PHYLOGENY, ANCESTRAL RANGE RECONSTRUCTION AND PARTIAL TAXONOMIC REVISION OF PACIFIC <u>CYRTANDRA</u> (GESNERIACEAE)

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of JOHN ROBERT CLARK find it satisfactory and recommend that it be accepted.

Chair

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Abstract

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Cyrtandra J.R. & G.Forster (Gesneriaceae) is one of the most widely dispersed plant genera in southeast Asia and the Pacific. Species concepts are variable and characters used to differentiate species are minimally useful between lineages. Molecular-based approaches for interpreting relationships between areas and for assigning taxonomic rank have been proposed. As part of this dissertation, I conducted three independent but related studies to address relationships in *Cyrtandra*: 1) I first used a preliminary phylogeny of *Cyrtandra* to compare four methods of ancestral range reconstruction: two developed for character-state reconstruction (Fitch parsimony and stochastic mapping), and two developed for ancestral range reconstruction (dispersal-vicariance analysis and dispersal-extinction-cladogenesis). The methods yielded conflicting results, dependent upon their respective assumptions. Likelihood-based methods allowed analytical interpretation of results useful in evaluating reconstructions. Dispersal-extinction-cladogenesis was the most applicable in my study, possibly due to its incorporating distance and timing of connections between areas. 2) I used molecular phylogenetic, diversification rates, and ancestral range analyses to construct a well-resolved evolutionary hypothesis for *Cyrtandra*. Results support a greater Fiji-Samoa region, corresponding with Takhtajan's 'Fijian Region,' as a major 'cross-roads' for *Cyrtandra* in the Pacific. I also compared existing taxonomy to my hypothesis to better understand its applicability. Current classifications are partially artificial; I suggest that sectional classifications should be revised to reflect monophyletic lineages. Also, species-level relationships should be closely studied in relation to these lineages. 3) Finally, I conducted a study/review of Hawaiian *Cyrtandra* as a model for phylogenetic-based revision in this genus. Morphological groupings, taxon distributions and taxonomy are well described for Hawaiian *Cyrtandra* but conflict with the current phylogenetic hypothesis. To stabilize taxonomy for future revision, a preliminary key to current sections is proposed and five species previously not treated are presented and classified. Results from this and the previous two studies indicate that a phylogenetics-based approach to taxon revisions is most appropriate for understanding evolutionary relationships in unwieldy genera such as *Cyrtandra*. Future studies in this genus will benefit from comprehensive, population-level molecular analysis in well-studied areas such as Hawai'i and from increased sampling in under-collected areas such as Fiji.

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DEDICATION

This dissertation is dedicated to the members and supporters of The Gesneriad Society, Inc., for their inspiration and support throughout this process.

ATTRIBUTIONS AND GENERAL INTRODUCTION

Although this dissertation has been the sole responsibility of me (JRC), much of this research has been collaborative in nature owing to the broad scale of the subject matter. *Cyrtandra* J.R. & G.Forster (Gesneriaceae), a large genus of great distribution, is arguably one of the most challenging genera of angiosperms in terms of understanding evolutionary and taxonomic relationships; a concerted research effort has been required to address this complex system. Near-future publication of the three chapters presented in the following pages will include co-authorship by several individuals who have provided invaluable expertise. These include Richard H. Ree, Ph.D., Curator, Botany Department, Field Museum of Natural History, Michael E. Alfaro, Ph.D., Assistant Professor, School of Biological Science, Washington State University, Matthew G. King, Ph.D., Post-doctoral Research Fellow, University of British Columbia (co-authors for Chapter 1), Warren L. Wagner, Ph.D., Department Chair, Department of Botany, National Museum of Natural History, and Eric H. Roalson, Ph.D., Assistant Professor, School of Biological Science, Washington State University (co-authors on Chapters 1, 2, and 3). Dr. Wagner, author of the *Manual of the Flowering Plants of Hawai'i*, provided the original impetus for this project and initiated aspects of Chapter 3 over 20 years ago.

In this study, I have constructed a top-down approach towards elucidating the detailed dispersal of *Cyrtandra* throughout its vast southeast Asian and Pacific range. First, in Chapter 1 I compare current ancestral range reconstruction methods and test their utility in retracing the evolutionary history of island plant lineages, a critical first-step in addressing questions in insular lineage radiations such as *Cyrtandra*. A comparative study of this nature has never been conducted until now, results from which have major implications in how insular ancestral range studies will be conducted in the future. In Chapter 2, I use this new found understanding on the application and utility of ancestral range reconstruction methods to conduct a detailed analysis on the dispersal and diversification of Pacific-wide *Cyrtandra*. I also begin to apply this new hypothesis of evolutionary relationships in *Cyrtandra* by interpreting broad-scale similarities and differences in morphological features across major terminal lineages in my phylogenetic hypothesis. A phylogenetics-based approach to taxonomic revision in *Cyrtandra* will be essential in developing a genus-wide classification and this effort represents the first of its kind to do so. Chapter 3 deals with a detailed summary of the current state of knowledge surrounding the Hawaiian lineage in preparation for a monographic revision of Hawaiian *Cyrtandra*. Hawai'i represents a monophyletic lineage within the Pacific clade and this area has more species of *Cyrtandra* per land area than any other Pacific *Cyrtandra* group. Hawaiian *Cyrtandra* represent an ideal lineage to examine in more detail patterns of diversification at the species and population level because the area is well collected and documented. Chapter 3 serves as a starting point to conduct future molecular-based studies in the phylogenetics and taxonomy of Hawaiian *Cyrtandra*. Additionally, methodologies outlined here can be applied to other major areas of diversity including the Fiji Islands. Ultimately, this and future detailed research through combined efforts across institutional and political boundaries is warranted because *Cyrtandra* is one of the most widespread plant genera in the world and understanding where and why it exists will be a major step towards a better understanding of plant evolution and Pacific biogeography.

CHAPTER ONE

A COMPARATIVE STUDY IN ANCESTRAL RANGE RECONSTRUCTION METHODS: RETRACING THE UNCERTAIN HISTORIES OF INSULAR LINEAGES

INTRODUCTION

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 an understanding of how and why lineages evolve (Lomolino, 2000). Emergent from this is a widely accepted view that 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). I 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 Hawaii (Maui, Molokai, Lanai, 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, are also conceivable (Ree et al., 2005). Acknowledging these alternatives raises the question of how phylogenetic data is best used to infer whether dispersal-mediated allopatry is indeed the predominant mode of insular lineage divergence.

A variety of methods have been used for inferring ancestral ranges on phylogenetic trees and can be categorized along two distinct lines: first, in how geographic ranges are treated conceptually, and second, in the optimality criterion used for choosing between alternative hypotheses. Conceptually, *character state reconstruction* methods implemented in ancestral range reconstruction restrict ranges to single areas and treat them as discrete 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 intermediate widespread ranges being integral to inferences about disjunctions. Ancestral ranges can be reconstructed using either of these two kinds of methods by employing either a parsimony-based algorithm or a likelihood-based algorithm (Table 1.1).

To date, methods for ancestral range reconstruction encompassing these categories have never been rigorously compared in an empirical system. In this study, I 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, 2008). My motivation is to test their performance in reconstructing ancestral range evolution in a well-resolved phylogeny of the insular angiosperm genus *Cyrtandra* J.R. & G.Forster (Gesneriaceae), using a prior hypothesis of dispersal-mediated allopatry as a common benchmark. I 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 (see Appendix 1 for distribution map and species numbers per area). *Cyrtandra* likely evolved in the Indo-Malayan region (Burtt, 2001) and, today, is found on most high islands of the Pacific. *Cyrtandra* species are morphologically diverse and include a variety of habit, fruit and flower characteristics. Species in the Pacific islands east of Papua New Guinea, however, are remarkably similar in being white flowered with fleshy berries and a predominantly understory shrub/small tree habit. Pacific *Cyrtandra* species are thought to be monophyletic based on morphology and molecular data (Gillett, 1973; Cronk et al., 2005) and most Pacific species inhabit very similar perennially wet upland tropical forests throughout the Pacific Islands.

Pacific *Cyrtandra* species are almost exclusively 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 are indicative of a dispersal-mediated allopatric divergence model. 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

The current study includes 61 taxa including two outgroup taxa (both *Aeschynanthus* L. species; Table 1.2). *Aeschynanthus* was selected as the appropriate outgroup based on current understanding of phylogenetic relationships among paleotropic gesneriads (Mayer et al., 2003; Cronk el al., 2005). Samples included represent lineages present on most major geological features in the Pacific and all attempts were made to include at least one specimen from principal lineages as defined by Gillett (1973) and Wagner et al. (1990; Table 1.1). Several putative taxa from Indonesia and Fiji were included that have not yet been identified to species, but initial analysis showed to be genetically distinct.

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 taxon, 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 denaturation @ 95°C; 34 cycles of 1 min @ 95°C, 1 min @ 48°C, 1 min @ 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 & Marcos, 1998) and ETS-B developed for *Mimulus* (Phrymaceae) by Beardsley and Olmstead (2002). PCR reaction conditions: initial denaturation @ 95°C; 34 cycles of 1 min @ 95°C, 1 min @ 50°C, 1 min @ 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 denaturation @ 95°C; 30 cycles of 1 min @ 95°C, 30 sec. @ 52°C, 30 sec. @ 72°C; followed by a 7 min extension at 72°C. Note: nine of the 61 taxa analyzed could not be sequenced for *psbA-trnH*. Preliminary analyses with and without these taxa did not alter topologies significantly (data not shown). The inclusion of these taxa 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, both ML and BI 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 ($\geq$ 75% bootstrap support and/or $\geq$ 95% posterior probability) among the various topologies were in conflict; therefore, the three genic regions were combined and analyzed. Results from these combined dataset ML and combined dataset BI analyses were used in the dating and ancestral range reconstruction methods.

Estimation of Phylogeny Divergence Times

I used the r8s program (v. 1.7.1; Sanderson, 2004) to estimate a chronogram for *Cyrtandra* based on the maximum likelihood tree using semi-parametric rate smoothing

(SPRS) by penalized likelihood and the truncated Newton algorithm (Sanderson 2002). 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 data sets 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).

Because a poor fossil record exits for the Lamiales with no known macrofossils for the Gesneriaceae (Wiehler, 1983), I used estimated ages of Pacific island groups as maximum age calibration points (Baldwin and Sanderson, 1998; Roalson et al., 2008). The extant Hawaiian Islands have an estimated age of 5.1 million years before the present (MYBP; Price and Clague, 2002), Fiji (40 MYBP; Evenhuis and Bickel, 2005), Samoa (24 MYBP; Hart et al., 2004), Micronesia [Kosrae] (<9 MYBP; Keating et al., 1984) and Marquesas (6 MYBP; Florence and Lorence, 1997).

Ancestral Range Analysis

Character state reconstruction methods.—In applying character state reconstruction methods to ancestral range reconstruction, geographic ranges are coded as discrete, single-area states, and widespread ranges spanning more than one area are not allowed.

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.

Stochastic mapping (SM), a Bayesian approach to character state reconstruction (Nielsen, 2002; Huelsenbeck et al., 2003), may also be applied to ancestral ranges. As with FP, single-area ranges can be coded as discrete states, but unlike FP, SM is based on a probabilistic model of transitions between these 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 prevalent 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.

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 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.

The dispersal-extinction-cladogenesis (DEC) model (Ree et al. 2005; Ree and Smith 2008) is to DIVA what stochastic mapping is to Fitch parsimony: 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). 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.

Testing the Methods

Given that my focus is on an empirical system, rather than simulated data with known evolutionary sequences, my evaluation of the performance of ancestral range methods is limited to how well their results match my prior expectations. With this in mind, I established two criteria for interpreting the 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 in relatively close proximity with one another if the Pacific area as a whole is considered. Furthermore, range limits for *C. samoensis* are unclear and current data suggest the lineage may contain cryptic species (M. Kiehn and J.R. Clark, unpublished data) with much smaller geographic ranges. Given the assuredly limited distribution of all other species of *Cyrtandra*, it is probable than ancestral species also had limited ranges. Thus, the range of *C. samoensis* can be used as a conservative maximum estimate of taxon 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, Hawaii and the Marquesas Islands, and can be used as conservative maximum ages for endemic lineages inhabiting these areas. Islands ages were used to infer clade origins and to accept or reject hypotheses whether or not reconstructions are less than the age of the islands.

Applying the models.—Ancestral range patterns were inferred using FP, SM, DIVA, and DEC. In all analyses, seven geographic areas were recognized: 1) a broadly defined "Indonesia" referring to the collective ranges of the more western taxa representing the grade of species within which the Pacific lineage is nested; 2) Fiji; 3) Hawaii; 4) Samoa; 5) Tonga; 6) Micronesia; and 7) Marquesas.

For FP analysis, geographic distribution was coded as a single multi-state character based on extant species distributions. Analysis was performed using MacClade 4.03 (Maddison and Maddison, 2001) using the single most likely tree from the likelihood analysis.

SM analysis was performed using SIMMAP 1.0b2 (Bollback, 2005) and a sub-sample of 1000 trees from the Bayesian posterior distribution. As in the FP analysis, areas were coded as a single multi-state character.

I used DIVA 1.1a with the ML tree, coding geographic range as the presence or absence of a species across regions (Ronquist, 1996). DIVA analysis was performed both unrestricted ("DIVA1") and restricted to a maximum range size of two areas ("DIVA2").

DEC analysis was performed using Lagrange version 2 (Ree and Smith, 2008) 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 2 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").

RESULTS

Maximum Likelihood Phylogeny Estimation

Combined analysis of ITS, ETS and *psbA-trnH* genic regions resulted in one most likely tree (-lnL = 8518.3163; ML phylogram, Figure 1.1). Resolution across the phylogeny, as indicated by bootstrap and posterior probability support indices, is high with most major clades being strongly supported. Monophyly of the Pacific clade (node 1; Figure 1.1) 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 = <75%; PP = 98%).

Several geographic areas are polyphyletic including Samoa with two well-supported clades (node 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 taxon nested with the Samoan clade (node 13). Terminal lineages, principally within island systems, are less resolved (Figure 1.1).

Estimation of Phylogeny Divergence Times

Figure 1.2 illustrates the results in the form of a chronogram from the r8s phylogeny divergence time estimates. The origin of the Southeast Asian “Indonesian” grade and the monophyletic Pacific clade is estimated at 35.2 MYBP (± 6.8 MYBP). Other noteworthy divergence dates include the South Pacific – Hawaiian stem lineage (18.7 ± 5.3 MYBP; node 2) and the origin of major crown group lineages including the Marquesas (5.9 ± 0.3 MYBP; node 14), one of two Samoan clades (10.5 ± 2.8 MYBP; node 7), the other Samoan clade (7.5 ± 3.4 MYBP; node 13), one Fijian clade (4.5 ± 2.6 MYBP; node 5) and the other major Fijian clade (8.1 ± 2.8 MYBP; node 11). Ages for lineage divergence events correspond with known geologic ages of other areas not constrained in this analysis (Figure 1.2) suggesting that the constraints imposed in this study offer a reasonable estimate of divergence times.

Ancestral Range Reconstruction Analysis

Ancestral ranges inferred using FP, SM, DIVA, and DEC are summarized in Table 1.3 and Figure 1.3. 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 1.3 while for clarity only the first reconstruction, in instances where more than one is inferred, is illustrated in Figure 1.3.

No single method conclusively resolved all fourteen nodes being considered. FP exhibits the least power in reconstructing ancestral ranges with eight nodes having multiple most parsimonious reconstructions. SM results are most congruent with the dispersal-mediated allopatry hypothesis and only five of fourteen nodes have more than one ancestral area reconstruction falling within a 95% confidence interval. Both DIVA analyses infer rather complex scenarios with wider ancestral ranges being inferred for increasingly interior nodes. DIVA1 results are the most conclusively resolved having only two nodes with more than one possible reconstruction while DIVA2 results have seven nodes not conclusively resolved. DEC results have the greatest number of unresolved nodes, nine of fourteen in DEC1, and with as many as five likely scenarios, those falling within 2 –lnL scores, for one single node. DEC2 results have slightly few unresolved nodes, eight of fourteen, and of the unresolved nodes no more than two reconstructions are likely in each.

FP results are difficult to elaborate on since most internal nodes, of those considered, are reconstructed with multiple equally parsimonious areas. For instance, nodes 1-4 all are reconstructed as Indonesia, Hawaii, Fiji, or Samoa.

SM yields results that are generally consistent with dispersal-mediated allopatry. Inconclusive reconstructions 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 Hawaii (>99% PP), while 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 Hawaii. The time criterion is not violated in any of the SM area reconstructions. SM results are also not in conflict with the 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-Hawaii-Samoa-Tonga-Micronesia-Marquesas, with vicariant divergence splitting Hawaii 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 taxa and in node 2, a Fiji-Hawaii and Hawaii-Samoa lineage is inferred. The daughter lineage Hawaii is only inferred to be

Hawaii, as in DIVA1, while the South Pacific lineage is inferred to be either Hawaii or Hawaii-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 my dating analysis (Figure 1.3; see Figure 1.2 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, and Indonesia-Fiji. For daughter lineages, Hawaii 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, since 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 taxa, 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 taxa inhabiting both Fiji and

Samoa may be a reasonable hypothesis, although such ranges are clearly at the outer limits of potential ranges for extant taxa.

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 methods are generally more compatible with prior expectations about the timing and mode of biogeographic evolution than those from parsimony methods. This suggests a clear division by optimality criterion, as opposed to the contrast I initially made between methods designed for characters versus geographic ranges.

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), with parsimony being 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 (Figure 1.2). 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 taxa 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*.

In DIVA1, increasingly widespread ancestral ranges are inferred on the phylogeny at deeper internal nodes. Because extant taxa 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, but this conflicts with the criterion that ancestral ranges in *Cyrtandra* were similar to current ranges. Restricting range size to a maximum of two areas (DIVA2) partially alleviates this conflict. For example, the ancestor of the Pacific clade is inferred to have inhabited both Fiji and Hawaii, an astounding range of over 4800 km – over five times the distance spanning any current range of extant species.

Moreover, Fiji originated no more than 40 MYBP and the extant Hawaiian Islands are much younger, about 5.1 MYBP. Even considering the statistical uncertainty of node age estimates, the possibility of a taxon present in both Fiji and Hawaii at the origin of the Pacific lineage is highly unlikely. Similar conflicts are apparent at nodes predating Micronesia (<9 MYBP) and the Marquesas (<6 MYBP). These results suggest that both FP and DIVA are inadequately capturing critical aspects of biogeographic evolution in Pacific *Cyrtandra*.

Likelihood Methods

Unlike FP and DIVA, SM yields inferences about the direction and timing of dispersal events (Figure 1.3) 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 Hawaii. 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).

This illustrates the influence of branch lengths in SM analysis. Overall, SM results for *Cyrtandra* appear to effectively reflect dispersal-mediated allopatry, despite this mode of divergence not being explicitly included in the underlying model. I speculate that this result may be commonly obtained in cases where lineage coalescence following dispersal is rapid in relation to the phylogenetic timescale. Although this may be true for Pacific *Cyrtandra*, and for "supertramp" clades in general (Cronk et al., 2005), simulation studies will certainly be needed to test the universality of this hypothesis.

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 conflicts with those of other methods (Figure 1.3). 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, one inference by DEC likely to be wrong is a widespread lineage inhabiting Fiji and Samoa that persists over several million years, an unlikely scenario given diversification rates inferred in the dating analysis (Figure 1.1). One explanation for this result is that DEC does not explicitly link range expansion with allopatric divergence. In effect, range-dependent diversification events, effectively dispersal-mediated allopatry events, are not "seen" by the model. A similar observation was made by Ree and Smith (2008) in applying the DEC model to the Hawaiian Islands diversification of *Psychotria* L. (Rubiaceae), prompting them to suggest that an explicit model for dispersal-mediated allopatry is needed. Restricting DEC to no more than two areas per node somewhat approximates this scenario, but not entirely. Both unrestricted and restricted models inferred this widespread lineage. Ancestral ranges inferred under DEC2 are less uncertain than DEC1, suggesting that the former model may better fit the data. Nodes for which multiple scenarios are plausible tend to have weak clade support, suggesting that topological uncertainty may also be affecting DEC results.

Cyrtandra Biogeography

Although preliminary in its nature, the current study implicates Fiji, and perhaps more accurately Fiji-Samoa, as a critical "crossroads" 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). While 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 1.3; nodes 2-9). 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 as a "cross-roads" in the initial diversification of the Pacific lineage as well as later divergence events originating from this region.

Additional taxon sampling from the Fiji-Samoa region and surrounding areas are needed to better elaborate on this Fiji-Samoa crossroads 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). Comprehensive field surveys are warranted to better understand species distributions/species numbers and to collect tissue samples for additional phylogenetic and ancestral range analyses.

CONCLUSIONS, CONSIDERATIONS AND FUTURE DIRECTIONS

This 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, 2008). 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 exceedingly important, as much so as distance and time. Methods such as BiSSE, as implemented in Mesquite (Maddison and Maddison, 2006), currently offer a means of modeling character-dependent birth and death of lineages. Integrating a birth-death model with DEC would allow tests of biogeographic hypotheses that explicitly include rates of lineage diversification, but at a likely cost of statistical power, with large trees being required for conclusive reconstructions of ancestral range dynamics. Further comparison of other biogeographic systems, such as continental or marine lineages, may help to guide model choice in studies of range evolution.

These studies would further illustrate the comparative performance of these and other methods, offer an alternative scenario to the dispersal-mediated allopatric model herein examined and offer additional environments in which ancestral range inheritance regimes may be drastically different.

Method	Model	Area states	Program; authors	Notes
Fitch parsimony [FP]	Character state reconstruction; area-cladogram comparison using parsimony optimization.	Discrete; one area reconstruction per node; areas coded as character states.	MacClade 4.03; Maddison and Maddison, 2001	Minimizes the number of changes required to explain an observed distribution of character states without reference to relative or absolute time; the directional costs of transitions between states are weighted equally.
Stochastic mapping [SM]	Character state reconstruction; a stochastic resampling of reconstructions using likelihood criterion in a Bayesian framework across a Bayesian analysis-generated set of topologies.	Discrete; one area reconstruction per node; areas coded as character states.	SIMMAP 1.0b2; Bollback, 2005	A probabilistic model of transitions between these states in continuous time; generates inferences of ancestral states by simulating evolutionary sequences on the phylogeny that yield the observed data at its tips; transitions are explicitly modeled as occurring along phylogenetic branches, with the probability of change being proportionate to evolutionary rate and branch length.
Dispersal vicariance analysis [DIVA]	Ancestral range reconstruction; area-cladogram, event-based method; parsimony optimization based on cost/benefit analysis.	Multiple; one to several areas can be inferred at any given node.	DIVA 1.1a; Ronquist, 1997	Minimizes the number of dispersal and local extinction events that are required to explain the current ranges of species; assumes that when a widespread ancestral lineage diverges, its range is subdivided by vicariance; assigns no cost to this event relative to dispersal and local extinction; branch lengths are not considered nor is any assumption made about where such events occur along a branch.
Dispersal-extinction-cladogenesis [DEC]	Ancestral range reconstruction; likelihood model-based method; a highly parameterized method employing likelihood criterion for assessing alternative area reconstructions.	Multiple; one to several areas can be inferred at any given node.	Lagrange 2.0; Ree and Smith, 2008	A continuous-time model for geographic range evolution; 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 branch length; has considerable flexibility in parameter specification incorporating prior evidence for connections between areas through time; DEC enumerates scenarios by which speciation causes descendant ranges to be inherited but it does not enforce vicariance on widespread ancestors.

Table 1.1. Summary table comparing the four ancestral range reconstruction methods applied in the current study.

Genus	*Specific Epithet*	Author(s)	ID No.	COLLECTOR	Collector No.	Origin
Cyrtandra	*tintinabula*	Rock	C0012	Perlman	17676	Hawai`i, Hawai`i
Cyrtandra	*wagneri*	Lorence and Perlman	C0013	Perlman	17673	Hawai`i, Hawai`i
Cyrtandra	sp.		C0016	Plunkett	1837	Fiji, Viti Levu
Cyrtandra	sp.		C0017	Plunkett	1838	Fiji, Viti Levu
Cyrtandra	sp.		C0018	Plunkett	1843	Fiji, Viti Levu
Cyrtandra	sp.		C0019	Plunkett	1875	Fiji, Viti Levu
Cyrtandra	sp.		C0020	Plunkett	1898	Fiji, Viti Levu
Cyrtandra	*wainihaensis*	Léveillé	C0021	Clark	549	Hawai`i, Kaua`i
Cyrtandra	*wawrae*	C.B. Clarke	C0022	Clark	550	Hawai`i, Kaua`i
Cyrtandra	*longifolia*	(Wawra) Hillebrand ex C.B. Clarke	C0023	Clark	551	Hawai`i, Kaua`i
Cyrtandra	*kauaiensis*	Wawra	C0026	Clark	556A	Hawai`i, Kaua`i
Cyrtandra	*pulchella*	Rich ex A. Gray	C0029	Lorence	8525	Samoa, Tau
Cyrtandra	*samoensis*	A. Gray	C0030	Lorence	8633	Samoa, Ofu
Cyrtandra	*samoensis*	A. Gray	C0031	RP	71221	Tonga
Cyrtandra	*ootensis var. mollissima*	Fosberg & Sachet	C0032	Wood	6563	Marquesas, Tahuata
Cyrtandra	*kusaimontana*	Hosokawa	C0033	Flynn	5995	Micronesia, Kosrae
Cyrtandra	*urvillei*	C.B. Clarke	C0034	Lorence	7838	Micronesia, Kosrae
Cyrtandra	*kealiae*	Wawra	C0035	Clark	566	Hawai`i, Kaua`i
Cyrtandra	*laxiflora*	H. Mann	C0037	Clark	568	Hawai`i, O`ahu
Cyrtandra	*hawaiensis*	C.B. Clarke	C0038	Clark	569	Hawai`i, O`ahu
Cyrtandra	*propinqua*	C. Forbes	C0039	Clark	570	Hawai`i, O`ahu
Cyrtandra	*calpidicarpa*	(Rock) St. John & Storey	C0040	Clark	571	Hawai`i, O`ahu
Cyrtandra	*kaulantha*	St. John & Storey	C0041	Clark	572	Hawai`i, O`ahu
Cyrtandra	*sandwicensis*	(Léveillé) St. John & Storey	C0045	Clark	576	Hawai`i, O`ahu
Cyrtandra	*grandiflora*	Gaudichaud	C0046	Clark	577	Hawai`i, O`ahu
Cyrtandra	*cordifolia*	Gaudichaud	C0048	Clark	579	Hawai`i, O`ahu
Cyrtandra	sp.		C0050	Clark	581	Hawai`i, O`ahu
Cyrtandra	*kealiae ssp. urceolata*	W.L. Wagner & Lorence	C0054	Perlman	18805	Hawai`i, Kaua`i
Aeschynanthus	*tricolor*	Hook.	C0055	MSBG	1974-1760-W	Indonesia
Aeschynanthus	*longicaulis*	Wallich ex R. Brown	C0056	MSBG	1974-2207-W	Indonesia
Cyrtandra	*feaniana*	F. Brown	C0059	Price	200	Marquesas, Hiva Oa
Cyrtandra	*ootensis* var. *ootensis*	F. Brown	C0060	Wood	10047	Marquesas, Hiva Oa
Cyrtandra	*ootensis* var. *molissima*	Fosberg & Sachet	C0061	Perlman	18399	Marquesas, Fatu Hiva
Cyrtandra	*thibaultii*	Fosberg & Sachet	C0062	Meyer	2541	Marquesas, Ua Pou
Cyrtandra	*ootensis* var. *mollissima*	Fosberg & Sachet	C0063	Wood	10266	Marquesas, Tahuata
Cyrtandra	*jonesii*	(F. Brown) Gillett	C0064	Wood	10484	Marquesas, Ua Huka
Cyrtandra	*nukukivensis*	Forest and Brown	C0065	Wood	10428	Marquesas, Ua Pou
Cyrtandra	cf. *richii*	A. Gray	C0068	Clark	646	Samoa, U`polu
Cyrtandra	*pogonantha*	A. Gray	C0071	Clark	649	Samoa, U`polu
Cyrtandra	*richii*	A. Gray	C0072	Clark	650	Samoa, Sava`i

Table 1.2. Taxa sampling list (1 of 2). 40 of 61 taxa total including two outgroup species (*Aeschynanthus* sp.). Taxa are ordered by ID No.

Genus	*Specific Epithet*	Author(s)	ID No.	COLLECTOR	Collector No.	Origin
Cyrtandra	***compressa***	**C.B. Clarke**	**C0074**	**Clark**	**652**	**Samoa, Sava`i**
Cyrtandra	***aurantiicarpa***	**Gillett**	**C0076**	**Clark**	**655**	**Samoa, Sava`i**
Cyrtandra	***coccinea***	**Blume**	**C0089**	**Hoover & Agus**	**ARs 167**	**Indonesia, Java**
Cyrtandra	**sp.**		**C0092**	**Hoover & Agus**	**ARs 173**	**Indonesia, Java**
Cyrtandra	**sp.**		**C0093**	**Hoover & Agus**	**ARs 175**	**Indonesia, Java**
Cyrtandra	**sp.**		**C0094**	**D**	**536**	**Indonesia**
Cyrtandra	**sp.**		**C0095**	**Wiriadinata, H.**	**12709**	**Indonesia, Java**
Cyrtandra	**sp.**		**C0096**	**HW**	**12713**	**Indonesia**
Cyrtandra	***picta***	**Blume**	**C0097**	**Wiriadinata, H.**	**12715**	**Indonesia**
Cyrtandra	***pendula***	**Blume**	**C0098**	**Wiriadinata, H.**	**12716**	**Indonesia**
Cyrtandra	***sulcata***	**Blume**	**C0100**	**Hoover & Agus**	**ARs 160**	**Indonesia, Java**
Cyrtandra	***spathulata***	**St. John**	**C0102**	**Clark**	**664**	**Hawai`i, Maui**
Cyrtandra	***grayana***	**Hillebrand**	**C0103**	**Clark**	**666**	**Hawai`i, Maui**
Cyrtandra	***munroi***	**C. Forbes**	**C0104**	**Clark**	**675**	**Hawai`i, Maui**
Cyrtandra	***grayi***	**C.B. Clarke**	**C0105**	**Clark**	**676**	**Hawai`i, Maui**
Cyrtandra	**sp.**		**C0112**	**Plunkett**	**1980**	**Fiji, Viti Levu**
Cyrtandra	***milnei***	**Seem. ex A. Gray**	**C0113**	**Clark**	**687**	**Fiji, Viti Levu**
Cyrtandra	***anthropophagorum***	**Seem. ex A. Gray**	**C0114**	**Clark**	**688**	**Fiji, Viti Levu**
Cyrtandra	***leucantha***	**A.C. Smith**	**C0116**	**Clark**	**693**	**Fiji, Viti Levu**
Cyrtandra	***occulta***	**A.C. Smith**	**C0117**	**Clark**	**694**	**Fiji, Viti Levu**
Cyrtandra	**cf. *occulta***	**A.C. Smith**	**C0119**	**Clark**	**702**	**Fiji, Viti Levu**

Table 1.2. Taxa sampling list (2 of 2). 21 of 61 taxa total including two outgroup species (*Aeschynanthus* sp.). Taxa are ordered by ID No.

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

Table 1.3. Summary and comparison of results from the four ancestral range reconstruction methods. Node numbers and method names are as described in the text and in Figure 2. Area reconstructions are represented in binary format (0=absent; 1=present) in the order of Indonesian grade, Fiji, Hawaii, Samoa, Tonga, Micronesia, Marquesas.

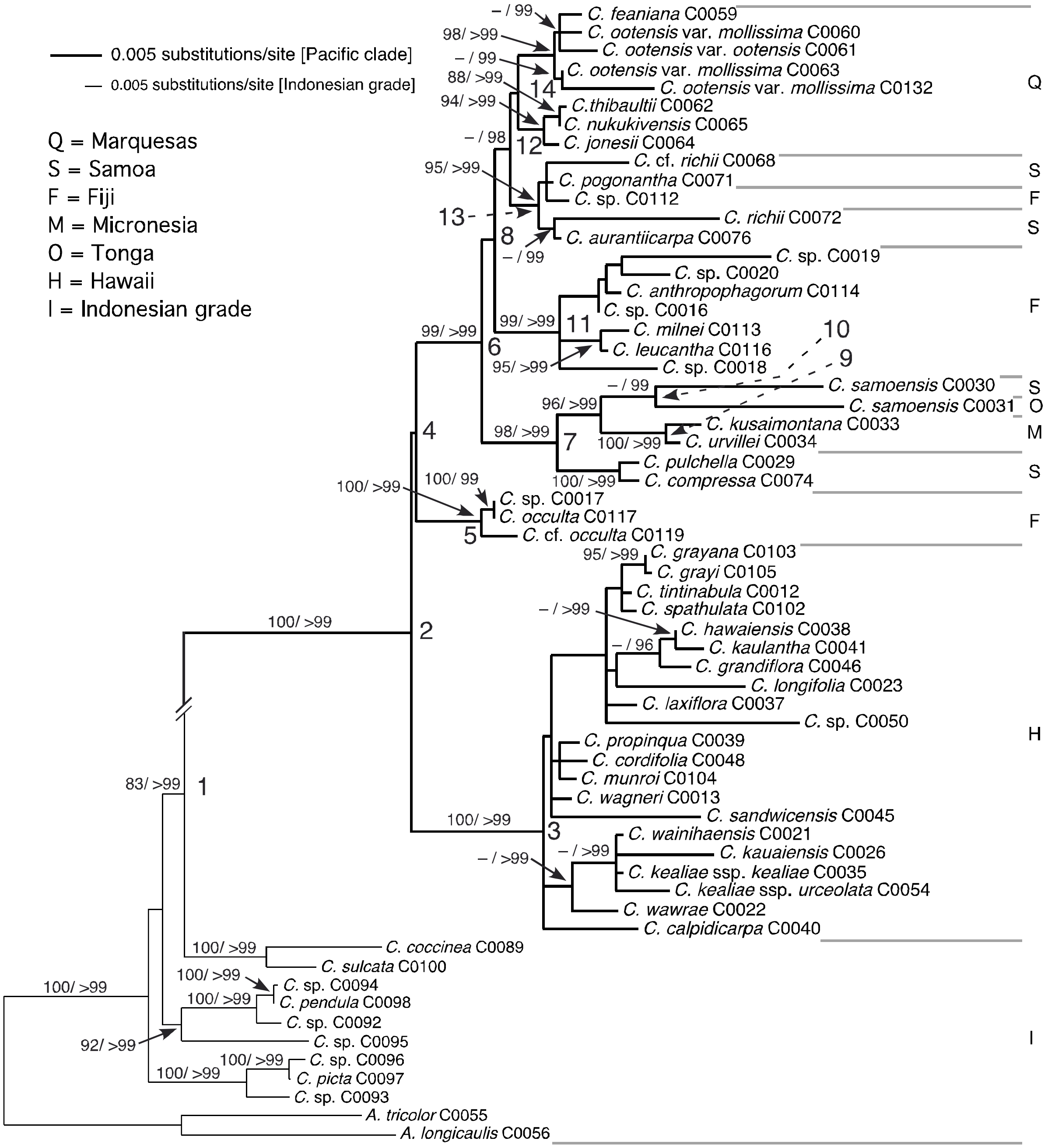

Figure 1.1. Maximum likelihood phylogram (-lnL = 8518.3163; single ML tree). Analysis of ITS, ETS and *psbA-trnH* regions; K80+G substitution model; letters to the right indicate geographic regions; numbers above nodes indicate branch support (bootstrap support ≥70% / Bayesian posterior probabilities ≥ 95%); Indonesian grade and outgroup taxa scaled down for de-emphasis; numbers by selected nodes (1-14) are for reference.

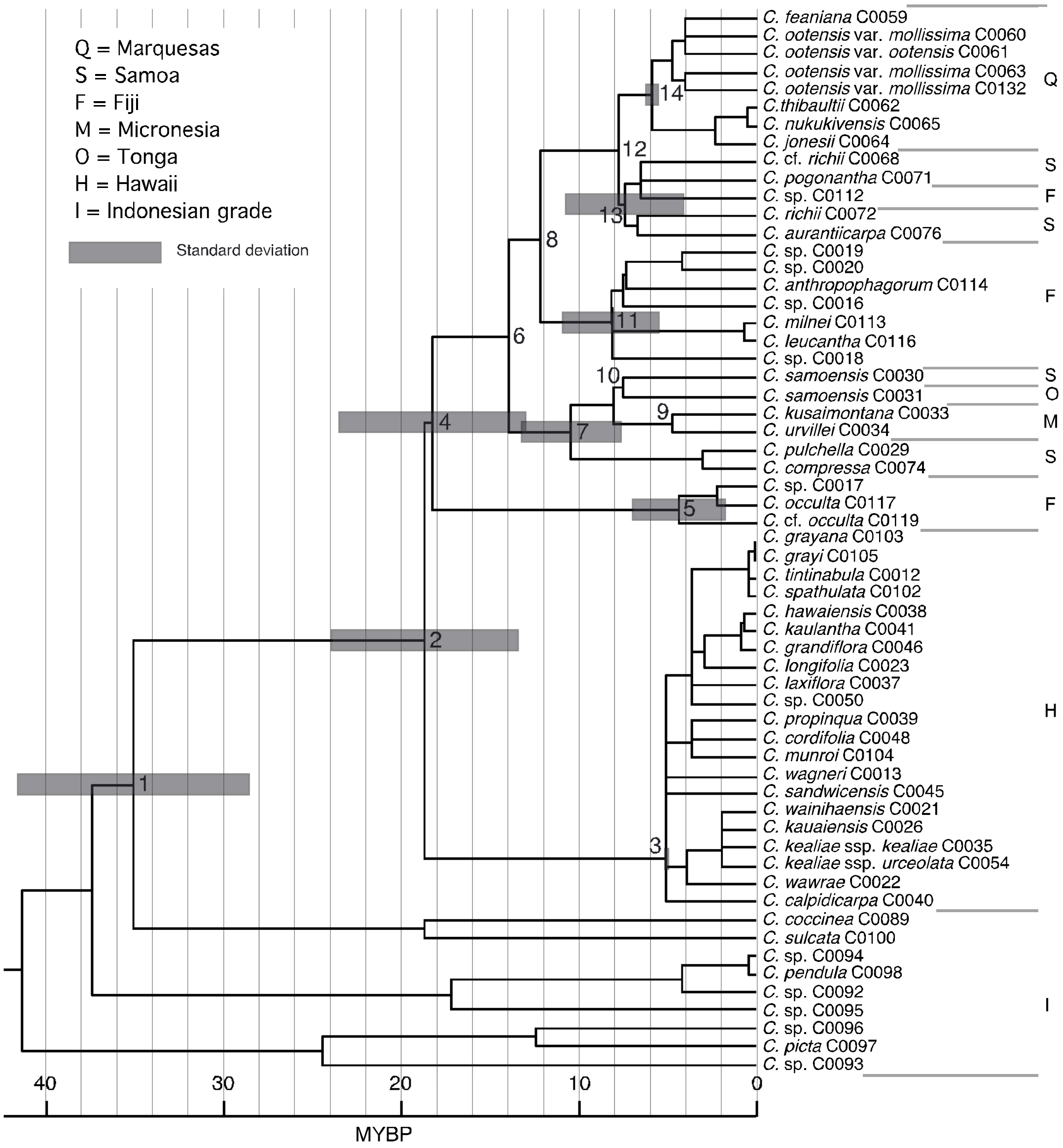

Figure 1.2. Chronogram based on penalized likelihood analysis of the ML tree calibrated using island ages referred to in the text; numbers at the bottom are ages in millions of years before the present (MYBP); gray bars represent standard deviations around selected nodes; letters indicate geographic area; numbers (1-14) are for reference; see text for details.

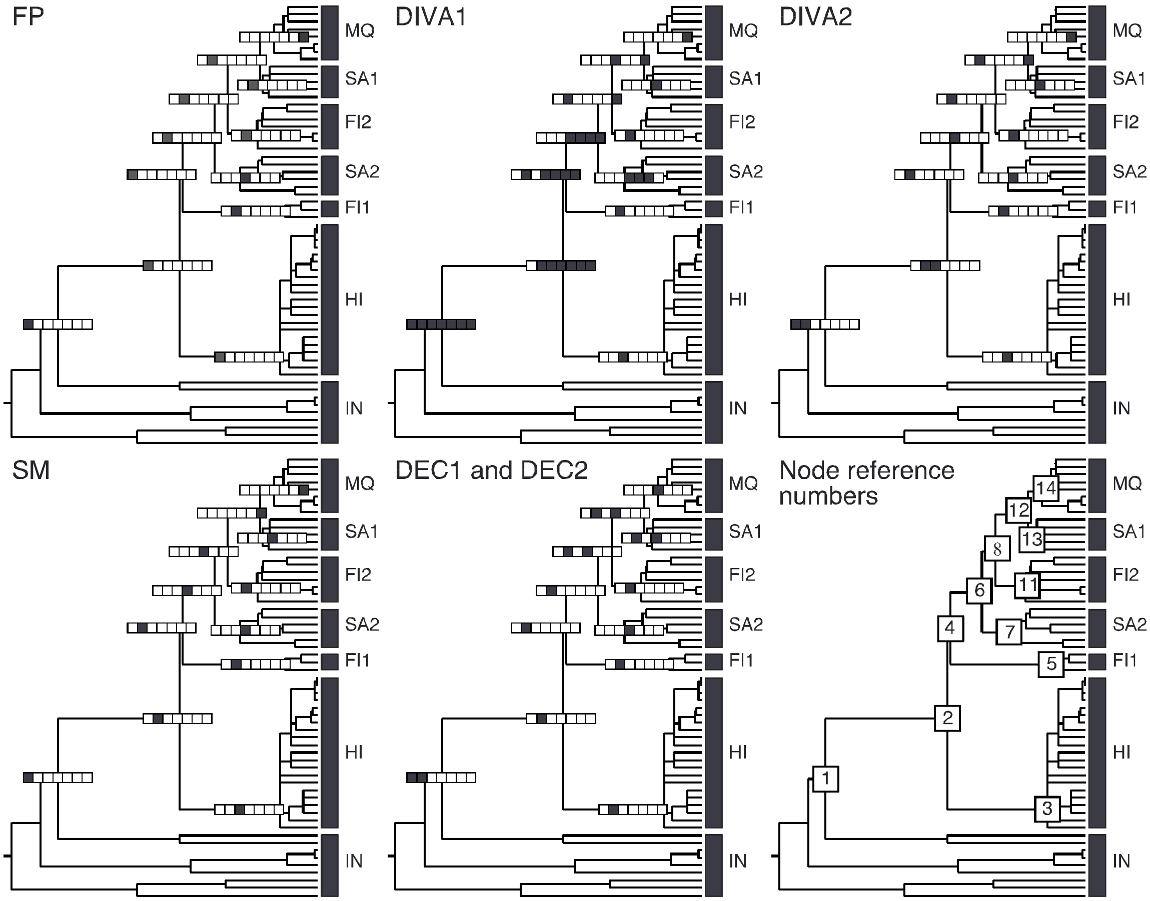

Figure 1.3. Summary and comparison of results from the four ancestral range reconstruction methods (details in table 1.3); FP = Fitch parsimony, DIVA1 = dispersal vicariance analysis (unrestricted), DIVA2 = dispersal vicariance analysis (restricted to $\leq$ 2 areas per node), SM = stochastic mapping; DEC1 = dispersal-extinction-cladogenesis (unrestricted), DEC2 = dispersal-extinction-cladogenesis (restricted to $\leq$ 2 areas per node); 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. See text for details.

CHAPTER TWO

PATTERNS OF DIVERSIFICATION AND ANCESTRAL RANGE RECONSTRUCTION IN THE SOUTHEAST ASIAN-PACIFIC ANGIOSPERM LINEAGE *CYRTANDRA* (GESNERIACEAE)

INTRODUCTION

The genus *Cyrtandra* J.R. & G.Forster 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 later dispersed throughout the Pacific. Species of *Cyrtandra* are morphologically diverse and include shrubs and small trees, and sometimes herbs, lianas and even epiphytes. Fruit are either hard capsules or fleshy berries and flowers are often white, although pink-, red- and yellow-flowered species exist. Species of *Cyrtandra* in the Pacific islands east of Papua New Guinea, however, are remarkably similar and are almost exclusively white-flowered with fleshy berries and a predominantly understory shrub or small tree habit. Most Pacific species inhabit very similar perennially wet upland tropical forests throughout the high islands. Because of the uniformity in habitat across a broad and diverse region, it does not appear that Pacific *Cyrtandra* radiated in response to ecological pressures (Cronk et al., 2005). Rather, the genus more likely diverged under a classic dispersal-mediated allopatric model (*sensu* Clark et al., *accepted*). Most species within *Cyrtandra* are narrowly distributed endemics occupying no more than a single archipelago, a single island or even a single valley, supporting the hypothesis of dispersal-mediated allopatric divergence. This divergence scenario has been inferred in other insular lineages with similar life histories (Price and Wagner, 2004).

Supra-generic Classification

Cyrtandra belongs to the subfamily Cyrtandroideae Endlicher (Burtt & Wiehler, 1995). The Cyrtandroideae includes approximately half of all gesneriad species and is believed to be monophyletic based on developmental, morphological and genetic analyses (Burtt and Wiehler, 1995; Mayer et al., 2003). The Cyrtandroideae includes perhaps 80 or more recognized genera (Weber, 2004) and has been studied at the tribal and generic level (e.g., Wang, 2002; Mayer et al., 2003). The sister lineage to the Cyrtandroideae is the Gesnerioideae + Coronantherioideae (Weber, 2004); the Gesnerioideae are principally neotropical (Zimmer et al., 2002) whereas the Coronantherioideae are distributed minimally in South America and predominantly in Australia and northeast into the eastern South Pacific. The Coronantherioideae appear to be of Gondwanan origin, inhabiting mostly landmass that were once part of or closely neighboring this ancient land mass (Raven and Axelrod, 1974).

Although both the Cyrtandroideae and the Coronantherioideae are prominent components of southeast Asian/northeast Australian floras, the major challenges of establishment and proliferation on remote islands of the Pacific appear to have posed insurmountable barriers to all members of either of these subfamilies except for *Cyrtandra*. For example, the closest relatives of *Cyrtandra*, *Aeschynanthus* (~160 species) and *Didymocarpus* (~180 species), are found as far east as the Solomon Islands, but no further. Similarly, *Coronanthera* is the only genus in the Coronantherioideae to have a range extending into the Solomon Islands with all other species in this subfamily restricted to the west of this island chain. Nearly half of all *Cyrtandra* species, approximately 300, occur east of the Solomon Islands (Burtt, 2001).

Phylogenetics

Cyrtandra has been the subject of molecular systematics studies since the late 1990's. Samuel et al. (1997) examined the chloroplast *atpB/rbcL* spacer region across 10 *Cyrtandra* species and several outgroup taxa. Using maximum parsimony analysis, their results suggested a paraphyletic Samoan clade and an unresolved relationship between Malaysian and Hawaiian taxa. Atkins et al. (2001) conducted an Indo-Malayan/Philippines study using maximum parsimony analysis of the nuclear ribosomal internal transcribed spacer region (ITS) sequences from 26 species. Their results hinted at a dynamic exchange of species in the region with a major division between Sundaland (Borneo and Peninsular Malaysia) and Philippine species. Palawan, the island system between these two regions, was represented as paraphyletic and nested within these two clades (Atkins et al., 2001). Recently, Cronk et al. (2005) presented a partial genus-wide *Cyrtandra* phylogeny also based on ITS. In this study, the authors analyzed sequence data from 36 species across the taxon's range. Based on maximum parsimony and Bayesian analyses, the hypothesis that Pacific *Cyrtandra* represent a single introduction from more mainland sources is strongly supported (Cronk et al., 2005) and a sister relationship between the Taiwanese taxon *C. umbellifera* and the Pacific clade is also inferred. Cronk et al. suggest that Hawai'i may have been the initial dispersal point into the Pacific with later dispersal and diversification from Hawai'i into the South Pacific.

Taxonomy

Nearly every researcher that has addressed the classification and taxonomy of *Cyrtandra* has commented on the extreme difficulty in character state assignments for this large genus (Hillebrand, 1883; Gillett, 1973; Wagner et al., 1990; Smith, 1991; Burtt, 2001). Previous divergence dating analysis has indicated that a large number of species have arisen over a relatively short time (Clark et al., *accepted*); a rapid species divergence resulting in potentially homoplastic character suites between lineages could be contributing to taxonomic issues encountered by students of *Cyrtandra*.

Cyrtandra has been challenging to classify at the supraspecific, subgeneric level (Gillett, 1973; Wagner et al., 1990; Burtt, 2001; Schlag-Edler, 2001; Cronk et al., 2005), although numerous regional subgeneric classifications have been proposed (Hawai'i, Hillebrand, 1888; New Guinea, Schlecter, 1923; west Malaysia, Kraenzlin, 1927; Hawai'i, St. John, 1966, 1987, Wagner et al., 1990; west Malaysia, Burtt, 1990). Currently, over 40 sections are recognized (Burtt, 2001) but no satisfactory genus-wide sectional classification exists.

Homoplasy in floral characters is common in the Gesneriaceae (Clark et al., 2006; Roalson et al., 2003; 2005; 2008), making morphological-based classifications exceptionally difficult in this family. Despite this, floral characters have historically been considered important and have predominantly been relied on for taxonomic assignment, particularly in *Cyrtandra* (Gillett, 1967, 1973; Wagner et al., 1990). For example, the only genus-wide classification of *Cyrtandra* by C.B. Clarke (1883; reviewed in Burtt, 2001) segregated the genus into two subfamilies based on calyx persistence or loss after anthesis.

The most recent sectional classification for Hawai'i *Cyrtandra* (Wagner et al., 1990) recognizes six sections based largely on the earlier Hawaiian classification of Hillebrand (1888) and are differentiated based first on calyx morphology, and secondarily on a combination of characters including floral symmetry, bracts and other floral characters. Additionally, general habit and other vegetative characters are also used in this classification (Wagner et al., 1990). By contrast, the most recent section named in *Cyrtandra*, section *Pleuroschisma* Hilliard & B.L.Burtt, was applied to nine Bornean species based on a single character, a unique fruit morphology characterized by two median septicidal splits at maturity (Hilliard et al., 2003). Across the genus, morphological characters such as foliar sclereids, pollen exine microstructure, leaf development, calyx morphology and persistence after anthesis, and other characters have been variously explored to better delineate sectional groupings in this unwieldy genus (for a review, see Kiehn, 2001).

Project Goals

A comprehensive analysis of samples of *Cyrtandra* across its range may prove useful in identifying distinct lineages for better sectional circumscription (Burtt, 2001; Kiehn, 2001). To this end, I am specifically addressing the following three questions: 1) What has been the historical pattern of range inheritance in *Cyrtandra* and how are major clades distributed across this range? 2) What are the underlying historical diversification patterns in *Cyrtandra* and how do these patterns correspond with current taxonomic rankings? 3) What morphological characters, if any, may represent synapomorphies for these major lineages and can these be useful in future revisions of current classifications? Although I will not propose any nomenclatural changes in this paper, I will make recommendations that may guide future taxonomic revisions of *Cyrtandra*.

MATERIALS AND METHODS

Taxon Sampling

The current study includes 88 taxa including two outgroup taxa (both *Aeschynanthus* L. species; Table 2.1). Sampling builds on the previous work of Clark et al. (*accepted*) and includes a more diverse and representative Malesian grade including species from Borneo, the Philippines and Taiwan. Samples included represent lineages present on most major geological features in the Pacific and all attempts were made to include at least one specimen from principal lineages as defined by Gillette (1973) and Wagner et al. (1990).

Phylogenetic Analysis

DNA extraction, genic region amplification, and sequencing were performed using protocols described by Clark et al. (*accepted*). Silica gel-dried leaf material was used for total genomic DNA extraction. Purified cycle sequence products analyzed on an Applied Biosystems Model 3730 Automated DNA Sequencer. For each taxon, forward and reverse sequencing reactions was 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. The internal transcribed spacer region, including ITS1, ITS2 and the 5.8S subunit, the 5’ end external transcribed spacer region (ETS), and the chloroplast *psbA-trnH* region were amplified using protocols described in Clark et al.

(*accepted*). For *Cyrtandra umbellifera*, a sample could not be secured for analysis; only the ITS sequence data available on GenBank was used in the current study (Table 2.1). Topological placement of this taxon did not vary between preliminary analysis of ITS alone and analysis of ITS in combination with ETS or in combination with ETS and *psbA-trnH*. This specimen was thus included in subsequent analyses.

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 100 heuristic bootstrap replicates executed over 100 random addition cycles with 10 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. The first 20% of trees were excluded as burn-in. Posterior probabilities did not differ between two identical runs with these trees removed indicating that stationarity was reached. 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 analysis 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 (≥ 70% bootstrap support) among the various topologies were in conflict; therefore, the three genic regions were combined and analyzed. Results from these combined ML and BI analyses were used in the dating and ancestral range reconstruction methods.

Estimation of Phylogeny Divergence Times

I used the r8s program (v. 1.7.1; Sanderson, 2004) to estimate a chronogram for *Cyrtandra* based on the maximum likelihood tree using semi-parametric rate smoothing (SPRS) by penalized likelihood and the truncated Newton algorithm (Sanderson, 2002). 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 data sets 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). Divergence dates were calibrated using island ages, as described in Clark et al. (*accepted*) including 35 million years before the present (MYBP) for the Indonesian grade/Pacific split, 6 MYBP for the origin of the Marquesas Islands, and 5.1 MYBP for the Hawaiian Islands.

Ancestral Range Analysis

I analyzed the chronogram from the r8s analysis using the dispersal-extinction-cladogenesis (DEC) model (Ree et al., 2005; Ree and Smith, 2008) as implemented in Lagrange (v.2; Ree and Smith, 2008) to infer ancestral areas. DEC is a continuous-time model for geographic range evolution that has proven more appropriate than alternative methods for ancestral range reconstruction in insular systems (Clark et al., *accepted*). Island systems were coded as 12 discrete areas: Borneo, Java, Sulawesi, Philippines, Taiwan, Fiji, Hawai'i, Samoa, Tonga, Micronesia, Society Islands, and the Marquesas. Based on results from the previous study, I restricted ancestral areas to no more than two areas per node. This restriction allows DEC to best approximate the possibility of dispersal-mediated allopatric divergence (*sensu* Clark et al., *accepted*), a scenario that is considered probable in insular systems. An inverse-scaled distance matrix was constructed to account for distance in the DEC analysis (see Clark et al., *accepted*; see Appendix 3 for the absolute, scaled, and inverse-scaled distance matrices).

Taxonomic Assignments

I compared the resultant phylogeny to three major classification schemes: 1) For the South Pacific, I consulted the only South Pacific treatment (Gillett, 1973). Although Gillett did not propose sectional rankings, he did suggest major species groupings that can be compared to my current phylogenetic hypothesis; 2) Fosberg and Sachet (1981) proposed two major lineages for Marquesan taxa; 3) The Hawaiian taxa have been divided into six sections (Wagner et al., 1990). Principal characters including inflorescence bract persistence and calyx symmetry and persistence have been noted as diagnostic for each of these lineages and were compared based on phylogenetic data generated via molecular characters independent of morphology.

RESULTS

Sequence Alignment

Aligned sequences were 740-bp for ITS (including the 5.8S subunit), 466-bp for ETS and 389-bp for *psbA-trnH* (excluding two ambiguous regions) for a total of 1595-bp of aligned sequence data. As noted by Cronk et al. (2005), all Hawaiian taxa share a common

12-bp insertion in the ITS region that is not found in other taxa sampled; my increased sampling compared to Cronk et al.'s study further supports that this insertion is unique to the Hawaiian taxa. A highly homoplastic 31-bp inversion was identified in the 3' end of *psbA-trnH*. This inversion has been identified in other genera in the Gesneriaceae (Clark et al., 2006). In *Cyrtandra*, the inversion can differ in direction even within conspecifics (e.g., *C. compressa* from Samoa). Additionally, *psbA-trnH* contains a highly variable AT repeat region near the 5' end ranging from 0-55 bp of ambiguously aligned data. Based on these two difficult DNA regions, I conducted preliminary analysis on *psbA-trnH* to interpret the affects of alignment and/or omission of these regions. Three variations on the *psbA-trnH* alignment were analyzed: 1) complete, with the inversion sequences separated (not aligned) and an aligned AT repeat, 2) reversed and complemented inversion sequences aligned and an aligned AT repeat, and 3) both the inversion and ambiguous AT repeat removed (data not shown). None of these alignments produced markedly different resolution of the phylogenies (as indicated by bootstrap support greater than 70%). However, of the various alignments, the sequence matrix with both regions removed exhibited the most phylogenetic structure and was subsequently used in the combined genic region analysis.

Phylogeny

Combined analysis of ITS, ETS and *psbA-trnH* genic regions resulted in one most likely tree (-lnL = 9782.6046; Figure 2.1). Identical analysis was conducted on ITS+ETS with *psbA-trnH* removed (-lnL = 8046.1170; Figure 2.2). These topologies varied little, having virtually identical taxon placement and most resolved relationships exhibiting similar support values (Figs. 2.1 and 2.2). However, the complete combined analysis had slightly higher support values for most nodes that differed between the two analyses. In addition, node 8 was supported (70% BS and 99% PP) only in the complete combined analysis and node 23 within the Hawaiian clade showed more resolved, supported structure than in the ITS+ETS analysis. Similar results were obtained with the inclusion of marginally informative (when analyzed independently) *Adh* sequence data when combined with ITS and ETS data in a previous study by Roalson et al. (2004).

Resolution across the complete combined dataset ML phylogeny (Figure 2.1), as indicated by bootstrap and posterior probability support indices, is high with most major clades strongly supported. These results parallel relationships inferred in the previous study with a less-inclusive taxon sampling (Clark et al., *accepted*). A grade of southeast Asian clades leading to a monophyletic Pacific clade is well supported (Figure 2.1). The grade includes a complex clade of Javan, Sulawesian, Taiwanese and Philippines taxa (clade 24) immediately sister to the Pacific clade; this relationship is well supported (93%BS; >99%PP). This differs from the relationships reported by Cronk et al. (2005) that reconstructed the Pacific clade sister to Taiwan that was in turn sister to the Philippine taxa sampled. In this study, the Pacific clade and the Javan-Sulawesian-Taiwanese- Philippines clade (clade 3) is sister to a distinct Javan clade (clade 25) that is in turn sister to Javan-Bornean clade (clade 26).

As in this previous study, several geographic areas are polyphyletic within the Pacific clade (clade 4; Figure 2.1) including Samoa with two well-supported clades (clades 10 and 14) with one Society Islands taxon nested within clade 10 and Tongan and Micronesian lineages nested within clade 14. Clade 14 is also noteworthy in that *C. samoensis* is paraphyletic; two samples from U'polu Island (Samoa) are sister to the two

Micronesian taxa (clade 17) and together this clade is sister to a clade including the Ofu Island (Samoa) and Tongan *C. samoensis* samples (clade 18). Fiji is also paraphyletic with two major lineages represented (clades 13 and 19) and one taxon nested within the Samoan clade 12 (in clade 10). The Marquesas Islands are monophyletic as are the Hawaiian Islands. The Marquesas are divided along two major clades with one outlier, *C. feaniana*, from Ua Pou that remains unplaced in a polytomy with the other two clades. Hawai'i has an over-arching structure including a well-supported Kaua'i clade (clade 22) and an O'ahu clade (clade 23). A third clade exists (clade 21) that is not supported and is made up of taxa from principally the island of Hawai'i and the Maui Nui complex.

Divergence Times and Ancestral Range Inheritance

The root age of the chronogram is estimated at 48 MYBP (Figure 2.3). This does not statistically differ from the root age estimate of 42 MYBP in the previous study (Clark et al., *accepted*). Other noteworthy divergence dates include the southeast Asian – Pacific split (39.2 ± 2.7 MYBP) and the origin of major crown group lineages including the Marquesas (5.8 ± 0.5 MYBP), Samoan clade 10 (11.6 ± 4.5 MYBP), Samoan clade 14 (12.0 ± 4.3 MYBP), Fijian clade 13 (11.4 ± 4.3 MYBP) and Fijian clade 19 (8.9 ± 4.4 MYBP). Unlike in the previous study, the divergence between Hawai'i and the South Pacific (21.7 ± 4.7 MYBP) and the Fiji clade 19/South Pacific clade 6 effectively collapses in dating analyses and no age estimates were inferred for node 5. Divergence event confidence intervals in more terminal taxa overlap substantially owing to short branches in the phylogeny and some degree of phylogenetic uncertainty. However, large confidence intervals around age estimates are often recovered in similar studies and do not preclude these data for subsequent analysis (Roalson et al., 2008).

DEC Ancestral Range Analysis

DEC results yield similar inferences to previous analyses (Clark et al., *accepted*). For node 2, the area is inferred to be a widespread Java-Fiji ancestor (Table 2.2; Figure 2.4). For daughter lineages node 25 and node 3, both are reconstructed as Java. The split between southeast Asia and the Pacific occurs at node 3; the daughter lineage node 24 is reconstructed also as Java and later spawns Sulawesian, Taiwanese and Philippine lineages. The other sister lineage, node 4, the Pacific lineage, is inferred to be Fiji.

Within the Pacific, the Hawaiian clade (node 20) is inferred to have originated by a divergence event within Fiji followed by dispersal and divergence within Hawai'i. Likewise, the South Pacific lineage originates in Fiji (node 5) and persists for some time (node 6). Data further suggests that a Fiji-Samoa lineage (beginning at node 7) persists also for a period. Major divergences originate from this Fiji-Samoa area including the Marquesas (node 9), a Samoan lineage (node 10), and one Fijian lineage (node 13). The other major Samoan lineage (node 14) is inferred to be independently derived from Fiji and includes Society Islands, Tongan and Micronesian lineages.

Recognizing Major Groupings

Given the current phylogeny, terminal clades can be compared based on morphological characteristics common within each. In this study, I identify these terminal groups according to the most inclusive, well-supported clades that can be easily distinguished from sister clades principally by morphological traits and partially by geographic area (Figure 2.1). A South Pacific clade consisting of six major groups: 1) Marquesas (clade 9), 2) Samoa 1 (clade 10), 3) Fiji 1 (clade 13), 4) Samoa 2 (clade 15), 5) *Cyrtandra samoensis* complex (clade 16), and 6) Fiji 2 (clade 19), and, lastly, a seventh distinct group, the Hawaiian clade (clade 20). The basal grade of taxa includes too sparse a sampling across a diverse group of species to make any detailed comments here. Relationships among these species have been addressed elsewhere (e.g., Atkins et al., 2001) and ongoing research is being conducted on these western-most species (T. Pennington, pers. comm.).

Several loosely defining characters including a sub-shrubby, fleshy-stemmed habit with universally white, fleshy fruit unify the *Marquesas group*. Two subgroups within this group are clearly delineated by calyx characters: the first subgroup, including *C. feaniana* and *C. ootensis* and varieties, has calyces divided nearly to the base; subgroup two includes *C. thibaultii*, *C. nukuhivensis*, and *C. jonesii* and is distinguished in having calyces divided ¾ the way to the base. The sister lineage, *Samoa 1 group*, can be distinguished from the Marquesan group in having a markedly woody shrub or small tree habit and distinctive orange fruit. These species are also defined in having either calyces divided asymmetrically, usually into an upper and lower beak, and inflorescence bracts deciduous (in the *C. richii* subgroup) or with five distinct lobes with a fused involucre bract forming a capitate inflorescence (in the *C. pogonantha* subgroup). One yet unidentified Fijian specimen is grouped in this Samoa 1 group, closely related to *C. pogonantha*. Its affinities and taxonomic placement are unclear.

The clade immediately sister to the Marquesan and Samoa 1 groups is the *Fiji 1 group*. This group is not clearly defined and includes a diverse assemblage of species. *Cyrtandra anthropophagorum* and possibly several other unidentified Fijian taxa are united in having a somewhat woody shrub habit, deciduous inflorescence bracts, and symmetrical calyces with lobes as long as the tube. The Fijian 1 group also includes *C. bidwillii* from the Society Islands that shares similar characteristics including the deciduous inflorescence bracts and symmetrical or nearly symmetrical calices, although corollas are noticeably larger in *C. bidwillii*. *Cyrtandra milnei* and *C. leucantha* are also included but differ markedly from the other species in the Fijian 1 group. These taxa can be defined based on the capitate inflorescence and markedly hirsute leaves and young stems. These latter species are also less woody than others in this clade.

The next major clade includes two groups identified in my rankings, the *Samoa 2 group* and the *Cyrtandra samoensis complex*. The Samoa 2 group is quite distinctive from other species of *Cyrtandra* in having large (>5 cm) campanulate corollas. These species also exhibit a markedly woody shrub habit, similar to species in the Samoa 1 group. All species sampled are from Samoa with the exception of an unidentified taxon from the Society Islands. The *Cyrtandra samoensis* complex includes *C. samoensis, the closely related C. kusaimontana,* and *C. urvillei* from Micronesia. These species share a common sub-shrub or shrub habit with fleshy stems. Flowers are born in often-dense axillary clusters; calyx lobes are distinct and have five symmetrical lobes.

The last group I recognize in the South Pacific clade is the *Fiji 2 group* containing *C. occulta* and two very similar species. This group shares many similarities with *C. milnei* and

C. leucantha in the Fijian 1 clade but differs in having infloresence bracts completely fused into a cup-shaped involucre, most similar to *C. pogonantha* in the Samoa 1 group.

Hawaiian lineages are more difficult to define morphologically, although three major subgroups are distinguished in the phylogeny: a Kaua'i subgroup, a O'ahu subgroup, and a broad O'ahu–Maui Nui–Hawai'i subgroup (Figure 2.5). These groups will be addressed further in the discussion.

DISCUSSION

Phylogeny of Cyrtandra

My phylogenetic hypothesis, using the most comprehensive sampling of Pacific species to date (88 taxa including at approximately 73 of the ~600 recognized species), is in line with current general hypotheses on relationships in *Cyrtandra* (Atkins et al., 2001 [30 taxa; 26 species]; Cronk et al., 2005 [36 taxa; 36 species]; Clark et al., *accepted* [61 taxa; 57 species]). Principally, a "western grade" of taxa from the southeast Asia-Borneo region leads to a monophyletic lineage east of the Philippines that extends throughout the Pacific Islands. Most major clades in the phylogeny are well supported in both bootstrap and Bayesian posterior probabilities.

The Pacific clade is strongly supported (100% BS; >99% PP), as is the sister relationship of this clade to a Javan-Sulawesi-Philippines-Taiwan clade (93% BS; >99%PP). This result differs from the hypothesis set forth by Cronk et al. (2005) in which *C. umbellifera* (from Taiwan) was sister to the Pacific clade. In their hypothesis, this Taiwan-Pacific clade was then sister to a Philippines group and then sister to an increasingly western grade of taxa (Cronk et al., 2005). Cronk et al. also noted that the Taiwanese and Philippine taxa sampled in their study shared the common Pacific morphology of being shrubs or trees and white-flowered/white-fruited and thus further supporting this relationship. However, this relationship was not strongly supported in their parsimony analysis and it is not entirely clear how this relationship was resolved. In my study (including 21 species common to both studies), the clade sister to the Pacific clade includes *C. umbellifera* (Taiwan) and *C. ferruginea* (Philippines), as well as the decidedly non-Pacific morph *C. serratifolia* (Java), a small herb with pink flowers. It should also be noted that in the analysis of ITS alone (data not shown), and in combined analysis of ITS+ETS without *psbA-trnH* (Figure 2.2), nearly identical topologies to the complete combined analysis were recovered. Foliar sclereids have been noted as absent from Pacific species, possibly representing a synapomorphy for this clade (Kiehn, 2001); more detailed examination of this and other micro-characters may be warranted to understand affinities between these allied species.

I also recovered in this study a far greater stratification in relationships among South Pacific taxa and within Hawai'i taxa than previous studies. In particular, distinct clades representing major geographic areas throughout the South Pacific are recovered with strong support, as are three distinct clades within Hawai'i. While some areas are strongly supported as monophyletic (e.g., Hawai'i and the Marquesas), areas such as Fiji and Samoa are polyphyletic and each includes two to several clades distributed across the phylogeny.

One major relationship that remains unresolved is the placement of clade 19, which includes the Fijian *C. occulta* and two similar Fijian species. In the combined dataset analysis, this clade is reconstructed sister to the remaining South Pacific, which is in turn is 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. Clade 19 is also resolved sister to the South Pacific in ITS analysis alone, and in the combined analysis of ITS and ETS. However, the clade is placed sister to Hawai'i in the ETS analysis, albeit with no branch support (data not shown). 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 relationship (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*).

Divergence Dates

The Gesneriaceae is one of the earliest diverging lineages of the Lamiales (Oxelman et al., 1999; Olmstead et al., 2000), and has been estimated as originating at around 71-74 MYBP based on analyses of divergence dates across the Asterids (Wikström et al., 2001). Crown group divergence dates are perhaps more contested, with recent age estimates pushed back to this stem lineage origin (71 MYBP; Bremer et al., 2004). *Cyrtandra* is one of the more recently derived lineages in the Gesneriaceae (Mayer et al., 2003) and its age must be somewhat later than these divergence dates. Dating of the split between *Cyrtandra* its sister genus *Aeschynanthus* could not be performed in this study because outgroup taxa must be pruned as part of the r8s analysis protocol (Sanderson, 2002). However, my results do provide ages for the terminal-most split between Borneo/Java, thought to be some of the oldest derived lineages in the genus (Burtt, 2001). This basal-most node is estimated at 48 MYBP (clade 1) suggesting a reasonable timeframe for the dispersal and diversification of *Cyrtandra*. This age estimate is also in line with parallel diversifications in the New World subfamily Gesnerioideae, tribe Gloxineae (20-30 MYBP), a slightly less species-rich but recently derived lineage in the Gesneriaceae (Roalson et al., 2008). Similarly, dates placed on successively more recent nodes correspond with origins of major landmasses. In particular, the split between the last southeast Asian lineage in my hypothesis (the Javan-Sulawesi-Philippines-Taiwan clade) and the South Pacific occurred around 40 MYBP (clade 3). This divergence slightly preceded the origin of Fiji (35 MYBP; Evenhuis and Bickel, 2005). The divergence estimate on clade 4 in the Pacific is estimated somewhat later than this, at ~22 MYBP, suggesting that *Cyrtandra* dispersal and diversification in the Pacific did not occur until well after ecological opportunities in this area arose.

Cyrtandra *Biogeography*

Fiji and surrounding areas have long been recognized as a major biogeographic interface centered between southeast Asian/Malesian and Pacific/Polynesian bioregions (Hedley, 1899; Takhtajan, 1986; Stoddart, 1992). Takhtajan (1969, 1986) recognized this area, encompassing Fiji and surrounding islands including the Santa Cruz Islands, Vanuatu, Niue, Samoa and Tonga, as the “Fijian Region.” A strong demarcation along the Tongan trench through the Fijian Region exists where floras west of this line (Hedley’s line; *sensu* Stoddart, 1992) are markedly more southeast Asian/continental (Gondwanan) in origin. East of Hedley’s line, these lineages are few and floras become increasingly less diverse and of more recent origin. To my knowledge, *Cyrtandra* represents the first plant lineage identified as having a focal “crossroads” (*sensu* Clark et al., *accepted*) of dispersal to far flung corners of the Pacific, including west to Micronesia, northeast to Hawai’i, and east to the Marquesas and the Society Islands, all originating from the Fijian Region.

The central role of the Fijian Region in the dispersal of Pacific *Cyrtandra* is supported in the DEC analysis, where both Fiji and sometimes Fiji-Samoa are strongly favored as potential areas for the origin of the Pacific clade (node 4). Additionally, Fiji and/or Samoa are often exclusively reconstructed at internal nodes in the Pacific clade, further supporting a central role of the Fijian Region. In the previous study, DEC reconstructs a persistent Fiji-Samoa lineage that spawned within-area lineages that later colonized more remote islands. The inclusion of additional taxa and one additional Pacific area (Society Islands) in this study did not alter this result, nor did the inclusion of more taxa in the southeast Asian grade (particular in the inclusion of the Taiwanese and Philippine taxa).

A general pattern of west to east stepping stone dispersal into the Pacific is seen in many taxa, both plant and animal (including humans), from more mainland southeast Asia into the Pacific and has been particular noted in plant dispersal and diversification (Stoddart, 1992). A similar hypothesis to ours was drawn based on morphology in Pacific species of *Cyrtandra* (Gillett, 1973), although Gillett did not consider Fiji or Samoa as central to this diversification but rather a route out into the Pacific.

A Fiji-Samoa crossroads hypothesis differs from the hypothesis proposed by Cronk et al. (2005) that implicated a Taiwan-to-Hawai'i route into the Pacific. Cronk et al. largely based their hypothesis on the sister relationship (in their study) of Taiwan to the Pacific and the old geologic age of the Hawaiian seamount chain (dating back to 91 MYBP; Price and Clague, 2002). However, an overwhelming amount of geological data suggests that a major gap of several million years existed between the formation of the extant Hawaiian high islands (5.1 MYBP) and previous islands that were appreciably above sea level (Price and

Clague, 2002). This window of no high islands precludes the persistence of upland terrestrial lineages on the Hawaiian Islands during the critical period. Alternatively, my hypothesis supports a Fijian origin for the Pacific clade; Fiji is of a much longer extant age, and in closer proximity to areas associated with the southeast Asian sister grade, further suggesting that a Fiji-first, not Hawai'i-first, hypothesis into the Pacific is more likely.

Notes on Taxonomy and Affinities

The Marquesas group and the two subgroups recognized in this study correspond with differences in calyx structure identified by Fosburg and Sachet (1981) that delineate the two subgroups. Similarly, the two subgroups in the sister *Samoa 1 group* (*C. richii* subgroup and *C. pogonantha* subgroup) are readily distinguished based on calyx and corolla morphology. Gillett (1973) differentiated between many taxa in the South Pacific species using calyx and corolla characters and floral parts indumentation (Gillett, 1973). However, Gillett did not infer a relationship between these two Samoan 1 subgroups despite their having uniquely orange fruit, the most readily identifiable character useful in separating these two groups; he instead placed more weight on floral traits that do not readily align these taxa.

The *Fiji 1 group* is not clearly defined and includes taxa from Gillett's (1967) "group 1" including *C. milnei* and *C. leucantha* and his "group 3" including *C. anthropophagorum*, as well as the non-Fijian *C. bidwillii* from the Society Islands, Gillett did not acknowledge any affinities between these Fijian species (1973). However, deciduous calyx lobes after anthesis appear to be one character that aligns these species and has been used by Gillett in other groupings and by other taxonomists in *Cyrtandra*. This character is rather homoplastic

(Burtt, 2001), as are many characters used in delineating these groupings, and cannot be extended beyond this clade.

Species in the *Samoa 2 group* differ markedly in calyx morphology and other characters and it is not surprising that Gillett did not mention any affinities between these taxa. However, the striking, large campanulate corollas shared by all three species hint at the underlying relationships. Based on this fact, other morphologically similar South Pacific species not sampled in this study may also be expected to belong in this group.

The *Cyrtandra samoensis complex* has been clearly defined by Gillett (1973). He recognized *C. samoensis* as a single species only tentatively, remarking that a lack of satisfactory characters exist with which to separate this complex. He also noted a close taxonomic affinity between *C. samoensis* and *C. urvillei* from Micronesia (included in this study) as well as several other taxa across a broad range in the Pacific. Gillett went as far as to suggest that these taxa might warrant synonymy. If synonymized, *C. samoensis sensu lato* would represent the greatest ranging single species in the genus *Cyrtandra*. My phylogeny supports the paraphyly of *C. samoensis* thus potentially warranting synonymy of allied species as Gillett suggested. Alternatively, as yet unidentified characters may be used to distinguish cryptic species currently lumped under *C. samoensis* (M. Kiehn and J.R. Clark, current research).

The Fiji 2 group containing *C. occulta* is in “group 1” of the Fijian species of *Cyrtandra* according to Gillett (1967). This taxonomic assignment allies this group more closely with the Fiji 1 group. However, its independent placement outside of a well-supported South Pacific clade makes this assessment untenable. Wagner et al. (1990) remarked on the similarity between *C. occulta* and species in the Hawaiian section

Verticillatae. This observation is noteworthy in that of all the South Pacific lineages, members of the Fiji 1 group are most genetically similar to the Hawaiian taxa in my study, further supporting Wagner's hypothesis.

I have sampled five of six sections of Hawaiian lineages (*sensu* Wagner et al., 1990). My phylogenetic hypothesis supports three major clades that partially correspond with these recognized sections (Figure 2.5). However, these groupings also include disparate sections being lumped together by area in well-supported clades and not with their respective sections across island areas.

Clade 23 includes eight specimens, seven of which belong to section *Verticillatae*. The eighth specimen, *C. filipes*, that is firmly nested within the clade, belongs to the closely allied section *Cylindrocalyces*. Zygomorphic calyces that are deciduous following anthesis unite all of these species.

Clade 21 in this phylogeny is slightly more complicated; a well-supported clade consisting of two species assigned to section *Apertae* is in a trichotomy with another clade containing three species all in section *Crotonocalyces*, and a third clade that consists primarily of species assigned to either section *Crotonocalyces* or section *Macrosepalae* (except for one specimen, *C. hawaiensis* from Moloka'i that belongs in section *Verticillatae*). This entire clade (with the exception of *C. hawaiensis*) can all be grouped based on persistent actinomorphic calyces; this character also distinguish this clade, clade 21, from clade 23. The *Apertae* clade can be distinguished from the others in this group in having open, branched cymes.

Clade 22 is a well-supported clade consisting entirely of specimens from Kaua'i. A single specimen from section *Cylindrocalyces* is sister to the remaining clade. This remaining group, much like clade 21, consists of specimens belonging to either Crotonocalyces or section *Macrosepalae*, again with one exception, *C. confertiflora* belonging to section *Verticillatae*.

Hybridization.–Natural, *in situ* hybridization has long been thought to occur in *Cyrtandra* (Gillett, 1973; Burtt, 2001; Kiehn, 2001) and nowhere is this more evident than in the Hawaiian species. Wagner et al. (1990) duly noted in their treatment of Hawaiian *Cyrtandra* that hybridization in many instances blurs the lines between sections, and may have even been causative in some speciation events. The fact that major clades across sections are grouped in molecular data analysis in several well-supported clades principally by area and not morphological traits may support this hypothesis.

CONCLUSIONS AND FUTURE DIRECTIONS

This study has contributed marked resolution to a general understanding of cladogenesis and dispersal in *Cyrtandra*, particularly in the Pacific. The Pacific clade is by all accounts a monophyletic unit. Ancestral range analysis points to Takhtajan's Fijian Region as the origin of this lineage, principally Fiji and Samoa, which served as a major crossroads to other, more remote areas of the Pacific. However, the connection of these Pacific taxa and more mainland, Malesian taxa remains largely unresolved. Further work is needed both in the Fijian Region to collect more *Cyrtandra* from this diverse area, and also from the underrepresented Borneo, Papua New Guinea, and Philippines regions. These areas hold hundreds of unsampled species, some of which may represent intermediary lineages between the Malesian and Pacific groups. Uncovering these possible lineages would help clarify the history of dispersal and divergence of *Cyrtandra* into the Pacific and would also be useful in understanding Pacific-wide biogeographic processes in general.

I have illustrated that recognizable taxonomic units can be effectively defined using first molecular techniques and then subsequent interpretation of these groupings based on morphological similarities. Monographic revisions need to be applied to each of the major areas recognized in this study. Areas such as Fiji remain remarkably under sampled, despite their importance in plant biogeography and warrant intensive phyto-inventory efforts.

Hawai'i is the most studied of all areas defined in this study and represents an excellent proving ground for application of molecular systematics approach to evaluating taxonomic groupings. However, population-level markers and analyses are needed to better understand population processes including hybridization and their influence on the evolutionary relationships of these lineages. In addition, a revised monograph of Hawaiian *Cyrtandra* is needed to summarize what is known about current distribution, ecology, and morphological patterns in Hawaiian *Cyrtandra*. A combination of these two approaches will ultimately result in a synthesis of evolution and taxonomy knowledge in Hawaiian *Cyrtandra* and may provide a model for future study and revisions within this genus.

Genus	*Specific Epithet*	Author(s)	ID No.	COLLECTOR	Collector No.	Origin
Cyrtandra	*tintinabula*	Rock	C0012	Perlman	17676	Hawai`i, Hawai`i
Cyrtandra	*wagneri*	Lorence and Perlman	C0014	Perlman	17675	Hawai`i, Hawai`i
Cyrtandra	*wagneri*	Lorence and Perlman	C0015	Lorence	8907	Hawai`i, Hawai`i
Cyrtandra	sp.		C0016	Plunkett	1837	Fiji, Viti Levu
Cyrtandra	sp.		C0017	Plunkett	1838	Fiji, Viti Levu
Cyrtandra	sp.		C0018	Plunkett	1843	Fiji, Viti Levu
Cyrtandra	sp.		C0019	Plunkett	1875	Fiji, Viti Levu
Cyrtandra	sp.		C0020	Plunkett	1898	Fiji, Viti Levu
Cyrtandra	*wainihaensis*	Léveillé	C0021	Clark	549	Hawai`i, Kaua`i
Cyrtandra	*wawrae*	C.B. Clarke	C0022	Clark	550	Hawai`i, Kaua`i
Cyrtandra	*longifolia*	(Wawra) Hillebrand ex C.B. Clarke	C0023	Clark	551	Hawai`i, Kaua`i
Cyrtandra	*kauaiensis*	Wawra	C0026	Clark	556A	Hawai`i, Kaua`i
Cyrtandra	*kauaiensis*	Wawra	C0028	Clark	558	Hawai`i, Kaua`i
Cyrtandra	*pulchella*	Rich ex A. Gray	C0029	Lorence	8525	Samoa, Tau
Cyrtandra	*samoensis*	A. Gray	C0030	Lorence	8633	Samoa, Ofu
Cyrtandra	*samoensis*	A. Gray	C0031	RP	71221	Tonga
Cyrtandra	*kusaimontana*	Hosokawa	C0033	Flynn	5995	Micronesia, Kosrae
Cyrtandra	*urvillei*	C.B. Clarke	C0034	Lorence	7838	Micronesia, Kosrae
Cyrtandra	*propinqua*	C. Forbes	C0039	Clark	570	Hawai`i, O`ahu
Cyrtandra	*sandwicensis*	(Léveillé) St. John & Storey	C0045	Clark	576	Hawai`i, O`ahu
Cyrtandra	*grandiflora*	Gaudichaud	C0046	Clark	577	Hawai`i, O`ahu
Cyrtandra	*cordifolia*	Gaudichaud	C0048	Clark	579	Hawai`i, O`ahu
Cyrtandra	*calpidicarpa*	(Rock) St. John & Storey	C0053	Clark	584	Hawai`i, O`ahu
Cyrtandra	*kealiae ssp. urceolata*	W.L. Wagner & Lorence	C0054	Perlman	18805	Hawai`i, Kaua`i
Aeschynanthus	*tricolor*	Hook.	C0055	MSBG	1974-1760-W	Indonesia
Aeschynanthus	*longicaulis*	Wallich ex R. Brown	C0056	MSBG	1974-2207-W	Indonesia
Cyrtandra	*feaniana*	F. Brown	C0059	Price	200	Marquesas, Hiva Oa
Cyrtandra	*ootensis* var. *ootensis*	F. Brown	C0060	Wood	10047	Marquesas, Hiva Oa
Cyrtandra	*ootensis* var. *molissima*	Fosberg & Sachet	C0061	Perlman	18399	Marquesas, Fatu Hiva
Cyrtandra	*thibaultii*	Fosberg & Sachet	C0062	Meyer	2541	Marquesas, Ua Pou
Cyrtandra	*ootensis* var. *mollissima*	Fosberg & Sachet	C0063	Wood	10266	Marquesas, Tahuata
Cyrtandra	*jonesii*	(F. Brown) Gillett	C0064	Wood	10484	Marquesas, Ua Huka
Cyrtandra	*nukukivensis*	Forest and Brown	C0065	Wood	10428	Marquesas, Ua Pou
Cyrtandra	*pogonantha*	A. Gray	C0066	Clark	644	Samoa, U`polu
Cyrtandra	cf. *pogonantha*	A. Gray	C0067	Clark	645	Samoa, U`polu
Cyrtandra	cf. *richii*	A. Gray	C0068	Clark	646	Samoa, U`polu
Cyrtandra	*richii*	A. Gray	C0072	Clark	650	Samoa, Sava`i
Cyrtandra	*richii*	A. Gray	C0073	Clark	651	Samoa, Sava`i
Cyrtandra	*compressa*	C.B. Clarke	C0074	Clark	652	Samoa, Sava`i
Cyrtandra	*compressa*	C.B. Clarke	C0075	Clark	653	Samoa, Sava`i
Cyrtandra	*aurantiicarpa*	Gillett	C0076	Clark	655	Samoa, Sava`i
Cyrtandra	*pogonantha*	A. Gray	C0081	Clark	660	Samoa, U`polu
Cyrtandra	*feaniana*	F. Brown	C0086	Wood	10804	Marquesas, Ua Pou
Cyrtandra	*coccinea*	Blume	C0089	Hoover & Agus	ARs 167	Indonesia, Java

Table 2.1. Taxa sampling list (1 of 2). 44 of 88 taxa total including two outgroup species (*Aeschynanthus* sp.). Taxa ordered by ID No.

Genus	*Specific Epithet*	Author(s)	ID No.	COLLECTOR	Collector No.	Origin
Cyrtandra	sp.		C0092	Hoover & Agus	ARs 173	Indonesia, Java
Cyrtandra	sp.		C0093	Hoover & Agus	ARs 175	Indonesia, Java
Cyrtandra	sp.		C0095	Wiriadinata, H.	12709	Indonesia, Java
Cyrtandra	*picta*	Blume	C0097	Wiriadinata, H.	12715	Indonesia
Cyrtandra	*pendula*	Blume	C0098	Wiriadinata, H.	12716	Indonesia
Cyrtandra	*sulcata*	Blume	C0100	Hoover & Agus	ARs 160	Indonesia, Java
Cyrtandra	*hawaiensis*	C.B. Clarke	C0101	Clark	661	Hawai`i, Maui
Cyrtandra	*spathulata*	St. John	C0102	Clark	664	Hawai`i, Maui
Cyrtandra	*grayana*	Hillebrand	C0103	Clark	666	Hawai`i, Maui
Cyrtandra	*munroi*	C. Forbes	C0104	Clark	675	Hawai`i, Maui
Cyrtandra	*grayi*	C.B. Clarke	C0105	Clark	676	Hawai`i, Maui
Cyrtandra	sp.		C0112	Plunkett	1980	Fiji, Viti Levu
Cyrtandra	*milnei*	Seem. ex A. Gray	C0113	Clark	687	Fiji, Viti Levu
Cyrtandra	*anthropophagorum*	Seem. ex A. Gray	C0114	Clark	688	Fiji, Viti Levu
Cyrtandra	*leucantha*	A.C. Smith	C0116	Clark	693	Fiji, Viti Levu
Cyrtandra	*occulta*	A.C. Smith	C0117	Clark	694	Fiji, Viti Levu
Cyrtandra	cf. *occulta*	A.C. Smith	C0119	Clark	702	Fiji, Viti Levu
Cyrtandra	aff. *bidwillii*	C.B. Clarke	C0130	Wood	11072	Society Islands, Hua Hine
Cyrtandra	sp.		C0131	Wood	11057	Society Islands, Hua Hine
Cyrtandra	*ootensis* var. *molissima*	Fosberg & Sachet	C0132	Wood	6563	Marquesas, Tahuata
Cyrtandra	*pickeringii*	A. Gray	C0134	Lorence	9528	Hawai`i, Kaua`i
Cyrtandra	*serratifolia*	H. Atkins & Cronk	C0136	Cubey and Scott	225	Indonesia, Sulawesi
Cyrtandra	*ferruginea*	Merrill	C0137	Cubey and Scott	226	Philippines, Luzon
Cyrtandra	sp.		C0139	Cubey and Scott	228	Indonesia, Sulawesi
Cyrtandra	cf. *mesilauensis*	B.L. Burtt	C0140	Cubey and Scott	229	Indonesia, Borneo
Cyrtandra	*falcifolia*	C.B. Clarke	C0141	Kiehn	940823-4/3	Samoa, U`polu
Cyrtandra	*samoensis*	A. Gray	C0142	Kiehn	940819-1/1	Samoa, U`polu
Cyrtandra	*falcifolia*	C.B. Clarke	C0143	Kiehn	940823-3/1	Samoa, U`polu
Cyrtandra	*samoensis*	A. Gray	C0144	Kiehn	940819-2/1	Samoa, U`polu
Cyrtandra	*filipes*	Hillebrand	C0145	Wood	7423	Hawai`i, Kaua`i
Cyrtandra	*procera*	Hillebrand	C0148	Oppenheimer	H110621	Hawai`i, Moloka`i
Cyrtandra	*macrocalyx*	Hillebrand	C0149	Oppenheimer	H110622	Hawai`i, Moloka`i
Cyrtandra	*platyphylla*	A. Gray	C0150	Oppenheimer	H100512	Hawai`i, Maui
Cyrtandra	*munroi*	C. Forbes	C0151	Oppenheimer	H120638	Hawai`i, Lana`i
Cyrtandra	*platyphylla*	A. Gray	C0152	Oppenheimer	H80514	Hawai`i, Maui
Cyrtandra	*bisserrata*	St. John	C0153	Wood	11386	Hawai`i, Moloka`i
Cyrtandra	*hawaiensis*	C.B. Clarke	C0154	Wood	11391	Hawai`i, Moloka`i
Cyrtandra	*hawaiensis*	C.B. Clarke	C0155	Roalson	1569-04	Hawai`i, O`ahu
Cyrtandra	*kaulantha*	St. John & Storey	C0156	Roalson	1570-14	Hawai`i, O`ahu
Cyrtandra	*laxiflora*	H. Mann	C0157	Roalson	1574-01	Hawai`i, O`ahu
Cyrtandra	*sessilis*	St. John & Storey	C0158	Roalson	1577-07	Hawai`i, O`ahu
Cyrtandra	*confertiflora*	(Wawra) C.B. Clarke	C0159	Roalson	1584-01	Hawai`i, Kaua`i
Cyrtandra	*calpidicarpa*	(Rock) St. John & Storey	C0164	Roalson	1576-7	Hawai`i, O`ahu
Cyrtandra	*umbelifera*	Merrill		Wagner		Taiwan, Ponso no Tao

Table 2.1. Taxa sampling list (2 of 2). 44 of 88 taxa total including two outgroup species (*Aeschynanthus* sp.). Taxa ordered by ID No.

nodes	areas	lnL	relative prob.
Node 1 [2|26]	[JF|J]	-107.40	0.37
	[J|BJ]	-108.00	0.21
	[J|J]	-108.10	0.18
	[JS|J]	-108.80	0.09
Node 2 [3|25]	[J|J]	-107.20	0.44
	[JF|J]	-107.40	0.37
	[JS|J]	-108.80	0.09
Node 3 [4|24]	[F|J]	-107.20	0.43
	[J|J]	-108.00	0.20
	[S|J]	-108.70	0.09
	[JF|J]	-109.00	0.08
Node 4 [5|19]	[F|F]	-106.80	0.64
	[FS|F]	-107.90	0.21
Node 5 [20|6]	[F|F]	-106.70	0.70
	[S|FS]	-108.60	0.11
Node 20 [–|23]	[H|FH]	-106.70	0.71
	[H|HS]	-108.30	0.15
Node – [21|22]	[H|H]	-106.50	0.89
Node 6 [7|14]	[FS|S]	-106.60	0.79
	[F|S]	-108.60	0.11
Node 7 [13|8]	[F|FS]	-106.90	0.61
	[F|S]	-108.00	0.21
	[F|F]	-108.30	0.15
Node 8 [9|10]	[S|FS]	-107.40	0.36
	[F|FS]	-107.70	0.28
	[S|S]	-107.90	0.23
	[F|F]	-108.70	0.10
Node 10 [12|11]	[FS|S]	-106.70	0.75
	[S|S]	-107.80	0.24
Node 14 [15|16]	[S|S]	-106.60	0.78
	[SI|S]	-108.20	0.16
Node 16 [18|17]	[S|S]	-106.50	0.86
	[SO|S]	-108.40	0.14

Table 2.2. DEC ancestral range reconstructions at numbered nodes of the chronogram. The "nodes" column refers to a node and its two daughter lineages as numbered in Figure 2.3. Area abbreviations (under "areas") are J=Java, B=Borneo, S=Sulawesi, T=Taiwan, P=Philippines, F=Fiji, S=Samoa, H=Hawaii, I=Society Islands, O=Tonga, M=Micronesia, Q=Marquesas. For example, the first row refers to the split at node 1 into its daughter lineages, node 2 and node 26; these are reconstructed as Java-Fiji and Java respectively (first among three additional likely reconstructions). The –lnL and relative probabilities are listed for each likely reconstruction. The "–" is a node arbitrarily resolved for analysis (see text).

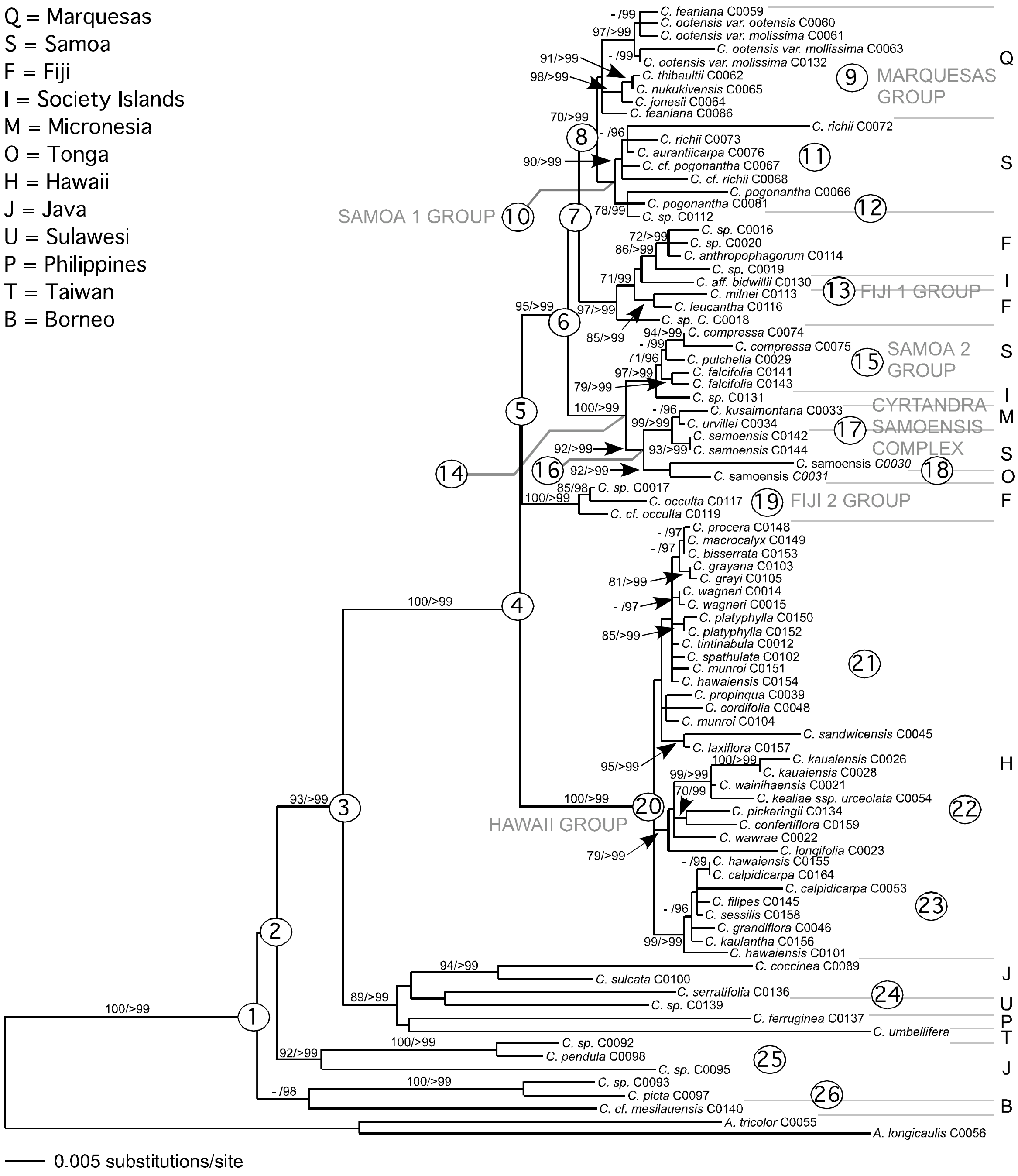

Figure 2.1. Maximum likelihood phylogram (-lnL = 9782.6046; single ML tree). Analysis of ITS, ETS and *psbA-trnH* regions; K80+G substitution model; letters to the right indicate geographic regions; numbers along branches indicate branch support (bootstrap support ≥70% / Bayesian posterior probabilities ≥ 95%); circled numbers by selected nodes (1-26), and GROUP NAMES, are for reference in the text.

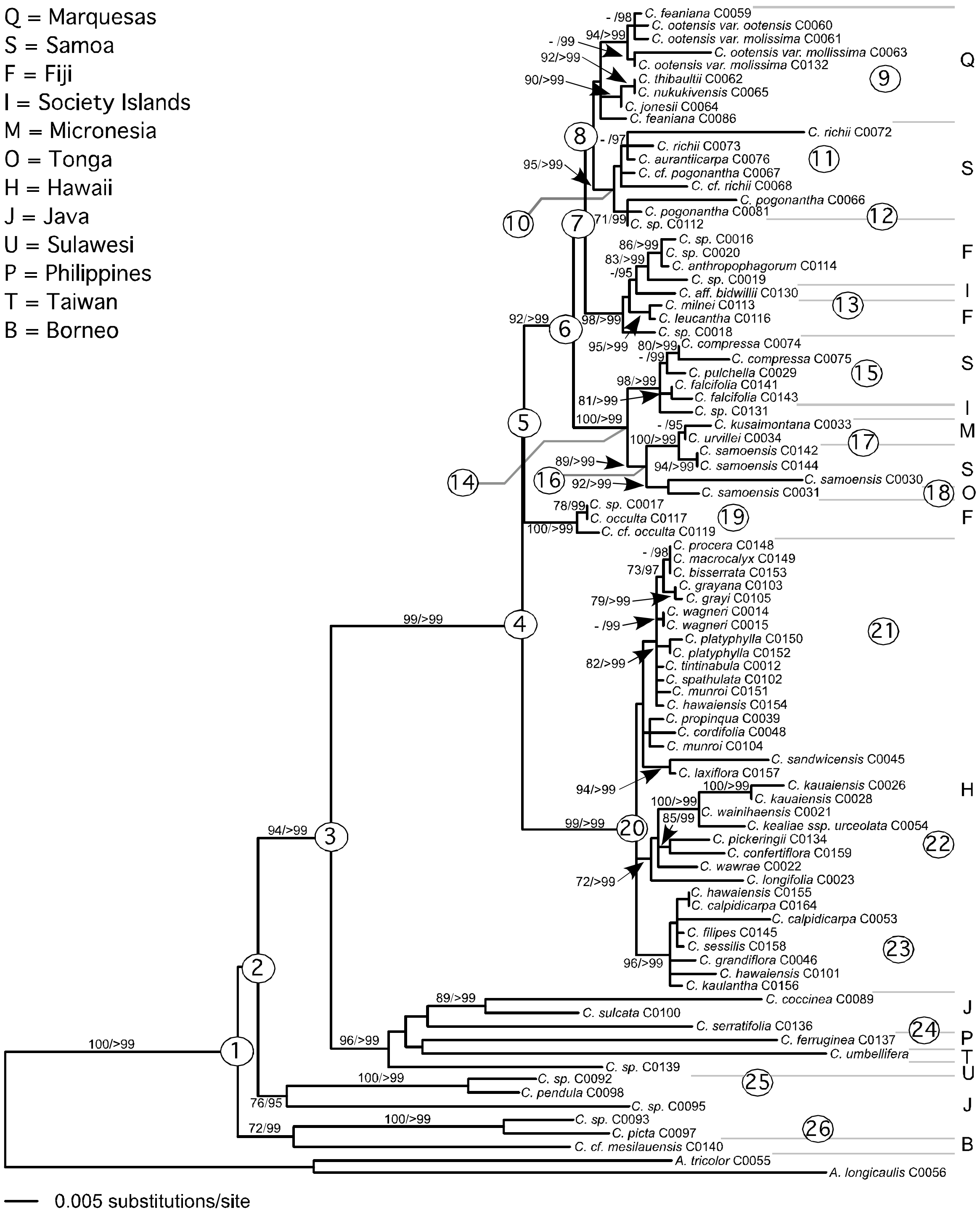

Figure 2.2. Maximum likelihood phylogram (-lnL = 8046.1170; single ML tree); analysis of ITS and ETS; K80+G substitution model; symbols like those in Figure 1.

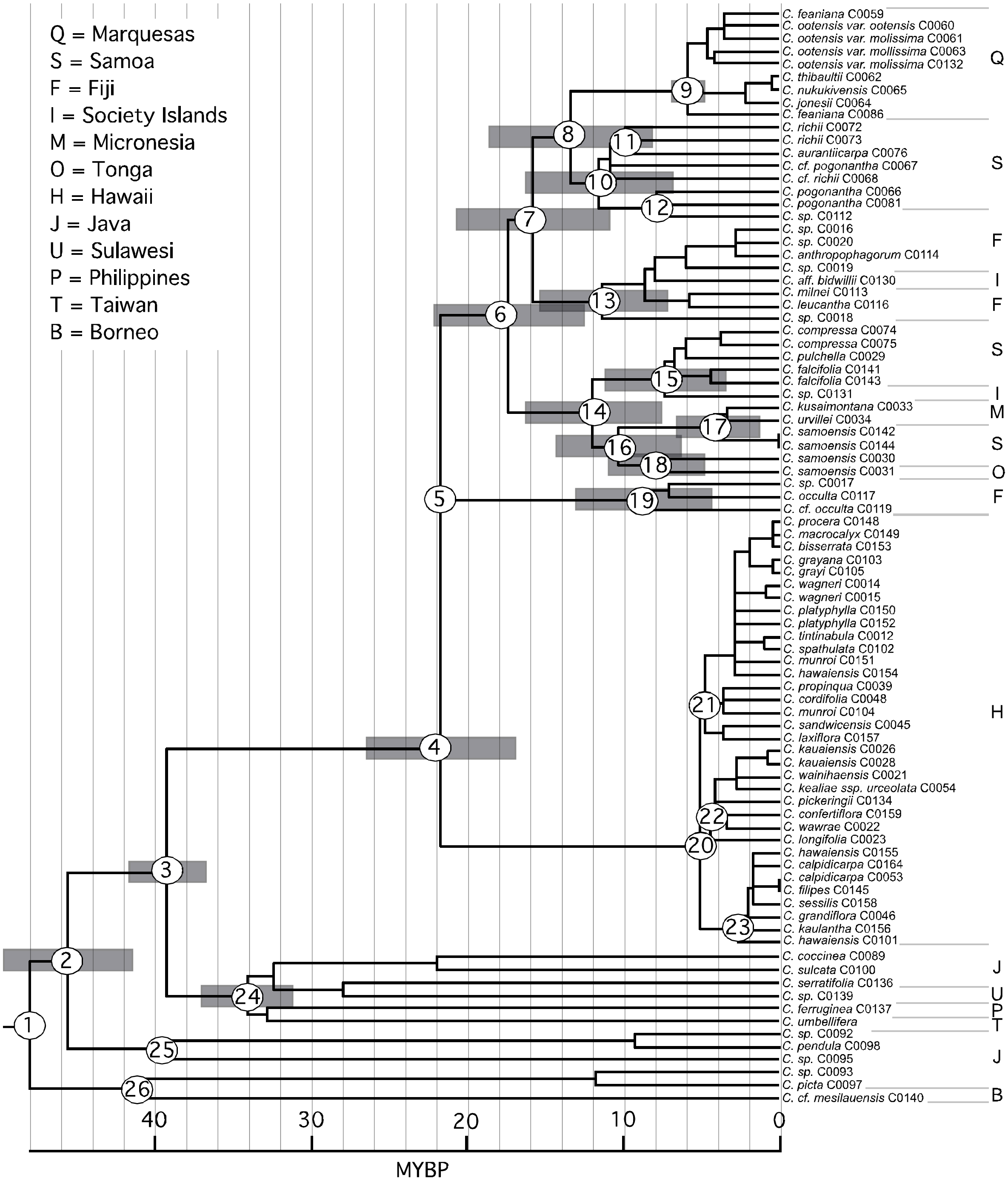

Figure 2.3. Chronogram based on penalized likelihood analysis of the ML tree calibrated using island ages referred to in the text; numbers at the bottom are ages in millions of years before the present (MYBP); gray bars indicate standard deviations around selected nodes; numbers by selected nodes (1-26) are for reference and are referred to in the text.

Figure 2.4. Simplified chronogram with areas inferred using DEC. Labeled nodes refer to taxonomic groups referred to in the Results. Schematics to the right indicate areas inferred at each numbered node, from left to right within the schematic, top = B,J,U,P,T,F; bottom = H,S,O,M,I,Q; NA = node not inferred.

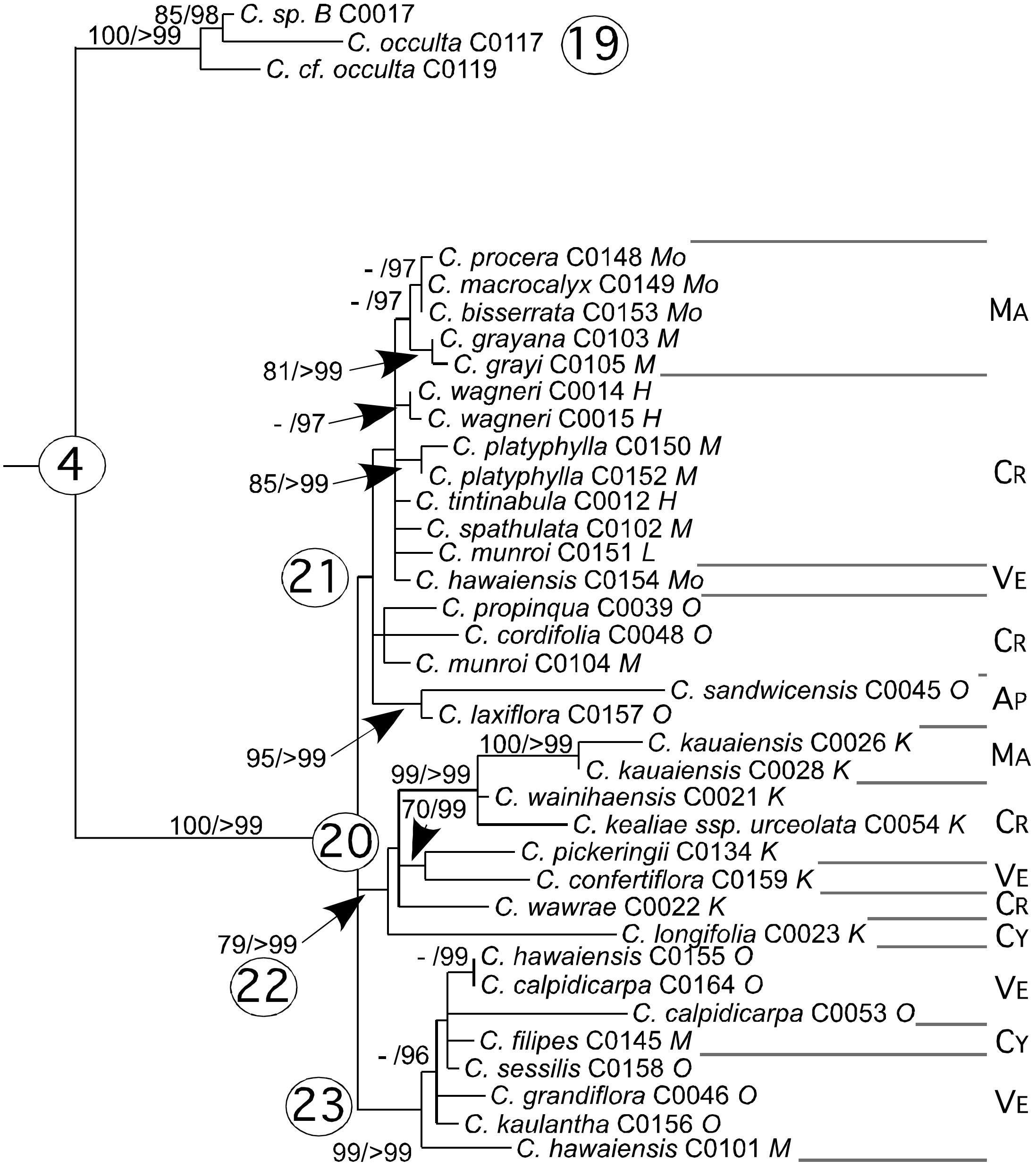

Figure 2.5. Detail of the Hawaiian clade of the combined maximum likelihood phylogram (Figure 1). Letters immediately following species names and identification numbers are abreviations of islands (Mo=Molokai, M=Maui, H=Hawai'i, L=Lana'i, O=O'ahu, K=Kaua'i). Letters in large and small capitals to the right indicate sectional placement based on Wagner et al. (1990; MA=*Macrosepalae;* CR=*Crotonocalyces;* VE=*Verticillatae;* AP=*Apertae;* CY=*Cylindrocalyces*).

CHAPTER THREE

A PRELIMINARY REVIEW OF HAWAIIAN *CYRTANDRA* IN PREPARATION FOR MONOGRAPHIC REVISION

INTRODUCTION

Cyrtandra J.R. & G.Forster (Gesneriaceae) includes as many as 600 species and has a paleotropical distribution in southeast Asia and throughout the Pacific islands including the Marquesas and Hawaiian Islands. *Cyrtandra* represents approximately 25% of the species within the Gesneriaceae and is a conspicuous understory species in many Old World tropical forests. The current phylogenetic hypothesis for *Cyrtandra* (Chapter 2) supports a southeast Asian origin of the genus (corroborating previous hypotheses; Burtt, 2001; Cronk et al., 2005; Clark et al., *accepted*) with later dispersal and diversification throughout the Pacific. The Pacific clade is strongly supported as being monophyletic in these studies and includes half of all species of *Cyrtandra* (±300 species). Phylogenetic dating of the Pacific clade suggests that this large number of species evolved in a relatively short window of time, within the last ±22 million years (Chapter 2). Although species of *Cyrtandra* are morphologically diverse across the entire range, with varied habits, fruits and flower types, the Pacific lineage is far less diverse (Cronk et al., 2005). Species of *Cyrtandra* in the Pacific clade are almost exclusively white-flowered with fleshy berries and a predominantly understory shrub or small tree habit. However, marked morphological diversity has been observed in Pacific *Cyrtandra*, particularly among Fijian and Hawaiian lineages (Gillett, 1967; Wagner et al., 1990). It is in Hawaii that the most comprehensive study of morphological diversity has been conducted (Wagner et al., 1990). As a result, *Cyrtandra* has probably been best studied in the Hawaiian Islands, and represents an ideal location to develop phylogenetic-based taxonomic methods that can be later applied to other, less-studied lineages within this genus.

A focused study of Hawaiian *Cyrtandra* is warranted in that Hawaiian *Cyrtandra* are strongly supported as monophyletic (Cronk et al., 2005; Clark et al., *in review*; see also Chapter 2). A revision of the genus is particularly warranted in that, despite current agreement that Hawaiian *Cyrtandra* are remarkably diverse, much debate exists over what constitutes a "species" in Hawai'i and definitive species numbers are not known. Harold St. John (see his summary, St. John, 1973) has recognized or described over 170 species of Hawaiian *Cyrtandra* and has argued that 100 or more additional species may exist (St. John to W.L. Wagner, pers. comm.). However, current species summaries for the genus (Wagner et al., 1990) recognize only 55 species. This discrepancy is only partially to blame on differing species concepts. More so, species boundaries are difficult to circumscribe in *Cyrtandra*, perhaps owing to the lineage's recent radiation (within the last 5 million years; see Chapter 2) and also possibly influenced by the large number of suspected hybrids in Hawaii (Wagner et al., 1990). Much work remains to be done to ascertain the relative influences of these factors and to better understand species concepts and relationships in Hawaiian *Cyrtandra*.

The purpose of this study is to summarize available information on Hawaiian *Cyrtandra* phylogenetics and species numbers/distributions, and to formalize hypotheses on origins, phytogeography and the role of hybridization in the diversification of this lineage. An overview of the six sections present in the Hawaiian Islands is given, as well as notes on the origins and potential monophyly of these sections. In this review, I include species descriptions for five species not previously included in the last published account of Hawaiian *Cyrtandra* (Wagner et al., 1990), and notes on these species' affinities and sectional placement are included. The ultimate purpose of this study is to lay the groundwork for a complete monographic revision of Hawaiian *Cyrtandra* that will serve as a foundation for future research in phylogenetic and taxonomic revision of this complex lineage of plants.

TAXONOMY AND CURRENT PHYLOGENETIC HYPOTHESIS

Numerous regional subgeneric classifications have been proposed for *Cyrtandra* (e.g., Hawai'i, Hillebrand, 1888; New Guinea, Schlecter, 1923; West Malaysia, Kraenzlin, 1927; Hawai'i, St. John, 1966, 1987, Wagner et al., 1990; West Malaysia, Burtt, 1990). Currently, two subgenera are generally recognized (C.B. Clarke, 1883; although Schlecter, 1923, recognized others in Papua New Guinea). Clarke based these subgenera, listed here, on calyx persistence after anthesis and calyx tube shape:

Cyrtandra subg. *Cyrtandra* (the correct name of this first subgenus, according to ICBN Art. 21.1) [=subg. *Macrocyathus* C. B. Clarke, A. DC. & C. DC., Monogr. phan. 5(1): 202, 227. 1883]; calyx lost after anthesis; calyx tube cylindrical.

Cyrtandra subg. *Brachycyathus* C. B. Clarke, A. DC. & C. DC., Monogr. phan. 5(1): 202, 203. 1883; calyx persistent; calyx tube short.

In general, Clarke's classification has been criticized for being artificial (Wagner et al., 1990) and has been largely ignored by many authors. Recently, B.L. Burtt (2001) has commented on the artificial nature of Clarke's subgenera. In particular, he argues that the calyx characters used by Clarke are "functionally a single character" in that a short calyx tube will remain intact because the developing fruit will not rupture the calyx. Likewise, a long calyx tube will rupture during fruit growth causing at least the partial removal of the calyx.

Burtt argues that this character can vary within closely related species (Burtt, 2001); my recent molecular studies support the paraphyly of these subgenera (Figure 2.5). In my phylogenetic hypothesis, the vast majority of South Pacific species (clade 5, Figure 2.1) are classified as subgenus *Cyrtandra.* However, species in Gillett's (1967) "Group 1" (subgenus *Brachycyathus*), including *C. occulta*, *C. milnei,* and *C. leucantha*, are distributed as sister to this clade (my Fiji 2 group including *C. occulta*) and nested within this greater subgenus *Cyrtandra* clade (species *C. leucantha* and *C. milnei* nested within my Fiji 1 group). In the Hawaiian clade, sister to the South Pacific clade, both subgenera are represented as well (Figure 2.5; *Verticillatae* and *Apertae* are subgenus *Cyrtandra*). It should be noted that, among Hawai'i species, species within each these subgenera loosely group together, baring three major exceptions (*C. hawaiensis* in clade 21 and *C. confertiflora* and *C. longifolia* in clade 22). The most recent sectional classification for Hawai'i (Wagner et al., 1990) recognizes 6 sections based largely on the earlier Hawaiian classification of Hillebrand (1888). These are first subdivided into two categories based on the character of calyx persistence after anthesis that is used in Clarke's subgenera (Wagner et al., 1990). Wagner et al. did not consider these to be subgenera but only recognized that this differentiation seemed to circumscribe natural groups within Hawai'i (Wagner et al., 1990).

The Hawaiian *Cyrtandra* have been treated taxonomically effectively six times (Clarke, 1883; Hillebrand, 1888; Rock, 1917, 1918, 1919a, 1919b; St. John, 1966, 1987a, 1987b, 1987c, 1987d, 1987e, 1987f, 1987g, 1987h; Wagner et al., 1990). According to

Wagner et al. (1990), Hillebrand's treatment bears the most resemblance to natural groupings, and the current species and sectional classifications are a version of this original treatment, modified to accommodate nomenclatural precedent of Clarke's original treatment (1883) and the addition of one new section based on the work of St. John (1966) for a total of six sections.

Wagner et al. (1990) speculated on the relative affinities between these sections and other lineages throughout the Pacific islands. Similarly, Gillett (1967, 1973) remarked similarly on affinities, often suggesting relationships between geographically distant species. Of the six sections in Hawai'i, Wagner et al. (1990) tentatively suggested that these lineages could be the result of 2-4 independent introductions to Hawai'i. However, my recent molecular-based phylogenetic analysis strongly refutes this hypothesis (see Chapter 2). Rather, the Hawaiian *Cyrtandra* represent a single, although highly polymorphic and potentially phylogenetically structured, single lineage. Affinities between the various sections in Hawai'i and other South Pacific species appear to be homoplastic in nature and not the mark of a common ancestry. The limits of the current sectional rankings, their affinities and origins, remain largely unresolved.

BIOGEOGRAPHY OF HAWAIIAN *CYRTANDRA*

Geology and Hawaiian Flora

The Hawaiian Islands represent an ancient lineage of oceanic islands that have formed along a geologic "hotspot." This hotspot is the result of a more or less continuous plume of hot magma from the Earth's mantle that remains stationary as the Pacific Ocean plate moves over it in a northwesterly direction. It is currently estimated that the Hawaiian

Islands chain, including now submerged seamounts and low elevation atolls, originated approximately 91 MYBP (Price and Clague, 2002). The extant "high islands" of Hawaii are substantially younger than this; current estimates suggest that these islands originated about 5.1 MYBP (Price and Clague, 2002).

Although it is highly probable that plant lineages inhabited the Hawaiian Islands throughout their history, most lineages now present in Hawaii are thought to have arrived since the formation of the now extant high islands (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 5.1-4.7 MYBP. This lull was essentially a bottleneck in Hawaiian lineages; subsequent colonization of Hawaiian Islands likely occurred from areas outside the chain. Today, the Hawaiian Islands flora is composed principally of southeast Asian, Australian and Pacific elements with some introductions originating from the Americas (Price and Wagner, 2004).

Origin of Hawaiian Cyrtandra

Like all species in Hawai'i, species of *Cyrtandra* had to have originated from long distance dispersal. Cronk et al. (2005) suggested that Hawaiian species were the result of dispersal from Taiwan to the now submerged islands that predate the extant high islands (2005). Although the potential exists for *Cyrtandra* to have inhabited these now extinct Hawaiian Islands, no data exists to support this hypothesis. To the contrary, my current phylogenetic hypothesis supports a Fijian origin for the Hawaiian lineage (Figure 2.1). In this hypothesis, a within-Fiji divergence event occurred sometime around 22 MYBP that resulted in the formation of two major Pacific lineages, the Hawaiian and the South Pacific clades (Figure 2.3). It appears that the Hawaiian lineage persisted in Fiji for several million years until the formation of the extant Hawaiian Islands at around 5.1 MYBP. This new ecological opportunity was then colonized by this Fijian lineage and subsequently diversified within Hawaii. My data suggests that the Hawaiian taxa are all that remain of this lineage, with no extant taxa remaining in Fiji. However, it should be noted that current sampling of Fijian species is sparse, with only ± 9 species of the almost 40 recognized species in Fiji included in the current analysis. The potential exists that some of these remaining taxa may represent closely related lineages to the Hawaiian group.

Within Hawai'i, it appears that *Cyrtandra* diversity across islands is largely the result of a classic stepping stone model from west to east, oldest to youngest islands. I base this hypothesis on my phylogenetic hypothesis that supports an early origin of Kaua'i lineages; Kaua'i taxa have the greatest genetic distance between similar taxa and constitute a single, well-supported lineage in the Hawaiian clade (Figure 2.5). This hypothesis has also been proposed for many other lineages in Hawai'i (e.g., *Psychotria* L., Neopokroeff et al., 2003) and has been corroborated in historical biogeography analysis of the islands (Wagner and Funk, 1995).

Dispersal Factors

Cyrtandra is unique among all Gesneriaceae in having colonized and speciated among the Pacific high islands (Cronk et al., 2005). The major challenge of dispersal, establishment, and proliferation on remote islands pose specific barriers that other

Gesneriaceae taxa have been unable to bridge. Two major characteristics of *Cyrtandra* may have facilitated long distance dispersal and the genus' establishment on remote oceanic islands: a generalist pollinator morphology and long distance dispersal-appropriate seed morphology.

Pollination.–Predominantly, islands harbor fewer numbers and smaller sized fauna that act as pollinators (principally insects), than neighboring landmasses. For example, studies have shown that insect diversity is greatly limited in the Hawaiian Islands, New Zealand and the Galapagos Islands (reviewed by Barret, 1998). This lack of pollinator diversity on islands thus favors plant species that are adapted to generalist pollinators. It is likely that attracting generalist pollinators may be advantageous in colonizing new areas. To the contrary, highly specialized corolla forms (e.g. strongly zygomorphic, long-tubular, nectar rewards, and brightly colored such as red) are generally thought to be the specialized result of pollinator co-evolution that may limit reproductive success in the absence of specific pollinators. Species of *Aeschynanthus*, the genus sister to *Cyrtandra*, are commonly characterized by long, tubular red corollas suggesting a specialization for bird pollination; this genus is not found east of the Solomon Islands. White flowers, as are characteristic of most species of *Cyrtandra*, are commonly thought to attract generalist pollinators. Although some species of *Cyrtandra* exhibit red color or other characters adapted to specific pollinators, no such morphologies are seen in remote island species within the genus (St. John, 1966; Gillett, 1967, 1973). The absence of such characters on island species of *Cyrtandra* thus lends support to the theory that a generalist-type flower morphology has led to the success of this genus in Pacific island ecosystems.

*Dispersal.–*Several modes of dispersal are possible in plants including wind or water (abiotic; including vegetation mat rafting), endozoochory (seeds inside fruitivorous birds and bats) and ectozoochory (clinging seeds [sticky or barbed] on birds or bats and seeds stuck in mud on birds feet) (Givnish, 1998). Each of these modes has a relative rate of efficacy depending upon the seed type and germination requirements of a particular species (Grant, 1998). Of these modes endozoochory and ectozoochory have been proposed for dispersal of *Cyrtandra*.

Although endozoochory may be a possible dispersal mode (St. John, 1966), there is current agreement that ectozoochory may be the more plausible scenario for long-distance dispersal in *Cyrtandra* (W.L. Wagner, pers. comm.). In this genus, many hundreds of small seeds are produced in large numbers of fleshy capsules or berries. Although these fruit are consumed by some migrating species of birds, it is unlikely that *Cyrtandra* seeds would survive passage through the guts of these animals. The small seeds with relatively thin seed coats are thought not to be suited to such conditions and would break down rapidly. Although considered to be a relatively infrequent dispersal mode as compared with other modes, ectozoochory satisfies the requirements for long-distance dispersal in light of the seed type of *Cyrtandra*. Shorebirds are one of the most probable vectors for ectozoochory dispersal of *Cyrtandra* throughout the Pacific and to the Hawaiian Islands.

*From shore to mountains.–*Currently, all species of Hawaiian *Cyrtandra* inhabit wet upland forests in Hawai'i and are not found at elevations at or near sea level, areas where shorebirds are logically found. This would initially suggest that the hypothesis of ectozoochory is implausible. However, very few species in Hawai'i below 300 meters are native only because of anthropogenic factors that have destroyed the native flora at these levels. In other areas of the Pacific, including Fiji and Samoa, species of *Cyrtandra* are found at lower elevations. One lineage in particular, the *C. samoensis* complex (discussed in detail in Chapter 2), is commonly found at sea level throughout its range in Micronesia, Niue, Tonga and Samoa. This lineage is also the most widespread *Cyrtandra*, inhabiting a range of over 3800 kilometers (although current species boundaries suggest a much smaller range for species within this complex). It is conceivable that this lineage's great range is a result of constant exposure to shorebird populations that relatively frequently disperse seeds of these species. This hypothesis can be extended to account for all island distributions of *Cyrtandra* as well as the high rates of endemism: 1) low-elevation lineages become dispersed over great distances; 2) from these, new lineages are spawned within island systems that become dependent upon more stringent, upland forest habitats over time; 3) this narrow ecological niche effectively restricts these new lineages to islands facilitating local endemic lineage diversification without marked dispersal to new areas.

HYBRIDIZATION

One possible explanation for the confusing array of species diversity in Hawai'i and possibly elsewhere is hybridization. Natural, *in situ* hybridization has long been thought to occur in *Cyrtandra* (Gillett, 1973; Burtt, 2001; Kiehn, 2001) and nowhere is this more evident than in the Hawaiian species. Wagner et al. (1990) duly noted in their treatment of Hawaiian *Cyrtandra* that hybridization in many instances blurs the lines between sections, and may have even been causative in some speciation events. The fact that major clades across sections are grouped in molecular data analysis in several well-supported clades principally by area, and not morphological traits, might support this hypothesis.

This proposed interaction between hybridization and phylogenetic structure in Hawaiian *Cyrtandra* is in agreement with the observed pattern of diverse clades on older islands (Kaua'i) with strong support, and younger Maui Nui-Hawai'i lineages with less support. In clade 22 (Figure 2.5), the Kaua'i clade, there is strong support for the monophyly of this lineage, despite its including four disparate sections. This would suggest that lineages present on Kaua'i, isolated over time from other islands, have introgressed significantly enough to now share common molecular signal (at least in the genic regions analyzed). In contrast with this, the more recent islands of Maui Nui show less genetic structure and specimens assigned to the various sections are scattered among one another on the phylogeny. The observed pattern is suggestive that hybridization may be occurring, obscuring defining boundaries between sections in a way that has not yet resolved itself as in older islands.

An explanation of introgressive hybridization suggests that sectional rankings, although recognizing real character suites in particular species, do not correspond with distinct evolutionary lineages. Rather, these characters suites may be characters that coalesce in different species groups over time, each drawing from a similar gene pool through introgression and general similarities in the genus as a whole. Under this explanation, Kaua'i exhibits the most genetic structure because lineages within this area, previously derived from a reticulated complex of species, have had the time to first coalesce and then once again diverge into distinct lineages. Species in Maui-Nui and elsewhere are in a state of flux between being a reticulated complex of lineages and being discrete sections. This process could be occurring multiple times in multiple areas, each resulting in several seemingly distinct lineages that appear to be most closely allied with similar lineages in different areas but are in fact of a common origin within that area. Similar processes may have occurred on older islands in the Pacific but this signal has been lost over time and is only now evident in the most recent expression of this, the Hawaiian Islands.

Increasingly, phylogenetic evidence suggests that reticulate introgression between multiple lineages has occurred in the ancestry of many taxa (McDade, 2000) and is recognized as a major factor in species evolution, particularly among plants (Tsukaya et al., 2003, McDade, 2000). Similar hypotheses on past introgression may be invoked to explain some of the difficult-to-resolve groupings among the South Pacific lineages. For example, the Samoa 1 group (Figure 2.1) contains two lineages, the *C. richii* subgroup and the *C. pogonantha* subgroup, that share little in common morphologically other than their orange fruit. One could speculate that two lineages, one orange-fruited and the other white-fruited, introgressed sufficiently over time to share a common fruit color and genetic signal. Analysis of only a few genic regions, millions of years after this event, now suggest that these lineages have a common ancestry, when in fact they do not (hypothetically).

Another possibility is that hybridization is a recent factor in Hawai'i and possibly elsewhere, brought on by anthropogenic factors. Following drastic anthropogenic environmental changes (which is indeed the case in Hawai'i), once isolated taxa are increasingly coming in contact with each other (Haig et al., 2004). These events can spark new hybridization and subsequent introgression between taxa in these new range overlaps. If occurring in Hawaiian *Cyrtandra*, morphological incongruence may be the result of recent hybridization and introgression; these signals could be confounding phylogenetic analysis and may be of particular concern in conservation. Introgression from common taxa may drastically affect the longevity of certain species of conservation concern by out-breeding depression (Haig et al., 2004) or even assimilation of narrowly distributed endemics by introduced or widespread taxa (McKinnon et al., 2004).

Evidence of Hybridization in Hawai'i

Regardless of its causes or role in evolution and diversification, hybridization is thought to be more extensive in species of *Cyrtandra* than in any other Hawaiian flowering plant group. *Cyrtandra* in the Hawaiian Islands is characterized by a particularly high level of sympatry within a relatively narrow ecological range in wet forests and mesic valleys. Sympatric species are usually members of different sections, with typically two to four species (but as many as eight species) occurring within a single valley. In virtually all mixed populations that have been examined closely one can find individuals that have intermediate morphological features, usually between species of different sections. Wagner et al. (1990) list 67 putative hybrid combinations in their treatment of the genus, these hybrids most often occurring between sections. Similar observations have been made in Samoan taxa (J.R. Clark and M. Kiehn, unpublished data) and in Fiji (J.R. Clark, unpublished data).

Alternatives to Hybridization

Hybridization need not be the only explanation for paraphyly of morphotypes in *Cyrtandra*. An alternative hypothesis, that the observed pattern is the result of convergences in form in different areas, also serves to explain this phenomenon. In this "convergence" hypothesis, similar morphological types are produced in disparate lineages due to possibly selective pressures, and/or random drift resulting in similar but non-homologous forms. In the Pacific, my data supports a rather recent divergence with limited genetic distance between species (Figure 2.1) suggesting that homoplasious characters resulting from a limited common gene pool may be a plausible explanation of convergent morphologies.

One striking example that lends credibility to this hypothesis is Gillett's "group 1" (1967) that has been discussed in detail in Chapter 2. These species are generally characterized by an involucre of bracts, partially or completely fused together, often pale-green or white, that surrounds the inflorescence. Wagner et al. (1990) mention that the Hawaiian section *Verticillatae*, with their large, conspicuous bracts, are quite similar to Gillett's group 1 species in Fiji. Likewise, *C. pogonantha* from Samoa (Gillett, 1973) and *C. reinwardtii* (Clarke) Bakh. (not sampled in this study; see Backer and Van Den Brink, 1965) from Java also share this feature. Molecular data suggests that these species are only distantly related and are more closely allied with species that do not share these same features. Ultimately, a convergence-based hypothesis and the previous hybridization-based hypotheses will need to be examined in more detail. Population-level lineage sampling is needed to explicitly test the influences of hybridization and/or other factors leading to common morphologies in disparate lineages in Hawai'i and elsewhere.

TAXONOMIC TREATMENT

CYRTANDRA J. R. & G. Forster, Char. Gen. Pl.: 3, *t. 3*. 1775. LECTOTYPE: *Cyrtandra biflora* J. R. & G. Forster. Lectotypified by H. St. John in Bernice P. Bishop Mus. Bull. 229:3. 1966. *Cyrtandra biflora* was one of the first collections of *Cyrtandra* made, collected in Tahiti on James Cook's first voyage of discovery in 1769. St. John gives a detailed discussion of the situation, but the salient points for selection of the lectotype are that the corolla tube of *C. biflora* is curved as stated in the Forster's short description and their "*t. 3*" is of that species. SYNONYMS (as first summarized in Burtt, 2001): *Whitia* Blume in Cat. Gewass. Buitenz. 16. 1823. TYPE: *W. oblongifolia* Blume; *Cyrtandropsis* Lauterbach in Lorentz, Nova Guinea 8:331. 1910. TYPE: *C. monoica* Lauterb.; *Cyrtandroidea* F.Brown in Bishop Bernice P. Bishop Mus. Bull. 180:323. 1935. TYPE: *C. jonesii* F. Brown; *Protocyrtandra* Hosokawa in Trans. Nat. Hist. Soc. Formosa 24:202. 1934. TYPE: *P. todaiensis* (Kanehira) Hosokawa. I here propose that the following should also be considered under synonymy: *Cyrtandra* subg. *Macrocyathus* C. B. Clarke, A. DC. & C. DC. in Monogr. phan. 5:202, 227. 1883 (no type specified); *Cyrtandra* subg. *Brachycyathus* [=subg. *Cyrtandra*] C. B. Clarke, A. DC. & C. DC. in Monogr. phan. 5: 202, 203. 1883 (no type specified). Current phylogenetic data suggests that these subgenera are paraphyletic and do not represent natural groupings. Each will need to be formal lectotypified in a future publication.

Perennial herbs, shrubs, small trees, sometimes lianas; terrestrial or sometimes epiphytic; stems rounded to angular, often four-sided, pubescent with multicellular hairs or glabrate; leaves simple, opposite or whorled, sometimes strongly anisophyllus, rarely alternate, upper surface usually hirtellous or glabrous, occasionally villous, lower surface velvety pilose, villous, more densely so with longer hairs on veins, or glabrous, petiolate or sessile, the base sometimes clasping the stem or perfoliate; flowers perfect, rarely unisexual, either in the upper leaf axils or cauliflorous or at the base of the stems or on adventitious roots, 1-numerous in cymes, pedunculate or sessile, peduncles with minute to large and foliaceous, distinct or occasionally connate bracts sometimes forming a cup-shaped involucre, those of the pedicels smaller; calyx 2-5(6)-lobed, the tube spathaceous or variously cleft into equal or unequal lobes, glabrous to variously hirsute, particularly in the inner lobes, deciduous or persistent in fruit; corolla fleshy or imbricate, usually bilabiate, white or yellow, occasionally green, orange, reddish, purplish, or variously streaked, campanulate to salverform, funnelform, or cylindrical, the tube straight or curved, the upper lip 2-lobed, the lower lip 3-lobed; nectary disk annular, often conspicuous and persistent around the ovary in fruit; stamens 2, inserted about halfway up the corolla tube, distally; staminodes 2-3 (0); fertile anthers coherent, positioned in the throat of the corolla tube, strongly protandrous; ovary superior, oblong-ovoid, pubescent or glabrous, the apex rounded or with a stylar beak; style present or absent; stigma capitate, 2-lobed (sometimes fused into a capitate disk appearing as one), the lobes usually elliptic; fruit a capsule or firm to fleshy berry, often green or brown if capsule, white or rarely orange or pinkish if berry, ovoid, ellipsoid to cylindrical, occasionally subglobose, often tipped by the persistent stylar beak, axile placentation; seeds numerous, minute, ellipsoid, usually less than about 1 mm long, the surface reticulate.

DISTRIBUTION: A genus of approximately 600 species from the Nicobar Islands to Malaya, throughout Malesia to the Philippines, Taiwan, southern Ryukyu Islands, southeast to Queensland and the Loyalty Islands, east to the high islands of the Pacific and the Hawaiian Islands, with centers of diversity in Papua New Guinea, Borneo, the Philippine Islands, and the Pacific Islands.

INFRAGENERIC CLASSIFICATION OF HAWAIIAN *CYRTANDRA*

In Hawai'i, the genus is represented by 60 species known from all major high islands excluding Ni'hau. No *Cyrtandra* are known from the low elevation atolls that make up the remainder of the Hawaiian Islands. Figure 3.1 summarizes the species and sections of Hawaiian *Cyrtandra* by island.

The current sectional classification is that proposed by Wagner et al. (1990), here modified to include five species not previously treated (three species that had been described but not originally treated and two species that have been described since the publication of the treatment). Currently, my phylogenetic hypothesis suggests that these sectional rankings, at least in part, may be artificial. Hawaiian sections were previously considered to extend beyond Hawai'i and encompass species from other regions (Wagner et al., 1990). Sectional names are principally those assigned by Hillebrand (1888) to Hawaiian lineages, but others include non-Hawaiian species (those with nomenclatural precedent by Clarke, 1883). These taxonomic issues will need to be resolved prior to any future subgeneric taxonomic revision in *Cyrtandra*. The sections are presented here 1) to include previously unassigned taxa for continuity, and 2) as a point of reference for future work. Sections are defined by a combination of features; one single salient feature cannot used to define these heterogeneous groups. This is compounded by hybridization and the presumed derivation of several species via hybridization (Wagner et al., 1990, 2001). Three of the six sections, *Apertae*, *Macrosepalae* and *Verticillatae*, require lectotypification to stabilize the names. This will be conducted in a later publication as part of the formal monograph of Hawaiian *Cyrtandra.* In the following treatment, island areas are included after each species abbreviated as Hawai'i (H), Maui (M if both West and east Maui; otherwise West Maui [WM] East Maui [EM]), Moloka'i (Mo), Lana'i (L), O'ahu (O), Kaua'i (K). The five species in bold are species not treated in the previous synopsis (Wagner et al., 1990); detailed, parallel accounts are given for these species following the *Preliminary Key to Sections*.

PRELIMINARY KEY TO THE SECTIONS OF HAWAIIAN *CYRTANDRA*

1. Calyx deciduous after anthesis, often zygomorphic, fusiform in bud, tipped by a long or short beak, the lobes often dissimilar in shape, unequal, usually tardily separating, if lobes equal, calyx retains other characters listed here, particularly in being deciduous after anthesis (e.g., *C. kaulantha*, *C. sessilis*).

2. Flowers in dense subcapitate clusters, peduncles short, rarely elongate (*C. grandiflora*) and inflorescences not congested; bracts usually large, sometimes connate; pedicels more or less concealed by the bracts (i.e., the bracts as long as or longer than pedicels); inner surface of calyx usually pubescent--sect. *Verticillatae* (11 species)

C. calpidicarpa (O), *C. confertiflora* (K), *C. crenata* (O), *C. cyaneoides* (K), *C. grandiflora* (O), *C. hawaiensis* (O, Mo, M, H), *C. heinrichii* (K), *C. kaulantha* (O), *C. oenobarba* (K, O), *C. olona* (K), *C. sessilis* (O)

2. Flowers in open cymes or solitary; bracts small, never connate, pedicels not concealed by bracts (i.e., the bracts usually shorter than pedicels or very narrow); inner surface of calyx glabrous--sect. *Cylindrocalyces* (3 species)

C. filipes (Mo, M), *C. longifolia* (K), *C. paludosa* (K, O, M, H)

1. Calyx actinomorphic or nearly so, not fusiform in bud, the lobes all similar or nearly so, subequal to equal, separate at anthesis, persistent after anthesis.

3. Leaves opposite, usually broadly elliptic to cordate or peltate, lower surface usually densely velvety pubescent; flowers usually 3-10 or more in open umbelliform cymes, usually densely shaggy pubescent, the bracts usually foliaceous, elliptic to ovate, 8-42(-100) mm long; calyx divided 1/8-3/4 its length, in three species to the base, the lobes deltate, ovate, or elliptic, both surfaces usually pubescent; corolla subcylindrical, nearly straight; ovary and style + pubescent--sect. *Crotonocalyces* (13 species)

C. cordifolia (O), *C. dentata* (O), *C. kealiae* (K), *C. munroi* (L), *C. oxybapha* (M), *C. pickeringii* (K), *C. platyphylla* (M, H), ***C. paliku* (K)**, *C. propinqua* (O), *C. tintinnabula* (H), *C. viridiflora* (O), *C. waianaeensis* (O), ***C. wagneri* (H)**, *C. wawrae* (K)

3. Leaves opposite or whorled, elliptic to suborbicular or obovate, lower surface sparsely to sometimes densely velvety pubescent; flowers 1-33 in open cymes or congested umbelliform cymes, sparsely to densely pubescent, the bracts not foliaceous, linear to lanceolate or ovate, 0.5-17 mm long; calyx divided 1/2-7/8 its length, rarely less, lobes linear to oblong or spatulate or foliaceous and elliptic to lanceolate, inner surface glabrous or pubescent in the upper 1/3-2/3; corolla subcylindrical to narrowly funnelform, usually curved, sometimes nearly straight; ovary and style glabrous to sparsely puberulent.

4. Flowers (3-)7-33 in open, branched cymes or by reduction solitary (*C. sandwicensis*); calyx cleft 1/3-1/2 its length or sometimes nearly to the base, the lobes deltate, inner surface glabrous; corolla subcylindrical, nearly straight, enlarging medially; leaves opposite--sect. *Apertae* (5 species)

C. garnotiana (O), *C. laxiflora* (O), *C. polyantha* (O), *C. rivularis* (O),
C. sandwicensis (O)

4. Flowers 1-12(-25) in congested to open, + umbelliform cymes; calyx cleft nearly to the base or if connate about 1/2 its length, then usually separating further after anthesis, the lobes linear, oblong, spatulate, or elliptic to lanceolate, inner surface glabrous to pubescent in the upper 1/3-2/3; corolla narrowly funnelform to cylindrical, usually curved, not enlarging medially; leaves opposite or whorled.

5. Leaves opposite or whorled, usually elliptic to elliptic-obovate; flowers 1-5(-10) in open cymes, bracts lanceolate to ovate or sometimes linear; calyx usually white or pale green, cleft to the base or only about 1/2 its length and usually irregularly separating further after anthesis, the lobes usually foliaceous, + revolute, elliptic, lanceolate, or spatulate, rarely linear-subulate--sect. *Macrosepalae*

C. biserrata (Mo, EM), ***C. ferripilosa* (EM)**, *C. grayana* (Mo, L, WM),
C. grayi (Mo, M), *C. halawensis* (Mo), *C. hashimotoi* (M), *C. hematos* (Mo),
C. kauaiensis (K), **C. kamoolauensis (K)**, *C. lessoniana* (O), *C. lysiosepala* (H),
C. macrocalyx (Mo), **C. nanawaleensis (H)**, *C. procera* (Mo), *C. pruinosa* (O),

C. hematos (Mo), *C. spathulata* (M), *C. waiolani* (O), *C. wainihaensis* (K)

5. Leaves opposite, usually ovate to broadly elliptic or suborbicular; flowers (1-)5-25 in often congested, umbelliform cymes, bracts linear, rarely lanceolate to elliptic; calyx usually green, cleft to the base, rarely 1/2-7/8 its length, the lobes not foliaceous nor revolute, linear, oblong, subulate, or linear-spatulate--sect. *Chaetocalyces*

C. giffardii (H), *C. gracilis* (O), *C. kalichii* (O), *C. kohalae* (H), *C. lydgatei* (Mo, L, M), *C. macraei* (O), *C. menziesii* (H), *C. subumbellata* (O)

ADDITIONAL SPECIES TREATMENTS

The following are five species that were not previously included in the manual (Wagner et al., 1990).

Section *Crotonocalyces*:

i. C. paliku W.L.Wagner, K.R.Wood, & Lorence, Novon 11: 146. 2001. TYPE: HAWAIIAN ISLANDS: KAUA`I: boarder of Kawaihau and Hanalei Districts, Makaleha Mountains, slopes below Kekoiki, N-facing cliffs, seeping basal rock face, 800-850 m, 22°08’58”N, 159°25’22”W, 9 February 1993, *K.R. Wood 2353* (PTBG!, HOLOTYPE).

Subshrub, 30-75 cm tall; 2- to 5-branched, each major stem erect, unbranched, or vigorous plants with 2 or 3 additional branchlets each up to ca. 25 cm long, conspicuously shaggy villous with dark reddish brown trichomes 3-5 mm long, 6-8 mm diameter, ringed with leaf scars; leaves opposite, clustered at upper 3 to 7 nodes, thick, those of a pair usually unequal, one 30-50% larger than the other, the blades strongly asymmetrical, usually falcate, elliptic to oblanceolate, 7-18 cm long, 4.5-9 cm wide, upper surface moderately villous, lower surface moderately long-villous, densely so along the veins, the hairs longer and more conspicuous towards the base, margins finely crenate-serrulate, the teeth widely spaced, apex acuminate, base attenuate to cuneate, inequilateral, one side (2-)10-25 mm shorter, petioles 2.5-4(-7) cm long, densely shaggy villous; flowers (1-)7-9 in cymes with 2 orders of branching, internodes, peduncles, and pedicels densely long-villous, peduncles 8-20 mm long, pedicels 11-15 mm long, lower-most bracts 4-10 mm long, ovate, usually obscured by the long-villous pubescence; calyx weakly zygomorphic, pale green, ellipsoid, 12-17 mm long, cleft to 1/3 its length, glabrous internally except a few hairs on the distal portion of the lobes, villous externally, persistent after anthesis, the tube 7-10 mm long, the lobes 5-7 mm long, triangular, slightly asymmetrical; corolla white, tube narrowly funnelform, slightly curved upward, 14-15 mm long, externally glabrous, glandular-puberulent internally on lobes and around throat, upper two lobes 5-10 mm long, lateral two lobes 8-12 mm long, lowermost lobe 8-12 mm long, ovate, subequal to slightly longer than the other four lobes; ovary glabrous; berries unknown, but immature fruit ca. 10 mm long, ellipsoid, glabrous. Seeds not seen.

This striking species is known only from the type locality characterized by steep, wet, north facing slopes of the Kekoiki and Makaleha Mountains, Kaua'i. Wagner et al. (2001) place this species in section *Verticillatae* based on its general habit similarities with *C. oenobarba* of this section. However, the species differs dramatically in many characters from other members of this section including in its striking indument of hairs, nearly actinomorphic, persistent calyx, and asymmetrical leaf bases. Wagner et al. (2001) suggest that *C. paliku* may be of hybrid origin between *C. oenobarba* and some unidentified species. However, all hybrids in the vicinity of *C. paliku* have quite distinct morphologies, none of which correspond with the peculiar herbaceous habit and other attributes that differentiate this species.

The vegetative habit, although rare in Pacific *Cyrtandra* and only known from one species of Hawaiian *Cyrtandra*, namely *C. oenobarba*, appears to be less aligning taxonomically than other features such as calyx structure and persistence. In the key to sections of *Cyrtandra* (Wagner et al., 1990), *C. paliku* readily keys to section *Crotonocalyces*. Indeed, *C. paliku* shares many features in common with the other members of this section, principally in corolla shape and persistence, but also in leaf and young stem morphology. Many species in this section including *C. kealiae* and *C. munroi* have markedly hirsute leaves with asymmetric bases similar to *C. paliku.* If *C. paliku* is a member of this section, the markedly different, low growing herbaceous habit could be hypothesized to have originated in response to the harsh conditions of the often bare, shaded but exposed rock faces that it inhabits. This would be another quite common example of reduced habit induced by wind shear related to these harsh and difficult sub alpine-like environments.

Unfortunately, as of this study, material has not become available for molecular analysis. Cronk et al. (2005) did include *C. paliku* in their analysis of ITS and this species is sister to all other taxa sampled. This relationship suggests an early derivation of this lineage within the Hawaiian *Cyrtandra*. Future molecular studies will be required to make a more informed hypothesis on the relationships of this curious species with other Hawaiian *Cyrtandra*.

DISTRIBUTION: Known only from the type locality.

ii. C. wagneri Lorence & Perlman, Novon 17: 357. 2007. TYPE: HAWAIIAN ISLANDS: HAWAI`I: North Hilo District, Laupahoehoe Natural Areas Reserve, banks of Kaiwilahilahi stream, both sides of stream, 200-2800 ft. [823-835 m], 19°56.416'N, 155°16.057'W, 20 June 2003, *S.P. Perlman, L. Perry & R. Warshauer 17591* (PTBG-040019!, HOLOTYPE).

Shrubs or small tree ca. 3 m tall; main stem usually solitary or sparsely branched, most parts golden- to dark-brown villosulous-hirtellous with spreading to antrorsely curved septate hairs, 2.5 cm dia.; leaves opposite, usually equal or subequal, symmetrical, chartaceous, elliptic to oblong-elliptic, 20-41 cm long, 8-18.5 cm wide, upper surface densely strigillose-hirtellous, lower surface moderately to densely velutinous-hirtellous with crinkled hairs, pubescence denser on veins and margin, venation prominent and raised, margin serrulate, teeth with tufts of hair, petioles 2.5-10 cm long; flowers cauligerous, borne near ground level or up to 2 m above, dichasial, erect, becoming pendulous with age, 2-3 branched cymes with 12-25 flowers each, primary axes 1-2.5 cm, secondary axes 1-2 cm, densely brown villosulous-hirtellous, bracts narrowly ovate to oblong, 4-9 mm long, 1.5-2 mm wide; flowers on robust pedicels, 10-15 mm long; calyx actinomorphic, green when fresh, 13-15 mm long, cleft to 7/8 its length, the lobes erect, lanceolate to linear-oblong, 12-14 mm long, 2-3 mm wide, apex acute, externally densely golden brown strigulose-hirtellous, internally strigulose in distal ½-1/3; corolla white, tube subcylindrical, flaring somewhat at mouth, slightly curved near middle, (12)15-22 mm long, externally strigulose-hirtellous with pale brown hairs, glabrous in basal 1/3, internally glabrous, upper three lobes suborbicular to broadly rhombic-ovate, (4)5-9 mm long, 6-12 mm wide, lower 2 lobes 5-8 mm long, 6-7 mm wide; anther 2-2,5 mm long, ovoid, filament 1.5-2 mm long, slightly coiled; ovary ovoid, glabrous or with a few scattered hairs, style 8-10 mm, pilosulous distally, stigma 3 mm, four-lobed; berries white, ovoid to ovoid-ellipsoid, 13-16 mm long, 7-8 mm in diameter; seeds pale brown, ellipsoid, 0.3 mm long.

This species is here tentatively placed in section *Crotonocalyces* based on its close affinities with *C. tintinnabula*, also endemic to the island of Hawai'i. Characters that differentiate *C. wagneri* from *C. tintinnabula* and other members of section *Crotonocalyces* include the large, equal, opposite leaves and somewhat spathulate calyx lobes that are deeply cleft nearly to the base (beyond 2/3 length, which is more commonly seen in *Crotonocalyces*). These latter characters closely align this species with section *Macrosepalae*.

Recent combined molecular analysis of the nuclear ITS, ETS and chloroplast *psbA-trnH* genic regions supports a close affinity of this species with other members of both sections *Crotonocalyces* and *Macrosepalae.* In fact, aside from two base transitions in ITS, no differences were observed between *C. wagneri* and *C. tintinnabula*. This alone does not suggest that these taxa are conspecific, but it does support their close relationship. Future studies are needed to look at the relationship of these and other members of these two sections.

DISTRIBUTION: Known only from the type locality.

Section *Macrosepalae*:

iii. Cyrtandra ferripilosa St. John, Phytologia 63: 497. 1987. TYPE: HAWAIIAN ISLANDS: MAUI: East Maui, Kuiki, Kapahulu Valley, U. S. Fish and Wildlife Service Forest Bird Survey Transect 18, Station 1, 1870 m, 22 Aug 1980, *F. R. Warshauer 3106* (BISH-520544! and a portion in spirit collection, HOLOTYPE).

Cyrtandra adusta St. John, Phytologia 63: 469. 1987. TYPE: HAWAIIAN ISLANDS: MAUI: East Maui, Kapahulu Valley, north of Palikea Stream, U.S. Fish and Wildlife Service Forest Bird Survey Transect 10, Station 11/12, 1865 m, 26 Jul 1980, *F. R. Warshauer 2889* (BISH portion in spirit collection, HOLOTYPE). The pressed specimen of this collection could not be located and was not returned by St. John to the main BISH collection.

Cyrtandra hanaensis St. John, Phytologia 65: 202. 1988. TYPE: HAWAIIAN ISLANDS: MAUI: East Maui, Hana Forest Reserve, along ridge trail above N rim of Kapahulu, under *Myrsine* sp., with *Clermontia* spp., *Metrosideros* [sp.], and *Pelea* [sp.], 6225 ft., 29 Jun 1973, *B. Harrison 286* (BISH-526959!, HOLOTYPE; BISH!, ISOTYPE).

Cyrtandra harrisonae St. John, Phytologia 65: 196. 1988. TYPE: HAWAIIAN ISLANDS: MAUI: East Maui, Hana Forest Reserve, on ridge trail above N rim of Kapahulu, under *Cheirodendron* and *Dicranopteris*, 5830 ft., 29 Jun 1973, *B. Harrison 285* (BISH-526957!, HOLOTYPE; BISH!, ISOTYPE).

Young plants pubescent; leaves opposite, those of a pair unequal, leaf blades subcoriaceous, elliptic to oblanceolate, subacuminate, base cuneate, hirsute, 6.8-19.7 cm long, 1.7-7.7 cm wide, petioles 1.5-4 cm long, pubescent; cymes 3-9 cm long, 6-flowered, pillous, pedicels 13-25 mm long; calyx 21-22 mm long, pubescent, lobes 15-16 mm long, lanceolate; corolla 15-16 mm long, pubescent.

This species appears to be very closely related to *Cyrtandra grayi* as a higher elevation replacement for it in Kapahulu Valley of East Maui; it is known only from a few specimens and it relationships and distribution should be studied. It is recognized here because it could not be accommodated in any of the related species of sect. *Macrosepalae* (*C. grayana, C. grayi, C. hashimotoi*, and *C. spathulata*). It differs from *C. grayi* in its overall more coriaceous leaves and having narrower, oblong and apparently greener calyx lobes.

DISTRIBUTION: East Maui, 1770-1900 m.

ADDITIONAL SPECIMEN EXAMINED. MAUI: **East Maui**. Kapahulu Valley, Haleakala National Park, West Camp, transect 4, 5200 ft, *Russell 550.*

iv. Cyrtandra kamoolaensis St. John, Phytologia 63: 499. 1987. TYPE: HAWAIIAN ISLANDS: KAUA`I: N side of north fork of Kamoola [Kamo`oloa] Stream, in gulch leading up to the ridge above the 40 ft. waterfall, 1760 ft. (536 m), rain forest with *Psychotria*, *Eugenia*, *Metrosideros*, *Gouldia*, *Pisonia*, and Urticaceae, 23 Oct 1976, *C. Christensen 70* (BISH521337! and a portion in spirit collection, HOLOTYPE).

Shrubs ca. 2 m tall; stems branched forming a bushy habit, the younger ones densely pilose; leaves opposite, slightly asymmetrical, chartaceous, elliptic, elliptic-ovate or obovate, 10-20 cm long, 4-8 cm wide, upper surface appressed pilose, more densely so along the veins, lower surface densely pilose along the principal veins, sparsely so along the higher order veins, the hairs golden brown, puberulent, apex acuminate, base narrowly cuneate, petioles 1-4.5 cm long; flowers solitary in the upper leaf axils, peduncles 5-9 mm long, densely appressed pilose, pedicels conspicuously swollen apically, 8-15 mm long, densely appressed pilose, bracts linear, 9-10 mm long, appressed pilose; calyx nearly actinomorphic, green, 32-34 mm long, ca. 6-11 mm wide, cleft to the base, the lobes leaf-like, slightly asymmetrical, narrowly oblanceolate, tapering to the base, prominently 3-nerved, higher order venation evident, unequal in width, both surfaces moderately appressed pilose, more densely so externally and toward the base internally, the margin sometimes with one or a few small teeth; corolla white, tube curved, ca. 16-18 mm long, densely pilose in the upper 2/3, upper lobes narrowly ovate, ca. 10 mm long, lower lobes oblong-ovate, ca. 8-11 mm long; ovary moderately puberulent; berries white, oblong-ellipsoid, ca. 2.8-3.2 cm long, ca. 1.6-1.8 cm in diameter, moderately puberulent. Seeds narrowly ellipsoid, sometimes somewhat irregular, 0.5 mm long, pale reddish brown, dark-tipped at both ends, the surface inconspicuously reticulate.

This distinctive species is poorly known.

DISTRIBUTION: Known only from Kamo`oloa Stream and upper `Apaeka`a Stream, 240-536 m, eastern Kaua'i.

v. Cyrtandra nanawaleensis St. John, Phytologia 63: 500. 1987. TYPE: HAWAIIAN ISLANDS: HAWAI`I: Puna District, Nanawale Forest Reserve, *L. K. Cuddihy 790053* (BISH-520476!, HOLOTYPE).

Cyrtandra comosa St. John, Phytologia 63: 496. 1987. TYPE: HAWAIIAN ISLANDS: HAWAI`I: Puna District, Malamaka Forest Reserve, 180 ft, 20 Jan 1981, *G. Clarke & J. Davis 554* (BISH-520685!, HOLOTYPE). There is an isotype in the herbarium of the Hawaii State Department of Land and Natural Resources (future status to be determined).

Cyrtandra fruticosa St John, Phytologia 63: 498. 1987, nom. illeg., non St. John, Phytologia 63: 470, 1987. *Cyrtandra spartoides* St. John, Phytologia 64: 488. 1988. TYPE: HAWAIIAN ISLANDS: HAWAI`I: Puna District, kipuka at Malamaka Forest Reserve, 610 ft, 22 Jan 1981, *G. Clarke 561* (BISH-520414!, HOLOTYPE; BISH! [2], ISOTYPES).

Cyrtandra disgrega St. John, Phytologia 65: 201. 1988. TYPE: HAWAIIAN ISLANDS: HAWAI`I: Nanawale, tall `Ohi`a [*Metrosideros* sp.] forest near *Cyrtandra* nos. 3411, 3412, 3447--3449, in open understory, Pohoiki Project voucher, field no. Hawai'i 84, 550 ft., Feb 1987, *W. Takeuchi & C. Imada 3414* (BISH-510487!, HOLOTYPE).

Cyrtandra hylematos St. John, Phytologia 65: 202. 1988. TYPE: HAWAIIAN ISLANDS: HAWAI`I: Nanawale, `Ohi`a [*Metrosideros* sp.] forest to 10+ m height, densely shaded understory with lush fern growth, numerous *Cyrtandra* distributed diffusely, Pohoiki Project voucher, field no. Hawai'i 82a, Feb 1987, *W. Takeuchi & C. Imada 3412* (BISH-510491!, HOLOTYPE; BISH!, ISOTYPE). The isotype has the same label information except it gives "Hawaii 82b" as the field number.

Cyrtandra infera St. John, Phytologia 65: 202. 1988. TYPE: HAWAIIAN ISLANDS: HAWAI`I: Nanawale Forest Reserve on well--drained a`a, under complete shade of hau [*Hibiscus tiliaceus*] canopy, in small graben type gulch, 300 ft., 28 Feb 1978 [1979], *Lisa Kuulei Croft 790240 [790040LKC]* (BISH-526961!, HOLOTYPE).

Cyrtandra kauensis St. John, Phytologia 65: 203. 1988. TYPE: HAWAIIAN ISLANDS: HAWAI`I: Ka`a, Kiolaka`a-Kea`a Homestead Addition, open *Metrosideros* forest with native trees--*Psychotria*, *Gouldia*, *Ilex*, *Coprosma*, *Myrsine*. *Cibotium* and *Dicranopteris* common, 2300 ft., 28 May 1981, *J. Davis 502* (BISH-526335!, HOLOTYPE).

Cyrtandra prolixa St. John, Phytologia 65: 204. 1988. TYPE: HAWAIIAN ISLANDS: HAWAI`I: Kona, lower Nanawale Forest Reserve, makai [ocean] side [at] transect E, 22 Mar 1979, *L.K. Cuddihy 790054* (BISH-526964!, HOLOTYPE).

Young stems hirsute; leaves opposite, petioles 2-5 mm long, pubescent, blades 4-15 cm long, 2-5.2 cm wide, elliptic acute, bases cuneate, hirsute; cymes 2 cm long, 3-flowered, pubescent, pedicels 5-8 mm long; calyx 7 mm long, pubescent, lobes 4-5 mm long, lanceolate, subobtuse; corolla 13 mm long, pubescent.

Not much is known about this rare species.

DISTRIBUTION: Nanawale Forest Reserve and surrounding areas.

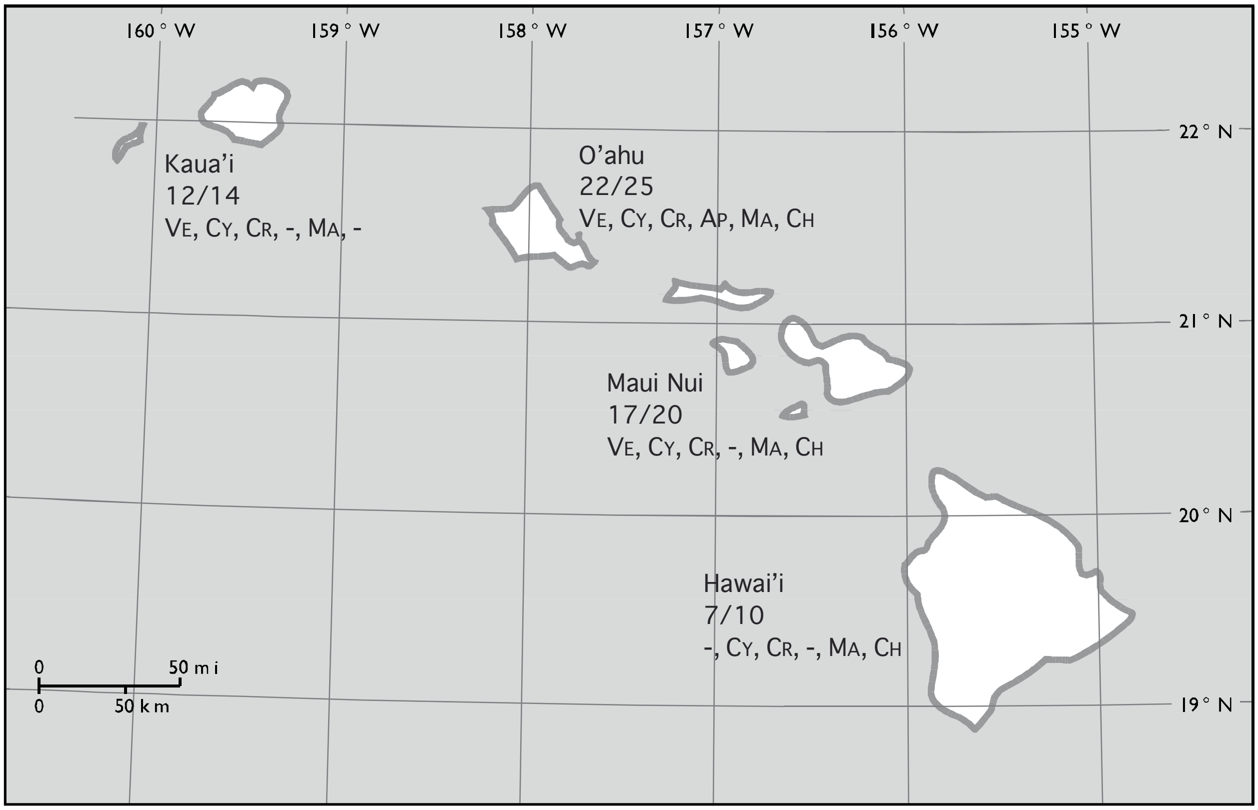

Figure 3.1. Distribution of species of *Cyrtandra* in the Hawaiian Islands. Numbers represent species found in each of four areas in the format *number endemic species/number of total species*; letters in small capitals designate sections represented in each area in the order VE=*Verticillatae,* CY=*Cylindrocalyces,* CR=*Crotonocalyces,* AP=*Apertae,* MA=*Macrosepalae,* CH = *Chaetocalyces*; a dash indicates that a section is not found in that area.

FINAL NOTES

This dissertation is only one part of a global effort to understand the origins, diversification and dispersal of *Cyrtandra*. Melanesians have been studying *Cyrtandra* on a regional basis, most notably in Fiji where Marika Tuiwawa and his daughter Senilolia have been looking at the distribution and taxonomy of Fijian *Cyrtandra.* Bill Burtt, Olive Hilliard, Michael Möller and Hannah Atkins, among several others at the Royal Botanic Gardens Edinburgh in Scotland, have a long history of Gesneriaceae taxonomy and systematics research in southeast Asia including in critical areas such as Borneo, Sulawesi and the Philippines. Work in Polynesia has been split among a wide range of researchers including Quentin Cronk at the University of British Columbia, Michael Kiehn at the University of Vienna, Art Whistler at the University of Hawaii, David Lorence and staff at the National Tropical Botanical Garden in Hawai'i, and numerous field and conservation biologists, including Hank Oppenheimer, in Hawai'i. These and other researchers continue to make great strides in understanding this diverse and challenging genus and are making valiant efforts to preserve it. It is my hope that this dissertation has both contributed towards the collective goal of solving the mysteries of this intriguing genus as well as generated novel approaches and useful hypotheses with which to continue this research legacy.

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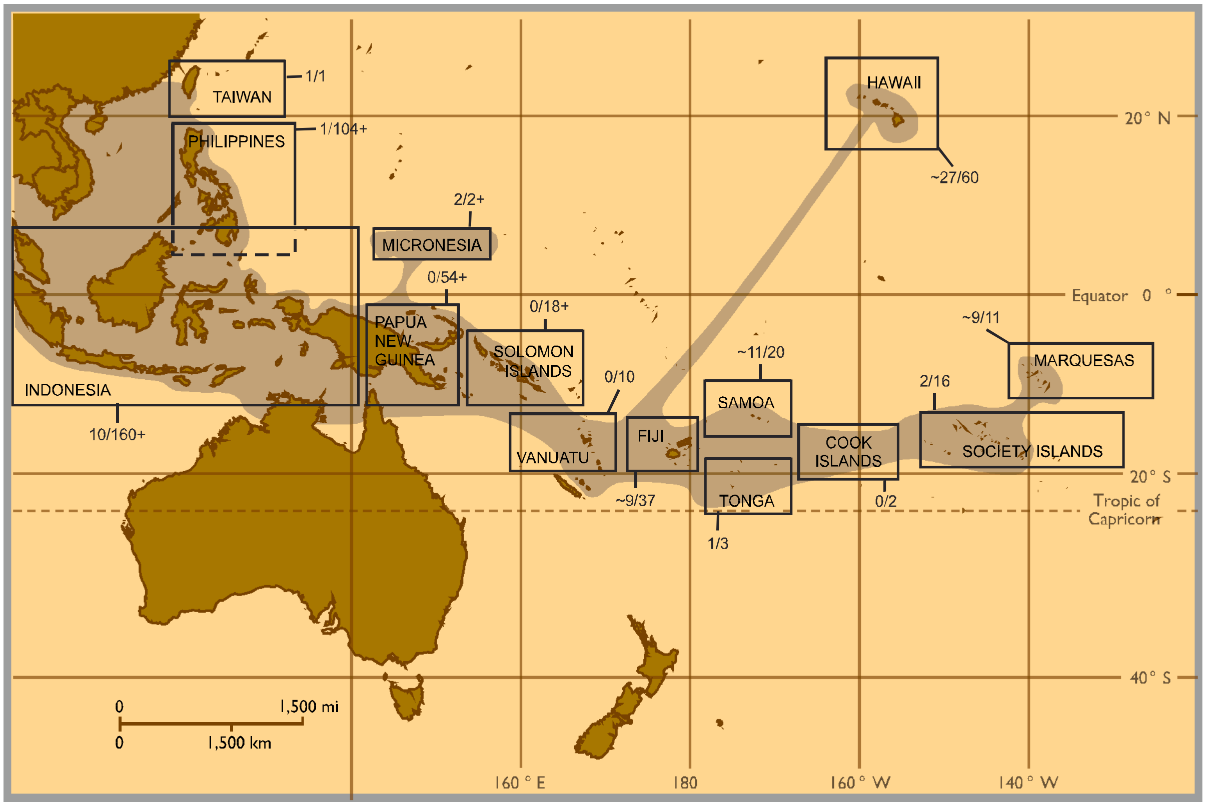

Appendix 1. Southeast Asian and Pacific distribution of *Cyrtandra*. Numbers before the forward slash are approximate species sampled in studies described in Chapter 2; numbers after slash are conservative estimates for species numbers in the defined areas.

Pacific Islands Distance Matrices

Absolute (in kilometers)

Location	IN	FI	HI	SA	TO	MI	MQ
IN		6635	8790	7463	7310	4496	10891
FI	6635		4871	961	761	3023	4456
HI	8790	4871		4181	5064	4568	3917
SA	7463	961	4181		884	3467	3523
TO	7310	761	5064	884		3776	3980
MI	4496	3023	4568	3467	3776		6519
MQ	10891	4456	3917	3523	3980	6519	

Scaled (by greatest absolute distance = IN-MQ=10891)

Location	IN	FI	HI	SA	TO	MI	MQ
IN		0.61	0.81	0.69	0.67	0.41	1.00
FI	0.61		0.45	0.09	0.07	0.28	0.41
HI	0.81	0.45		0.38	0.46	0.42	0.36
SA	0.69	0.09	0.38		0.08	0.32	0.32
TO	0.67	0.07	0.46	0.08		0.35	0.37
MI	0.41	0.28	0.42	0.32	0.35		0.60
MQ	1.00	0.41	0.36	0.32	0.37	0.60	

Scaled Inverse (1-scaled value)

Location	IN	FI	HI	SA	TO	MI	MQ
IN		0.39	0.19	0.31	0.33	0.59	0.00
FI	0.39		0.55	0.91	0.93	0.72	0.59
HI	0.19	0.55		0.62	0.54	0.58	0.64
SA	0.31	0.91	0.62		0.92	0.68	0.68
TO	0.33	0.93	0.54	0.92		0.65	0.63
MI	0.59	0.72	0.58	0.68	0.65		0.40
MQ	0.00	0.59	0.64	0.68	0.63	0.40	

IN=Indonesia:	03°29'58.26"N	122°27'24.63"E
FI=Fiji:	16°35'20.98"S	179°24'51.89"E
HI=Hawaii:	20°33'28.13"N	157°31'36.66"W
SA=Samoa:	13°46'02.29"S	172°05'46.00"W
TO=Tonga:	21°10'44.35"S	175°11'53.67"W
MI=Kosrae, Micronesia:	05°21'00.96"N	162°58'32.00"E
MQ=Marquesas:	09°21'10.42"S	140°03'02.53"W

Appendix 2. Distance matrices used to calculate distance penalization parameter in the DEC analysis; first matrix contains actual distances between areas in kilometers; second matrix contains each actual distance scaled by the greatest distance (10891 km); the third matrix contains the inverse of each scaled distance.

Pacific Islands Distance Matrices

Absolute (in kilometers)

Location	B	J	U	P	T	F	H	S	O	M	I	Q
B		1664	957	918	1855	7263	9283	8086	7938	5057	10588	11496
J	1664		1277	2384	3486	7599	10554	8525	8152	6052	10756	12047
U	957	1277		1239	2698	6647	9282	7527	7273	4816	9926	11023
P	918	2384	1239		1643	6519	8372	7295	7225	4161	9848	10642
T	1855	3486	2698	1643		7612	8278	8244	8361	4820	10850	11251
F	7263	7599	6647	6519	7612		4871	961	761	3023	3330	4456
H	9283	10554	9282	8372	8278	4871		4181	5064	4568	4933	3917
S	8086	8525	7527	7295	8244	961	4181		884	3467	2606	3523
O	7938	8152	7273	7225	8361	761	5064	884		3776	2656	3980
M	5057	6052	4816	4161	4820	3023	4568	3467	3776		6057	6519
I	10588	10756	9926	9848	10850	3330	4933	2606	2656	6057		1847
Q	11496	12047	11023	10642	11251	4456	3917	3523	3980	6519	1847	

Scaled by greatest distance (J:Q=12047)

Location	B	J	U	P	T	F	H	S	O	M	I	Q
B		0.14	0.08	0.08	0.15	0.60	0.77	0.67	0.66	0.42	0.88	0.95
J	0.14		0.11	0.20	0.29	0.63	0.88	0.71	0.68	0.50	0.89	1.00
U	0.08	0.11		0.10	0.22	0.55	0.77	0.62	0.60	0.40	0.82	0.91
P	0.08	0.20	0.10		0.14	0.54	0.69	0.61	0.60	0.35	0.82	0.88
T	0.15	0.29	0.22	0.14		0.63	0.69	0.68	0.69	0.40	0.90	0.93
F	0.60	0.63	0.55	0.54	0.63		0.40	0.08	0.06	0.25	0.28	0.37
H	0.77	0.88	0.77	0.69	0.69	0.40		0.35	0.42	0.38	0.41	0.33
S	0.67	0.71	0.62	0.61	0.68	0.08	0.35		0.07	0.29	0.22	0.29
O	0.66	0.68	0.60	0.60	0.69	0.06	0.42	0.07		0.31	0.22	0.33
M	0.42	0.50	0.40	0.35	0.40	0.25	0.38	0.29	0.31		0.50	0.54
I	0.88	0.89	0.82	0.82	0.90	0.28	0.41	0.22	0.22	0.50		0.15
Q	0.95	1.00	0.91	0.88	0.93	0.37	0.33	0.29	0.33	0.54	0.15	

Scaled inverse (1-scaled value)

Location	B	J	U	P	T	F	H	S	O	M	I	Q
B		0.86	0.92	0.92	0.85	0.40	0.23	0.33	0.34	0.58	0.12	0.05
J	0.86		0.89	0.80	0.71	0.37	0.12	0.29	0.32	0.50	0.11	0.00
U	0.92	0.89		0.90	0.78	0.45	0.23	0.38	0.40	0.60	0.18	0.09
P	0.92	0.80	0.90		0.86	0.46	0.31	0.39	0.40	0.65	0.18	0.12
T	0.85	0.71	0.78	0.86		0.37	0.31	0.32	0.31	0.60	0.10	0.07
F	0.40	0.37	0.45	0.46	0.37		0.60	0.92	0.94	0.75	0.72	0.63
H	0.23	0.12	0.23	0.31	0.31	0.60		0.65	0.58	0.62	0.59	0.67
S	0.33	0.29	0.38	0.39	0.32	0.92	0.65		0.93	0.71	0.78	0.71
O	0.34	0.32	0.40	0.40	0.31	0.94	0.58	0.93		0.69	0.78	0.67
M	0.58	0.50	0.60	0.65	0.60	0.75	0.62	0.71	0.69		0.50	0.46
I	0.12	0.11	0.18	0.18	0.10	0.72	0.59	0.78	0.78	0.50		0.85
Q	0.05	0.00	0.09	0.12	0.07	0.63	0.67	0.71	0.67	0.46	0.85	

B=Borneo:	5°47'23.77"N	117°16'33.51"E
J=Java:	7°19'56.90"S	109°54'23.59"E
U=Sulawesi:	2°19'42.93"S	120°17'52.86"E
P=Philippines:	7°39'52.92"N	125°22'24.01"E
T=Taiwan:	22°03'22.71"N	121°32'27.16"E
F=Fiji:	16°35'20.98"S	179°24'51.89"E
H=Hawaii:	20°33'28.13"N	157°31'36.66"W
S=Samoa:	13°46'02.29"S	172°05'46.00"W
O=Tonga:	21°10'44.35"S	175°11'53.67"W
M=Kosrae, Micronesia:	05°21'00.96"N	162°58'32.00"E
I=HuaHine, Society Islands:	23°22'57.14"S	149°28'35.24"W
Q=Marquesas:	09°21'10.42"S	140°03'02.53"W

Appendix 3. Distance matrices used to calculate distance penalization parameter in the DEC analysis; first matrix contains actual distances between areas in kilometers; second matrix contains each actual distance scaled by the greatest distance (12047 km); the third matrix contains the inverse of each scaled distance.