along phylogenetic branches, with the probability of change being proportionate to evolutionary rate and branch length. Bayesian methods have become increasingly explored in phylogenetics research (Alfaro et al., 2003; Alfaro and Holder, 2006), largely because they facilitate accounting for uncertainty in model parameters, including the phylogeny itself. By simulating evolutionary sequences of states across a posterior probability distribution of phylogenies, instead of conditioning on a single topology, SM can incorporate phylogenetic uncertainty into the inference of ancestor-descendant range transitions. SM analysis was performed using SIMMAP 1.0b2 (Bollback, 2005) on a sub-sample of 1000 trees from the Bayesian posterior distribution.

Explicit ancestral range reconstruction methods.— Dispersal-vicariance analysis (DIVA; Ronquist, 1997) is a method for inferring the most parsimonious ancestral ranges on a phylogeny by minimizing the number of dispersal and local extinction events that are required to explain the current ranges of species. DIVA employs a presence-coding model that allows for multi-area inferences at internal nodes. However, unlike presence-coding state reconstruction methods, DIVA avoids "no-state" assignments (*sensu* Mickevich and Mitter, 1981) by assigning a three-dimensional cost matrix (Hardy and Linder, 2005); DIVA assumes that when a widespread ancestral lineage diverges, its range is subdivided by vicariance and assigns no cost to this event relative to dispersal and local extinction. As with FP, branch lengths do not affect the inference of dispersal or local extinction events between ancestors and descendants, nor is any assumption made about where such events occur along a branch. Dispersal-mediated allopatry would here be reconstructed as dispersal followed by vicariance. We used DIVA 1.1a with the same ML tree used in FP analysis (Ronquist, 1996). DIVA analysis was performed both unrestricted ("DIVA1") and restricted to a maximum range size of two areas ("DIVA2").

The dispersal-extinction-cladogenesis (DEC) model (Ree et al. 2005; Ree and Smith 2008a) is a continuous-time model for geographic range evolution in which dispersal events cause range expansion, local extinction events cause range contraction, and the probability of each kind of event along a phylogenetic internode is proportionate to its rate and time (branch length). In DEC, areas are coded as present or absent for each area under consideration. The DEC model allows for considerable flexibility in parameter specification, e.g., allowing constraints to be imposed on dispersal rates according to prior evidence for connections between areas through time. Like DIVA, DEC enumerates scenarios ("ancestral states") by which speciation causes descendant ranges to be inherited from the ancestral range, but it differs from DIVA in not enforcing vicariance on widespread ancestors. It is important to note that DEC does not include speciation rate as a free parameter and assumes that the geographic pattern of divergence (within versus between areas) is independent of dispersal rates. This precludes direct inference of dispersal-mediated allopatry, in which divergence between areas is effectively instantaneous following dispersal. DEC analysis was performed using Lagrange version 2 (Ree and Smith, 2008b) using the chronogram from the SPRS analysis. Polytomies on this tree were resolved and minimal lengths (10e-4) assigned to new branches using Mesquite 1.12 (Maddison and Maddison, 2006). Model parameters were modified in two ways: (1) dispersal to islands before their temporal origin was set to zero and (2) the dispersal rate between islands was inversely scaled by a factor indicating relative distance (see Appendix 1, available online at http://www.systematicbiology.org, for the relative-distance matrix). As in the DIVA analysis, maximum range size was both unrestricted ("DEC1") and restricted to no more than two areas ("DEC2").

Evaluating the Methods

Given that our focus is on an empirical system, rather than simulated data with known evolutionary histories, our evaluation of the performance of ancestral range methods is limited to how well their results match our prior expectations. With this in mind, we established two criteria for interpreting our results:

(1) Ancestral ranges should be narrow, reflecting range extents of extant species. All information available indicates that species of *Cyrtandra* are rarely widespread and in the few instances when they are, species rarely extend beyond archipelagos. In fact, the only known Pacific species found on multiple island groups is *C. samoensis* (found on Samoa, Tonga, and Niue; Gillett, 1973). These areas are relatively close to one another if the Pacific area as a whole is considered. Furthermore, range limits for *C. samoensis* are unclear and current data suggest that this lineage may contain cryptic species (M. Kiehn

and J.R. Clark, unpublished data), each with far smaller geographic ranges. Given the limited distribution of all other species of *Cyrtandra*, it is probable that ancestral species also had limited ranges. Thus, the range of *C. samoensis* can be used as a conservative maximum estimate of species ranges in this group.

(2) A lineage resultant from a single colonization event and endemic to a particular area must post-date the geologic formation of that area (Baldwin and Marcos, 1998; Price and Clague 2002). Geologic ages for southeast Asian and Pacific islands are relatively well established for many archipelagos including Fiji, Samoa, Hawai'i, and the Marquesas Islands and can be used as conservative maximum ages for endemic species lineages inhabiting these areas. Reconstructions that exceed these maximum ages can be rejected.

RESULTS

Maximum Likelihood Phylogeny Estimation

Combined analysis of ITS, ETS, and *psbA-trnH* genic regions resulted in one most likely tree (Fig. 2; TreeBASE submission SN4027-19303). Most major bifurcations are strongly supported by both bootstrap and posterior probability support indices. Monophyly of the Pacific clade (node 1; Fig. 2) is well supported (BS = 100%; PP $\geq$ 99%). Similarly, the Hawaiian lineage is well supported as monophyletic (node 3; BS = 100%; PP $\geq$ 99%), as is the South Pacific lineage (node 6; BS = 99%; PP $\geq$ 99%), excluding the sister Fijian clade (node 5). Placement of this latter clade is the least supported of all internal nodes and is not supported in either ML or BI analyses. The Marquesan clade is well supported only in the BI analysis (node 14; BS = s < 75%; PP = 98%). Several geographic areas are polyphyletic including Samoa with two well-supported clades (nodes 7, 13), with Tongan and Micronesian lineages nested within one of these (node 7), Fiji with two distinct lineages represented (nodes 5, 11), and one species nested with the Samoan clade (node 13). Terminal lineages, principally within island systems, are less resolved (Fig. 1).

Estimation of Phylogeny Divergence Times

Figure 3 illustrates the results in the form of a chronogram from the r8s divergence estimates. The origin of the southeast Asian "Indonesian" grade and the monophyletic Pacific clade is estimated at 35.2 Ma (± 6.8 Ma). Other noteworthy divergence dates include the South Pacific–Hawaiian stem lineage (18.7 ± 5.3 Ma; node 2) and the origin of major crown group lineages, including one of two Samoan clades (10.5 ± 2.8 Ma; node 7), the other Samoan clade (7.5± 3.4 Ma; node 13), one Fijian clade (4.5 ± 2.6 Ma; node 5), and the other major Fijian clade (8.1 ± 2.8 Ma; node 11). Ages for lineage divergence events correspond with known geologic ages of other areas not constrained in this analysis (Fig. 3), suggesting that the constraints imposed in this study offer a reasonable estimate of divergence times. For example, divergence dates for lineages containing Samoan species do not exceed the maximum age estimates for these islands (24 Ma; Hart et al., 2004). Similarly, Micronesian species are estimated to have diverged more recently than the origin of these islands (Micronesia [Kosrae] <9 Ma; Keating et al., 1984).

Ancestral Range Reconstruction Analysis

Ancestral ranges inferred using FP, SM, DIVA, and DEC are summarized in Table 3 and Figure 4. The methods differ dramatically in their resolution, as indicated by the number of parsimonious (as in FP and DIVA), probable (as in SM), or likely (as in DEC) reconstructions per node and by the often-opposing range inheritance scenarios reconstructed under each method. For the six separate methods, all possible reconstructions are presented in Table 3, whereas for clarity only the first reconstruction is illustrated in Figure 4.

No method compared in this study resolved all fourteen nodes under consideration. FP analysis resulted in eight nodes having multiple most parsimonious reconstructions. SM results are most congruent with our dispersal-mediated allopatry hypothesis and nine of fourteen nodes are resolved as a single, probable ancestral area reconstruction. Both DIVA analyses infer rather complex scenarios with wider ancestral ranges being inferred for increasingly interior nodes. DIVA1 results are the most decisively resolved having only two nodes with more than one possible reconstruction while DIVA2 results have seven nodes not conclusively resolved. DEC1 results have the greatest number of unresolved nodes: 9 of 14, with one node having as many as five scenarios falling within two log-likelihood units of the maximum. DEC2 results have slightly fewer unresolved nodes, 8 of 14, and each of the unresolved nodes have no more than two likely reconstructions.

FP results are difficult to elaborate on since most internal nodes are reconstructed with multiple equally parsimonious areas. For instance, nodes 1 to 4 all are reconstructed as Indonesia, Hawai'i, Fiji, or Samoa, and no clear inference of dispersal-mediated allopatry can be made. Alternatively, SM, the only other method to use multistate, single-character coding, yields results that are generally consistent with dispersal-mediated allopatry. Inconclusive reconstructions using SM include node 2, inferred to be Fiji (85% PP) or Samoa (10% PP). Of the two daughter lineages, the Hawaiian clade is inferred to be Hawai'i (>99% PP), whereas the South Pacific can be either Fiji (91% PP) or Samoa (9%PP). Four possible range inheritance scenarios thus exist, the most probable being a Fijian origin for node 2, followed by a dispersal event leading to the Hawaiian lineage with persistence of the South Pacific lineage in Fiji. Other less probable scenarios would require additional dispersal events between Fiji and Samoa and/or Samoa and Hawai'i. The time criterion is not violated in any of the SM area reconstructions. SM results are also not in conflict with the

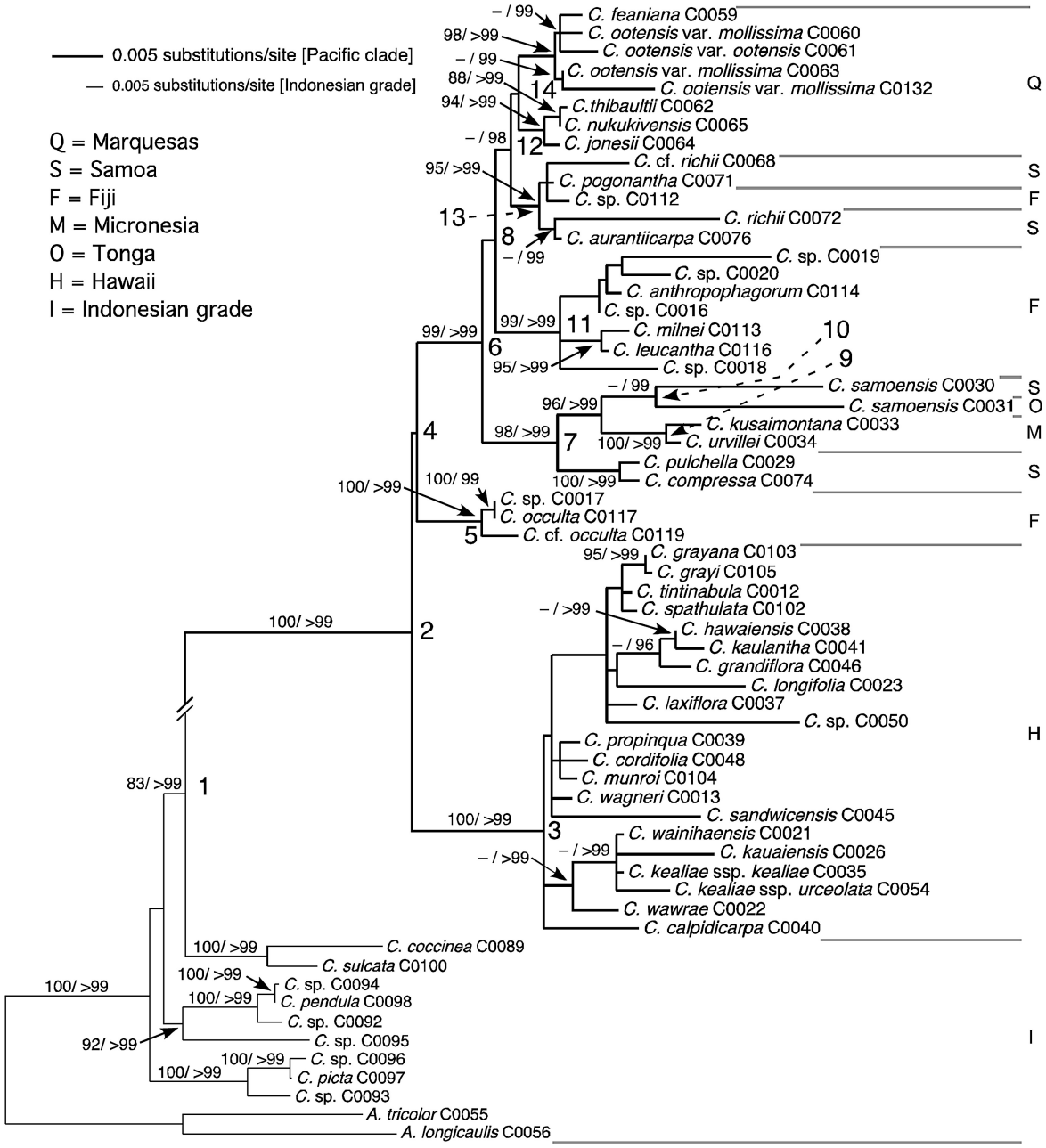

FIGURE 2. Single most likely phylogram from the maximum likelihood analysis of the internal transcribed spacer regions 1 and 2, including the 5.8s subunit (ITS), external transcribed spacer region (ETS) and the chloroplast genic region *psbA-trnH*. Maximum likelihood analysis performed using the K80+G substitution model. Letters to the right indicate geographic regions. Numbers above nodes indicate branch support (bootstrap support ≥70%/Bayesian posterior probabilities ≥95%). The Indonesian grade and outgroup taxa are scaled down for de-emphasis. Numbers by selected nodes (1–14) and numbers following species names (e.g., C0113) are for reference. Please see Table 2 and the text for details.

range extent criterion since only single areas per node are allowed.

DIVA1 results favor widespread ancestral ranges, e.g., node 2 is reconstructed as Fiji-Hawai'i-Samoa-Tonga-Micronesia-Marquesas, with vicariant divergence splitting Hawai'i off from the remainder of the South Pacific islands. DIVA2 results, although constrained to no more than two areas at any one node, still infer widespread species and in node 2, a Fiji-Hawai'i and Hawai'i-Samoa lineage is inferred. The daughter lineage Hawai'i is only inferred to be Hawai'i, as in DIVA1, whereas the South Pacific lineage is inferred to be either Hawai'i or Hawai'i-Samoa. In general, these results conflict with the range extent and time criteria in that broad ranges are reconstructed as well as interior lineages are inferred with areas that did not exist for some time, based on our dating analysis (Fig. 4; see Fig. 3 for lineage age estimates).

Both DEC1 and DEC2 results yield similar inferences, differing mainly in the number of plausible reconstructions at nodes that are not resolved with certainty. In DEC1, for node 2, five possible ancestral ranges are inferred: Fiji, Indonesia, Fiji-Samoa, Samoa,

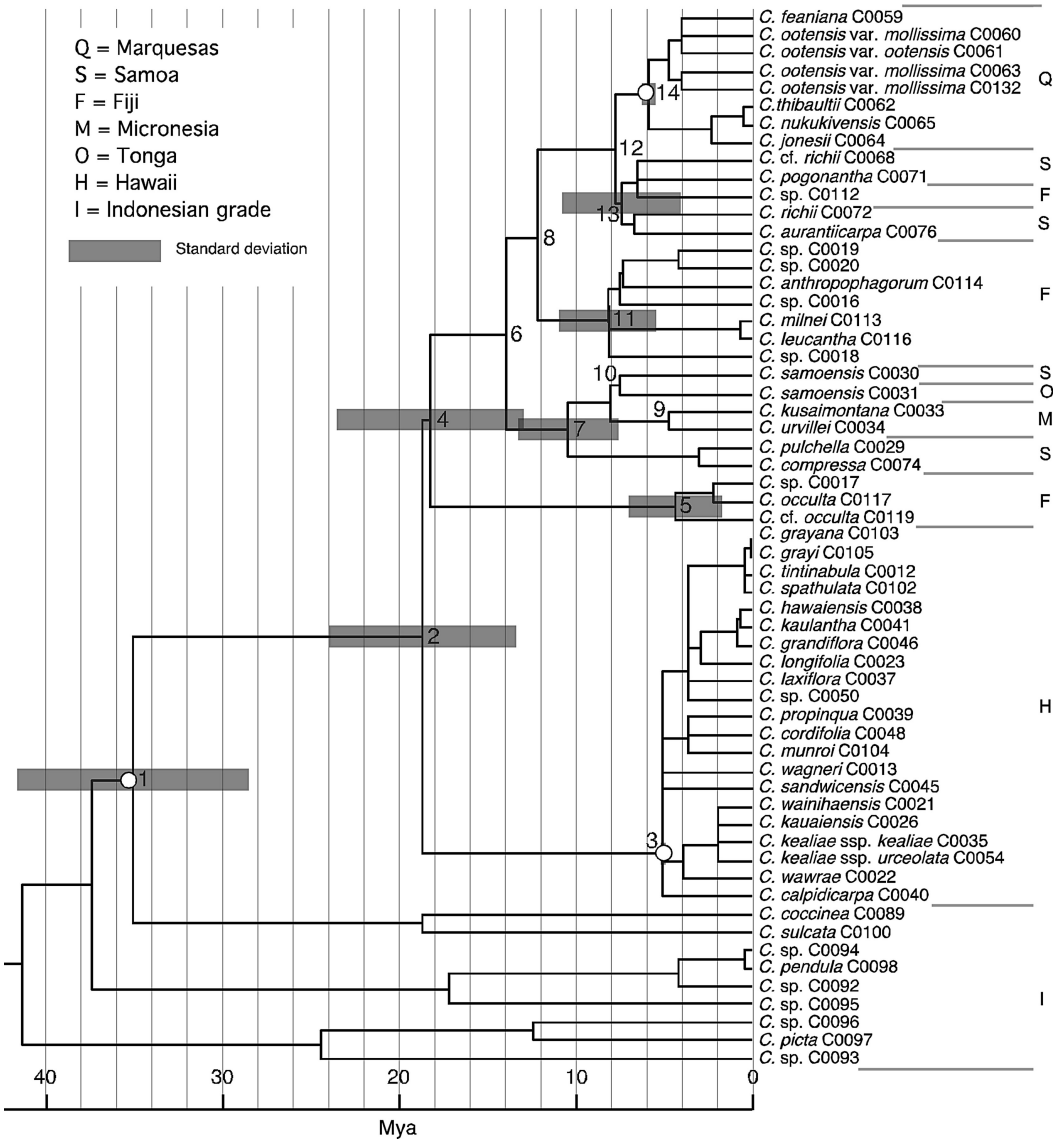

FIGURE 3. Chronogram based on penalized likelihood analysis of the ML tree calibrated using island ages. The three calibration points are each indicated by an open circle (◯); please see the text for details on the calibration constraints. Numbers at the bottom are ages in millions of years. Gray bars represent standard deviations around selected nodes. Letters indicate geographic area. Numbers by selected nodes (1–14) and numbers following species names (e.g., C0113) are for reference. Please see Table 2 and the text for details.

and Indonesia-Fiji. For daughter lineages, Hawai'i is inferred as either Fiji or Samoa and the South Pacific as Fiji or Fiji-Samoa. For DEC2 analysis, node 2 is inferred as being either Fiji or Indonesia. The daughter lineages are reconstructed identical to those in DEC1. Both analyses strongly support Fiji at node 2 having a within-area divergence event leading to the Hawaiian lineage and the South Pacific lineage. Less likely scenarios would involve more lineages that span areas and more dispersal events to account for the range inheritance scenario.

The general agreement of DEC with the range extent and time evaluation criteria is expected, because these two parameters are explicitly incorporated into the model. Despite this, there does exist a marginal conflict with the distance evaluation criterion: the greatest range inferred (Fiji-Samoa) is slightly greater than for known ranges of extant species, 961 km versus 884 km, a difference of 77 km. If the distance maximum is considered a hard constraint, then these results should be rejected. However, island distances were calculated from relatively centralized but arbitrary points within archipelagos; ancestral species inhabiting both Fiji and Samoa may be a reasonable hypothesis, although such ranges are at the outer limits of potential ranges for extant species.

DISCUSSION

This comparative study illustrates two main points: (1) ancestral range reconstructions can differ dramatically, depending on the underlying assumptions of the reconstruction methods; (2) for Pacific *Cyrtandra*, results from likelihood and Bayesian methods are generally more compatible with prior expectations about the timing and mode of biogeographic evolution

TABLE 3. Summary and comparison of results from the four ancestral range reconstruction methods. The "node" column refers to numbered nodes in Figure 2. FP = Fitch parsimony; DIVA1 = dispersal vicariance analysis (unconstrained); DIVA2 = dispersal vicariance analysis (constrained to ≤2 areas per node); SM = stochastic mapping (post. prob. = posterior probabilities for reconstructions); DEC1 = dispersal-extinction-cladogenesis (unconstrained); DEC2 = dispersal-extinction-cladogenesis (constrained to ≤2 areas per node). The −lnL scores are for the optimal reconstructions in DEC analyses. Area reconstructions are represented in binary format (0 = absent; 1 = present) in the order of Indonesian grade, Fiji, Hawaii, Samoa, Tonga, Micronesia, Marquesas. In instances of more than one reconstruction, two or more alternative reconstructions may have been reconstructed that are equally parsimonious, probable or likely, dependent upon the method. See text for details.

Node	FP	SM	post. prob.	DIVA1	DIVA2	DEC1	−lnL	DEC2	−lnL
1	1000000	1000000	>0.99	1111111	1100000	1100000	−63.90	1100000	−64.25
					1010000	1000000	−64.53	1000000	−64.87
					1001000	1001000	−65.25		
						1101000	−65.29		
2	1000000	0100000	0.85	0111111	0110000	0100000	−64.02	0100000	−64.39
	0100000	0001000	0.10		0011000	1000000	−64.83	1000000	−65.18
	0010000					0101000	−65.37		
	0001000					0001000	−65.48		
						1100000	−66.00		
3	1000000	0010000	>0.99	0010000	0010000	0100000	−63.86	0100000	−64.19
	0100000					0001000	−64.91	0001000	−65.57
	0010000								
	0001000								
4	1000000	0100000	0.91	0101111	0100000	0100000	−63.86	0100000	−64.19
	0100000	0001000	0.09		0101000	0101000	−64.91	0101000	−65.57
	0010000								
	0001000								
5	0100000	0100000	>0.99	0100000	0100000	0100000	−63.65	0100000	−64.00
6	0100000	0001000	0.83	0001111	0001000	0100000	−63.65	0100000	−64.00
	0001000	0100000	0.13	0101111	0101000	0101000	−64.21	0101000	−65.01
7	0001000	0001000	0.99	0001110	0001000	0001000	−63.37	0001000	−63.81
8	0100000	0001000	0.75	0100001	0100000	0101000	−63.37	0101000	−63.81
	0001000	0000001	0.24	0101001	0101000			0100000	−65.79
9	0000010	0000010	>0.99	0000010	0000010	0000001	−63.77	0000001	−63.77
10	0001000	0100000	>0.99	0001100	0100000	0001100	−63.77	0001000	−63.77
						0001000	−63.97		
11	0100000	0100000	>0.99	0100000	0100000	0100000	−63.52	0100000	−64.02
	0001000								
12	0100000	0000001	0.73	0001001	0100001	0101000	−63.52	0101000	−64.02
	0001000	0001000	0.27		1001001	0100000	−64.74	0001000	−65.28
	0000001					0001000	−64.90		
13	0100000	0001000	>0.99	0001000	0001000	0101000	−63.91	0101000	−64.48
	0001000				0101000	0100000	−64.20		
						0001000	−64.93		
14	0000001	0000001	>0.99	0000001	0000001	0001000	−63.91	0001000	−64.48
						0100000	−64.20	0100000	−64.77

than those from parsimony methods—in part because priors were more readily incorporated into likelihood methods such as DEC. This highlights the importance of considering optimality criterion in choosing among existing methods for biogeographic inference.

Parsimony-Based Methods

The suitability of parsimony versus likelihood has been debated for phylogeny reconstruction (Kolaczkowski and Thornton, 2004; Sober, 2004; Gadagkar and Kumar, 2005), character evolution (Cunningham, 1999; Pagel, 1999; Huelsenbeck et al., 2003), and ancestral range reconstruction (Nepokroeff et al., 2003; Ree et al., 2005). Parsimony has been criticized for its tendency to underestimate the number of character transitions on long phylogenetic branches and when rates of evolution are high (Felsenstein, 1973; Nielsen, 2002) and for the difficulty of evaluating the statistical certainty of most-parsimonious ancestral state reconstructions (Nielsen, 2002; Nepokroeff et al., 2003; Huelsenbeck et al., 2003). Geographic ranges of Pacific *Cyrtandra* exhibit the biogeographic equivalent of homoplasy (Fig. 2). The multiple equally parsimonious histories inferred by FP suggests that having a relatively large number of states (seven areas) between which transition costs are assumed to be flat and not considering relative opportunity for change along branches yields low potential to detect phylogenetic signal in these data. FP can provide clearer ancestral estimates when the topological distribution of geographic ranges is less complex; e.g., for species of *Santalum* L. across the Pacific, Australian species are predominant across the tips of the phylogeny, and Australia is correspondingly reconstructed as the ancestral range (Harbaugh and Baldwin, 2007). By contrast, no single area is so commonly represented for Pacific *Cyrtandra*. Alternative ways of coding areas, such as presence coding using multiple, binary coded states to represent areas (see Hardy and Linder, 2005) may circumvent this issue. However, the point of multi-state, single character coding as conducted in this study is to effectively enforce a dispersal-mediated allopatry scenario for range inheritance inference.

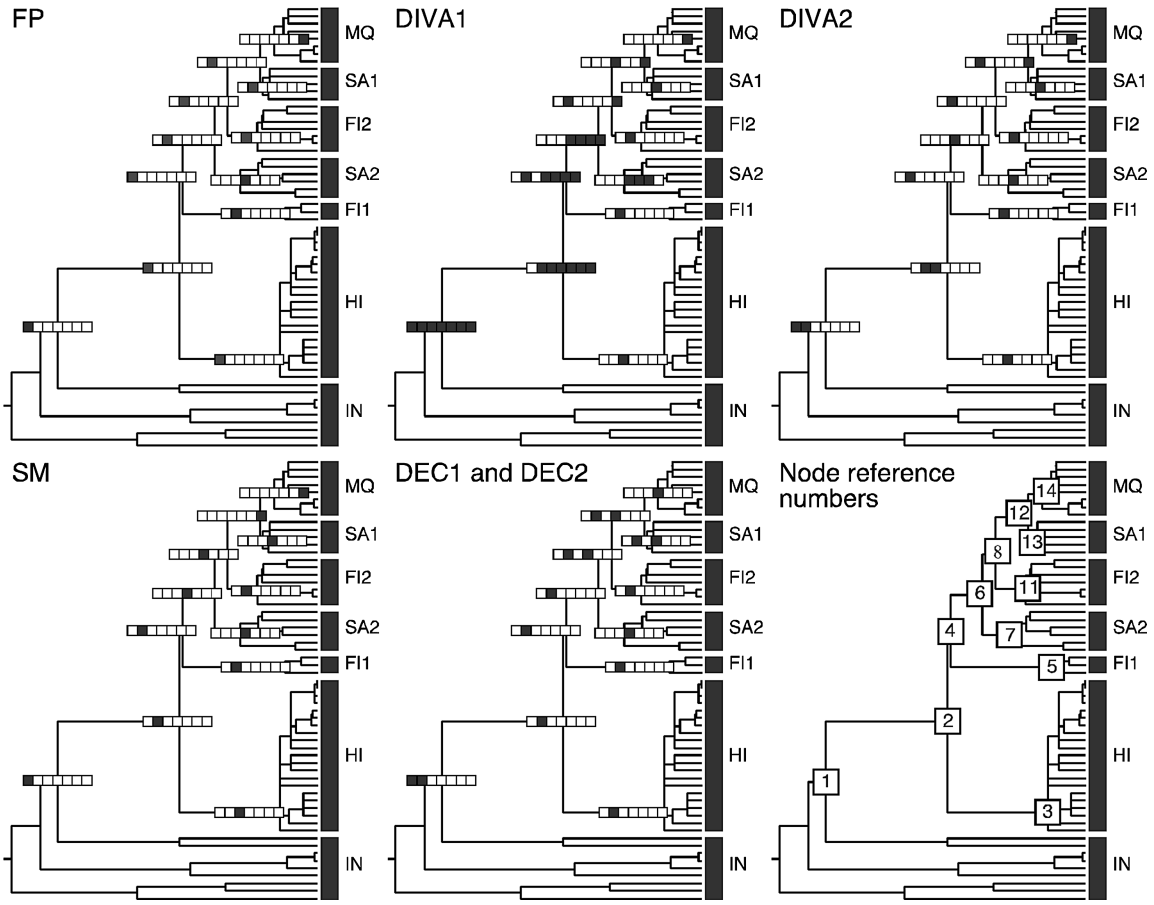

FIGURE 4. Summary and comparison of results from the four ancestral range reconstruction methods (details in Table 3). Area reconstructions are represented by open or shaded blocks (open=absent; shaded=present) in the order of Indonesian grade, Fiji, Hawaii, Samoa, Tonga, Micronesia, Marquesas. In instances of more than one reconstruction, only the first reconstruction is shown for simplicity. FP = Fitch parsimony, DIVA1 = dispersal vicariance analysis (unrestricted), DIVA2 = dispersal vicariance analysis (restricted to ≤ 2 areas per node), SM = stochastic mapping; DEC1 = dispersal-extinction-cladogenesis (unrestricted), DEC2 = dispersal-extinction-cladogenesis (restricted to ≤ 2 areas per node). Node reference numbers are those nodes referred to in Figures 2 and 3 and in the text.

Unlike FP, DIVA uses presence coding and considers vicariance scenarios at nodes to infer ancestral range inheritance (Hardy and Linder, 2005). In DIVA1, increasingly widespread ancestral ranges are inferred on the phylogeny at deeper internal nodes. Because extant species have small ranges, and vicariance has zero cost, it is generally more parsimonious to reconstruct ancestors with widespread ranges that progressively fragment by vicariance than for ancestors with more narrow ranges to evolve by dispersal and extinction events. However, this conflicts with our assumption that ancestral ranges in *Cyrtandra* were similar in extent to current ranges. Restricting range size to a maximum of two areas (DIVA2) does not alleviate this conflict. For example, the ancestor of the Pacific clade is inferred to have inhabited both Fiji and Hawai'i, an improbable range of over 4800 km—over five times the distance spanning any current ranges of extant species. By not restricting ranges to single areas, as in our FP coding, improbably wide ancestral ranges are disproportionately inferred, arguing against the usefulness of presence coding in our system.

The Fiji-Hawai'i ancestral range is rendered more unlikely by considering that Fiji originated no more than 40 Ma and the extant Hawaiian Islands are much younger, forming about 5.1 Ma. Even considering the statistical uncertainty of node age estimates, the hypothesis of an ancestral species being present in both Fiji and Hawai'i at the time of origin of the Pacific lineage is highly unlikely. Similar conflicts are apparent at nodes predating Micronesia (<9 Ma) and the Marquesas (<6 Ma). These results suggest that both FP and DIVA are inadequately capturing critical aspects of biogeographic evolution in Pacific *Cyrtandra*.

Likelihood- and Bayesian-Based Methods

Unlike FP and DIVA, SM yields inferences about the direction and timing of dispersal events (Fig. 3) that do not conflict with temporal origins of areas and suggest a stepping-stone pattern from west to east. From the Indonesian grade, Fiji is the first area colonized with subsequent dispersal events to Samoa and Hawai'i. Samoa is then inferred as the ancestral range for the remaining South Pacific clade. Dispersals from Samoa back to Fiji and also to Tonga, Micronesia, and the Marquesas are inferred. A less intuitive result is that the second Samoan clade (node 13) is inferred to arise from

an east to west colonization event from the Marquesas (73% posterior support), a scenario that requires more dispersal events than if it were ancestrally Samoan (27% posterior support).

These results illustrate the influence of branch lengths in SM analysis. Overall, SM appears to effectively reflect dispersal-mediated allopatry in *Cyrtandra*, despite this mode of divergence not being explicitly included in the underlying model. We speculate that this result may be commonly obtained in cases where lineage isolation and divergence following dispersal is rapid in relation to the phylogenetic timescale. Although this may be true for Pacific *Cyrtandra*, simulation studies will certainly be needed to test the generality of this pattern.

It may be less appropriate to apply SM in continental systems, or in smaller-scale insular studies (e.g., within the Hawaiian Islands), in which lineages are more likely to be widespread, and allopatric divergence following range expansion may be slower. The DEC method allows for alternative scenarios of geographic divergence beyond dispersal-mediated allopatry. For *Cyrtandra*, DEC results suggest a stepping-stone pattern similar to the SM results. DEC infers Fiji to be the area of origin for the Hawaiian lineage, a result that differs from those of other methods (Fig. 4). Similarly, DEC infers a Samoan origin of the Marquesan clade. These results are not as consistent with dispersal-mediated allopatry as those inferred by SM. In contrast to SM, the hypothesis of a Marquesan origin for the Samoan clade descendant from node 13 is not supported. Rather, this node is reconstructed as Fijian-Samoan, like many of the other interior nodes. Taken together, DEC results suggest a centralized role of a Fiji-Samoa complex from which other more remote areas of the Pacific were colonized.

The flexibility of the DEC model in allowing external knowledge to inform inferences is highlighted here by comparing it with less flexible methods that yield relatively inconclusive results (FP) or results that conflict with such knowledge (DIVA). Nevertheless, the inference by DEC of a widespread lineage inhabiting Fiji and Samoa that persists over several million years is highly suspect. One explanation for this result is that DEC does not explicitly link range expansion with allopatric divergence, so range-dependent divergence events such as dispersal-mediated allopatry events cannot be discerned by the model. Ree and Smith (2008) observed this in applying the DEC model to the Hawaiian diversification of *Psychotria* L. (Rubiaceae), and they suggested that an explicit model for dispersal-mediated allopatry is needed. Restricting DEC to no more than two areas per node somewhat approximates this scenario. Ancestral ranges inferred under DEC2 are less uncertain than DEC1, suggesting that the more constrained model may better fit the data.

Biological Significance

Cyrtandra biogeography.—Although preliminary in its nature, the current study implicates Fiji, and perhaps more accurately Fiji-Samoa, as a critical interface between the western distribution of species of *Cyrtandra* and the monophyletic Pacific Islands clade. In DIVA, SM, and DEC analyses, both Fiji and Samoa are strongly favored as potential areas for the origin of the Pacific clade, node 2. These results conflict with a hypothesis of a Hawaiian origin for the Pacific clade (Cronk et al., 2005). Whereas DIVA reconstructs these areas inclusive within a broad-ranging lineage covering distant areas, SM and DEC both reconstruct Fiji and Samoa almost exclusively at internal nodes in the Pacific clade (Figure 3, nodes 2 to 9). Whereas SM reconstructs directional dispersals to and from this area with subsequent dispersals to new areas, DEC reconstructs a persistent Fiji-Samoa lineage that spawned within-area lineages that later colonized more remote islands. Despite these differences, agreement among these methods centers on the pivotal role of Fiji-Samoa in the initial diversification of the Pacific lineage as well as later divergence events originating from this region.

Phylogenetic uncertainty.—One major relationship that remains unresolved is the placement of the Fiji 1 clade that includes *C. occulta* and two similar Fijian species. Resolution of this relationship in particular may be critical for further evaluation of the Fiji-Samoa hypothesis. In the combined dataset analysis, this clade is reconstructed sister to the remaining South Pacific, which together are sister to the Hawaiian clade. However, the branch separating Hawai'i and the South Pacific clade is exceedingly short and no support exists for this relationship. Efforts have been made to characterize additional genic regions, including the nuclear *GBSSI*, nuclear *G3pdh*, three anonymous nuclear regions, and several chloroplast regions, including *trnL-trnF, trnT-trnD, trnC-ycf6, ycf6-psbM, psbB-psbH,* and *psaI-accD*, in an attempt to improve the resolution of this and other unresolved relationships (data not shown). To date, regions tested have been either not sufficiently variable enough to include in phylogenetic analysis or were recalcitrant to characterization (*trnT-trnD, trnC-ycf6*).

In addition to improving branch length and node support, additional species sampling from the Fiji-Samoa region and surrounding areas is needed to better elaborate on this Fiji-Samoa hypothesis. There are approximately 60 known species from Fiji and Samoa alone, only a sampling of which is included in the current study; no specimens were included from the neighboring Solomon Islands or Vanuatu. These areas have been surveyed little for *Cyrtandra*, and complete species distributions and actual species numbers are not fully known (Gillett, 1973; Smith, 1991). Long branches in our phylogeny, such as those leading to the Pacific and Hawaiian lineages, may be an artifact of incomplete sampling in areas such as Fiji. Alternatively, extinctions along these branches might be responsible. Comprehensive field surveys are warranted to better understand extant species distributions/species numbers and to collect tissue samples for additional phylogenetic analysis needed to resolve this issue.

Hybridization.—Increasingly, phylogenetic evidence suggests that reticulate introgression between multiple lineages has occurred in the ancestry of many species (McDade, 2000) and is recognized as a major factor in species evolution, particularly among plants (Tsukaya et al., 2003, McDade, 2000). Natural, in situ hybridization

has long been thought to occur in *Cyrtandra* (Gillett, 1973; Burtt, 2001; Kiehn, 2001). However, no conflict in our three genic region datasets was observed that would suggest hybridization is an issue in resolving major relationships in *Cyrtandra*. Where hybridization may be an issue, at tip lineages, poor support exists for these relationships and any inference of hybridization is conjectural. Ultimately, the effects of hybridization will need to be examined in more detail, through population-level sampling and analysis, to elaborate on the biological significance of the results presented from this study. However, we have made an effort to exclude any individuals that morphologically appear to be of hybrid origin and conclusions drawn here deal with the relationship of major lineages, not individual species, and therefore we do not expect hybridization to be affecting the results of this study.

Conclusions, Considerations, and Future Directions

Our study suggests that ancestral range reconstruction yields more intuitive results when relevant sources of information such as distance between areas, divergence times, and topological uncertainties are considered. A stochastic mapping procedure, coupled with a DEC model, may allow the relative strengths of both SM and DEC to be incorporated into an improved method for ancestral range reconstruction (Ree and Smith, 2008a). This would facilitate the incorporation of phylogenetic uncertainty by mapping range evolution over a posterior distribution of trees. Future development of model-based ancestral range analysis must surely focus on including diversification rates. Currently, no methods use stochastic birth-death models to account for "invisible nodes" resulting from lineage extinction (Ronquist, 2002). Accounting for these "ghost lineages" may be important, as much so as accounting for distance and time. In *Cyrtandra*, it has been suggested that local extinction has played an important role in the diversification of this genus (Cronk et al., 2005). Models such as BiSSE (Maddison et al., 2007) include character-dependent birth and death rates of lineages, and analogous range-dependent birth-death models are needed. Future comparison of methods using different biogeographic systems, such as continental or marine lineages, would further illustrate the comparative performance of these and other methods.

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Condylostylus sp. (Diptera; Dolichopodidae) harvesting pollen from *Cyrtandra richii* (Gesneriaceae), U'polu Island, Samoa. Photo by John R. Clark. Insect identification by William Turner (Entomology, Washington State University).