# Patterns of diversification and ancestral range reconstruction in the southeast Asian–Pacific angiosperm lineage *Cyrtandra* (Gesneriaceae)

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## ARTICLE INFO

## ABSTRACT

The genus *Cyrtandra* is the largest in the Gesneriaceae family and is one of the most widely dispersed plant genera in southeast Asia and the Pacific. Species of *Cyrtandra* are morphologically diverse but characters are often homoplastic causing considerable difficulty in defining monophyletic classification units. In this study, we used molecular phylogenetic analysis of 88 taxa representing approximately 70 species to construct a well-resolved evolutionary hypothesis for *Cyrtandra*. Diversification rates analysis and ancestral range analysis were also conducted to infer timing of major lineage divergences and geographic origin of these lineages, principally among Pacific species. Using these data, we compared existing classification schemes to better understand the applicability of current taxonomy. Divergence time estimates support a diversification of the Pacific clade at approximately 20 MYBP. Although the origin of the Pacific lineage remains unresolved, ancestral range reconstruction analysis supports Fiji as the most likely "first-step" into the Pacific with subsequent dispersals to Hawai'i, and other archipelagos. A greater Fiji–Samoa region, corresponding with Takhtajan's Fijian Region, is implicated as a major Pacific region interface and possibly a center of origin for expansion of *Cyrtandra* throughout the Pacific. Among South Pacific taxa sampled, several supported clades in our evolutionary hypothesis are characterized by distinct morphological traits possibly warranting sectional rankings. Relationships among Hawaiian taxa are less resolved and the distributions of species within this clade do not consistently correspond to existing sectional rankings. More detailed, population-level research is needed to clarify these relationships. We argue that future sectional classifications should correspond with monophyletic lineages and that species-level relationships should be more closely studied within these lineages.

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## 1. 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 allopatry model (*sensu* Clark et al., 2008). Most species within *Cyrtandra* are narrowly distributed endemics occupying no more than a single archipelago, a single island or even a single valley, further supporting this hypothesis. Dispersal-mediated allopatric divergence has been inferred in other insular plant lineages with similar life histories (Price and Wagner, 2004).

### 1.1. Supra-generic classification

*Cyrtandra* belongs to the subfamily Cyrtandroideae Endlicher (Burtt and 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 et al.,

2002; Mayer et al., 2003; Möller et al., 2009). 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 western South Pacific. The Coronantherioideae are hypothesized to be of Gondwanan origin, inhabiting mostly landmasses that were once part of or closely neighboring this ancient continent (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, other closely related large genera such as *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 species of *Cyrtandra*, approximately 250, occur east of the Solomon Islands (Burtt, 2001).

### 1.2. Phylogenetics

*Cyrtandra* has been the subject of molecular systematics studies since the late 1990s. Samuel et al. (1997) examined the chloroplast *atpB/rbcL* spacer region across 10 species of *Cyrtandra* 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. Sampled taxa from Palawan, the island system between these two regions, are paraphyletic and are nested within the Sundaland and Philippine clades (Atkins et al., 2001). More 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 species *C. umbellifera* and the Pacific clade is also inferred. Cronk et al. argue that Hawai'i may have been the initial dispersal point into the Pacific with later dispersal from Hawai'i into the South Pacific.

### 1.3. Taxonomy

Nearly every researcher that has addressed the classification and taxonomy of *Cyrtandra* has commented on the extreme difficulty in delineating species within this large genus (Clarke, 1883; Hillebrand, 1888; Rock, 1917; 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., 2008); a rapid species divergence resulting in potentially homoplastic character suites between lineages could be contributing to taxonomic issues in *Cyrtandra*.

*Cyrtandra* has been a challenge to classify at the supraspecific, subgeneric level (Gillett, 1973; Wagner et al., 1990; Burtt, 2001; Schlag-Edler and Kiehn, 2001; Cronk et al., 2005), although numerous regional subgeneric classifications have been proposed (Hawai'i, Hillebrand, 1888; New Guinea, Schelecter, 1923; west Malaysia, Kraenzlin, 1927;

Hawai'i, St. John, 1966, 1987a,b,c,d,e,f,g,h; 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* (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 Hawaiian *Cyrtandra* (Wagner et al., 1990) recognizes six sections based largely on the earlier classification of Hillebrand (1888). Sections are defined first on calyx morphology and secondarily on a combination of characters including flower symmetry, bracts and other floral characters. Habit and vegetative characters also are 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 calyx persistence after anthesis have all been variously explored to better delineate sectional groupings in this unwieldy genus (for a review, see Kiehn (2001)).

### 1.4. Project goals

Analysis of a broadly sampled subset of species of *Cyrtandra*, representing major lineages in the genus, may prove useful in identifying distinct lineages for better sectional circumscription (Burtt, 2001; Kiehn, 2001). To this end, we are 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 may represent synapomorphies for these major lineages and can these be useful in future revisions of current classifications? Although we will not propose any nomenclatural changes in this paper, we will make recommendations that may guide future taxonomic revisions of *Cyrtandra*.

## 2. Materials and methods

### 2.1. Taxon sampling

We sampled 88 specimens representing approximately 70 species including two outgroup taxa (both *Aeschynanthus* L. species; Table 1). Sampling builds on the previous work of Clark et al. (2008) and includes Bornean, Philippine and Taiwanese specimens for a more diverse and representative southeast Asian–Malesian grade. Sampled Pacific taxa correspond to lineages present on most high islands and all attempts were made to include at least one specimen from principal lineages as defined by Gillett (1973) and Wagner et al. (1990). For a distribution map of the genus *Cyrtandra* along with approximate species numbers for major regions discussed in this paper, see Fig. 1 in Clark et al. (2008).

### 2.2. Phylogenetic analysis

DNA extraction, genic region amplification, and sequencing were performed using protocols described by Clark et al. (2008). Silica

**Table 1**
Taxon sampling list for 88 individuals sampled in the current study including two outgroup species (*Aeschynanthus* sp.).

| Species | ID No. | COLLECTOR and No.; voucher | Origin | ITS | ETS | *psbA-trnH* |
|---|---|---|---|---|---|---|
| *A. longicaulis* Wall. ex R.Brown | C0056 | MSBG 1974-2207-W | Indonesia | EU919959 | EU919898 | EU920018 |
| *A. tricolor* Hook. | C0055 | MSBG 1974-1760-W | Indonesia | EU919958 | EU919897 | EU920017 |
| *C. anthropophagorum* Seem. ex A. Gray | C0020 | Plunkett 1898; US | Fiji, Viti Levu | EU919936 | EU919875 | GQ475120 |
| *C. anthropophagorum* Seem. ex A. Gray | C0114 | Clark 688; SEL | Fiji, Viti Levu | EU919987 | EU919926 | EU920042 |
| *C. aurantiicarpa* G.W. Gillett | C0076 | Clark 655; SEL | Samoa, Savai'i | EU919971 | EU919910 | EU920030 |
| *C. aff. bidwillii* C.B. Clarke | C0130 | Wood 11072; PTBG | Society Islands, Hua Hine | GQ475176 | GQ475089 | GQ475139 |
| *C. biserrata* H. St. John | C0153 | Wood 11386; PTBG | Hawai'i, Moloka'i | GQ475194 | GQ475107 | GQ475157 |
| *C. calpidicarpa* (Rock) H. St. John & Storey | C0053 | Clark 584; SEL | Hawai'i, O'ahu | GQ475168 | GQ475080 | GQ475123 |
| *C. calpidicarpa* (Rock) H. St. John & Storey | C0164 | Roalson 1576-7; WS | Hawai'i, O'ahu | GQ475201 | * | GQ475164 |
| *C. coccinea* Blume | C0089 | Hoover & Agus ARs 167; US | Indonesia, Java | EU919972 | EU919911 | GQ475131 |
| *C. compressa* C.B. Clarke | C0074 | Clark 652; SEL | Samoa, Savai'i | EU919970 | EU919909 | EU920029 |
| *C. compressa* C.B. Clarke | C0075 | Clark 653; SEL | Samoa, Savai'i | GQ475172 | GQ475085 | GQ475128 |
| *C. confertiflora* (Wawra) C.B. Clarke | C0159 | Roalson 1584-01; WS | Hawai'i, Kaua'i | GQ475200 | GQ475113 | GQ475163 |
| *C. cordifolia* Gaudich. | C0048 | Clark 579; SEL | Hawai'i, O'ahu | EU919955 | EU919894 | EU920014 |
| *C. falcifolia* C.B. Clarke | C0141 | Kiehn 940823-4/3; WU | Samoa, U'polu | GQ475184 | GQ475097 | GQ475147 |
| *C. falcifolia* C.B. Clarke | C0143 | Kiehn 940823-3/1; WU | Samoa, U'polu | GQ475186 | GQ475099 | GQ475149 |
| *C. feaniana* F.Br. | C0059 | Price 200; PTBG | Marquesas, Hiva Oa | EU919960 | EU919899 | EU920019 |
| *C. feaniana* F.Br. | C0086 | Wood 10804; PTBG | Marquesas, Ua Pou | GQ475174 | GQ475087 | GQ475130 |
| *C. ferruginea* Merr. | C0137 | Cubey and Scott 226; E | Philippines, Luzon | GQ475181 | GQ475094 | GQ475144 |
| *C. filipes* Hillebr. | C0145 | Wood 7423; PTBG | Hawai'i, Kaua'i | GQ475188 | GQ475101 | GQ475151 |
| *C. grandiflora* Gaudich. | C0046 | Clark 577; SEL | Hawai'i, O'ahu | EU919954 | EU919893 | EU920013 |
| *C. grayana* Hillebr. | C0103 | Clark 666; SEL | Hawai'i, Maui | EU919982 | EU919921 | EU920039 |
| *C. grayi* C.B. Clarke | C0105 | Clark 676; SEL | Hawai'i, Maui | EU919984 | EU919923 | EU920040 |
| *C. hawaiensis* C.B. Clarke | C0101 | Clark 661; SEL | Hawai'i, Maui | GQ475175 | GQ475088 | GQ475133 |
| *C. hawaiensis* C.B. Clarke | C0154 | Wood 11391; PTBG | Hawai'i, Moloka'i | GQ475195 | GQ475108 | GQ475158 |
| *C. hawaiensis* C.B. Clarke | C0155 | Roalson 1569-04; WS | Hawai'i, O'ahu | GQ475196 | GQ475109 | GQ475159 |
| *C. cf. hawaiensis* C.B. Clarke | C0158 | Roalson 1577-07; WS | Hawai'i, O'ahu | GQ475199 | GQ475112 | GQ475162 |
| *C. jonesii* (F.Br.) G.W. Gillett | C0064 | Wood 10484; PTBG | Marquesas, Ua Huka | EU919965 | EU919904 | EU920024 |
| *C. kauaiensis* Wawra | C0026 | Clark 556A; SEL | Hawai'i, Kaua'i | EU919940 | EU919879 | EU919999 |
| *C. kauaiensis* Wawra | C0028 | Clark 558; SEL | Hawai'i, Kaua'i | GQ475167 | GQ475079 | GQ475121 |
| *C. kaulantha* H. St. John & Storey | C0156 | Roalson 1570-14; WS | Hawai'i, O'ahu | GQ475197 | GQ475110 | GQ475160 |
| *C. kealiae* ssp. urceolata W.L.Wagner & Lorence | C0054 | Perlman 18805; PTBG | Hawai'i, Kaua'i | EU919957 | EU919896 | EU920016 |
| *C. kusaimontana* Hosok. | C0033 | Flynn 5995; PTBG | Micronesia, Kosrae | EU919945 | EU919884 | EU920004 |
| *C. laxiflora* H. Mann | C0157 | Roalson 1574-01; WS | Hawai'i, O'ahu | GQ475198 | GQ475111 | GQ475161 |
| *C. leucantha* A.C. Sm. | C0116 | Clark 693; SEL | Fiji, Viti Levu | EU919988 | EU919927 | GQ475136 |
| *C. longifolia* (Wawra) Hillebr. ex C.B. Clarke | C0023 | Clark 551; SEL | Hawai'i, Kaua'i | EU919939 | EU919878 | EU919998 |
| *C. macrocalyx* Hillebr. | C0149 | Oppenheimer H110622; BISH | Hawai'i, Moloka'i | GQ475190 | GQ475103 | GQ475153 |
| *C. cf. mesilauensis* B.L.Burtt | C0140 | Cubey and Scott 229; E | Indonesia, Borneo | GQ475183 | GQ475096 | GQ475146 |
| *C. milnei* Seem. ex A. Gray | C0113 | Clark 687; SEL | Fiji, Viti Levu | EU919986 | EU919925 | GQ475135 |
| *C. munroi* C.N. Forbes | C0104 | Clark 675; SEL | Hawai'i, Maui | EU919983 | EU919922 | GQ475134 |
| *C. munroi* C.N. Forbes | C0151 | Oppenheimer H120638; BISH | Hawai'i, Lana'i | GQ475192 | GQ475105 | GQ475155 |
| *C. nukuhivensis* F.Br. | C0065 | Wood 10428; PTBG | Marquesas, Ua Pou | EU919966 | EU919905 | EU920025 |
| *C. occulta* A.C. Sm. | C0017 | Plunkett 1838; US | Fiji, Viti Levu | EU919933 | EU919872 | EU919992 |
| *C. occulta* A.C. Sm. | C0117 | Clark 694; SEL | Fiji, Viti Levu | EU919989 | EU919928 | GQ475137 |
| *C. cf. occulta* A.C.Smith | C0119 | Clark 702; SEL | Fiji, Viti Levu | EU919990 | EU919929 | GQ475138 |
| *C. ootensis* F.Br. var. *molissima* Fosberg & Sachet | C0061 | Perlman 18399; PTBG | Marquesas, Fatu Hiva | EU919962 | EU919901 | GQ475124 |
| *C. ootensis* F.Br. var. *molissima* Fosberg & Sachet | C0132 | Wood 6563; PTBG | Marquesas, Tahuata | GQ475178 | GQ475091 | GQ475141 |
| *C. ootensis* F.Br. var. *mollissima* Fosberg & Sachet | C0063 | Wood 10266; PTBG | Marquesas, Tahuata | EU919964 | EU919903 | EU920023 |
| *C. ootensis* F.Br. var. *ootensis* F.Br. | C0060 | Wood 10047; PTBG | Marquesas, Hiva Oa | EU919961 | EU919900 | EU920020 |
| *C. pendula* Blume | C0098 | Wiriadinata, H. 12716; US | Indonesia | EU919979 | EU919918 | EU920037 |
| *C. pickeringii* A. Gray | C0134 | Lorence 9528; PTBG | Hawai'i, Kaua'i | GQ475179 | GQ475092 | GQ475142 |
| *C. picta* Blume | C0097 | Wiriadinata, H. 12715; US | Indonesia | EU919978 | EU919917 | EU920036 |
| *C. platyphylla* A. Gray | C0150 | Oppenheimer H100512; BISH | Hawai'i, Maui | GQ475191 | GQ475104 | GQ475154 |
| *C. platyphylla* A. Gray | C0152 | Oppenheimer H80514; BISH | Hawai'i, Maui | GQ475193 | GQ475106 | GQ475156 |
| *C. pogonantha* A. Gray | C0066 | Clark 644; SEL | Samoa, U'polu | GQ475169 | GQ475081 | GQ475125 |
| *C. pogonantha* A. Gray | C0081 | Clark 660; SEL | Samoa, U'polu | GQ475173 | GQ475086 | GQ475129 |
| *C. pogonantha* A. Gray | C0112 | Plunkett 1980; US | Samoa, U'polu | EU919985 | EU919924 | EU920041 |
| *C. cf. pogonantha* A. Gray | C0067 | Clark 645; SEL | Samoa, U'polu | GQ475170 | GQ475082 | GQ475126 |
| *C. procera* Hillebr. | C0148 | Oppenheimer H110621; BISH | Hawai'i, Moloka'i | GQ475189 | GQ475102 | GQ475152 |
| *C. propinqua* C. Forbes | C0039 | Clark 570; SEL | Hawai'i, O'ahu | EU919950 | EU919889 | EU920009 |
| *C. pulchella* Rich ex A. Gray | C0029 | Lorence 8525; PTBG | Samoa, Tau | EU919941 | EU919880 | EU920000 |
| *C. richii* A. Gray | C0072 | Clark 650; SEL | Samoa, Savai'i | EU919969 | GQ475083 | EU920028 |
| *C. richii* A. Gray | C0073 | Clark 651; SEL | Samoa, Savai'i | GQ475171 | GQ475084 | GQ475127 |
| *C. cf. richii* A. Gray | C0068 | Clark 646; SEL | Samoa, U'polu | EU919967 | EU919906 | EU920026 |
| *C. samoensis* A. Gray | C0030 | Lorence 8633; PTBG | Samoa, Ofu | EU919942 | EU919881 | EU920001 |
| *C. samoensis* A. Gray | C0031 | RP 71221; PTBG | Tonga | EU919943 | EU919882 | GQ475122 |
| *C. samoensis* A. Gray | C0142 | Kiehn 940819-1/1; WU | Samoa, U'polu | GQ475185 | GQ475098 | GQ475148 |
| *C. samoensis* A. Gray | C0144 | Kiehn 940819-2/1; WU | Samoa, U'polu | GQ475187 | GQ475100 | GQ475150 |
| *C. sandwicensis* (H. Lév.) H. St. John & Storey | C0045 | Clark 576; SEL | Hawai'i, O'ahu | EU919953 | EU919892 | EU920012 |
| *C. serratifolia* H.J. Atkins & Cronk | C0136 | Cubey and Scott 225; E | Indonesia, Sulawesi | GQ475180 | GQ475093 | GQ475143 |
| *C.* sp. | C0016 | Plunkett 1837; US | Fiji, Viti Levu | EU919932 | EU919871 | GQ475117 |
| *C.* sp. | C0018 | Plunkett 1843; US | Fiji, Viti Levu | EU919934 | EU919873 | GQ475118 |
| *C.* sp. | C0019 | Plunkett 1875; US | Fiji, Viti Levu | EU919935 | EU919874 | GQ475119 |
| *C.* sp. | C0092 | Hoover & Agus ARs 173; US | Indonesia, Java | EU919973 | EU919912 | EU920031 |
| *C.* sp. | C0093 | Hoover & Agus ARs 175; US | Indonesia, Java | EU919974 | EU919913 | EU920032 |
| *C.* sp. | C0095 | Wiriadinata, H. 12709; US | Indonesia, Java | EU919976 | EU919915 | EU920034 |

**Table 1** (continued)

| Species | ID No. | COLLECTOR and No.; voucher | Origin | ITS | ETS | *psbA-trnH* |
|---|---|---|---|---|---|---|
| C. sp. | C0131 | Wood 11057; PTBG | Society Islands, Hua Hine | GQ475177 | GQ475090 | GQ475140 |
| C. sp. | C0139 | Cubey and Scott 228; E | Indonesia, Sulawesi | GQ475182 | GQ475095 | GQ475145 |
| C. spathulata H. St. John | C0102 | Clark 664; SEL | Hawai'i, Maui | EU919981 | EU919920 | EU920038 |
| C. sulcata Blume | C0100 | Hoover & Agus ARs 160; US | Indonesia, Java | EU919980 | EU919919 | GQ475132 |
| C. thibaultii Fosberg & Sachet | C0062 | Meyer 2541; PTBG | Marquesas, Ua Pou | EU919963 | EU919902 | EU920022 |
| C. tintinnabula Rock | C0012 | Perlman 17676; PTBG | Hawai'i, Hawai'i | EU919930 | EU919869 | GQ475114 |
| C. umbellifera Merr. | | | Taiwan, Ponso no Tao | AH006052 | * | * |
| C. urvillei C.B. Clarke | C0034 | Lorence 7838; PTBG | Micronesia, Kosrae | EU919946 | EU919885 | EU920005 |
| C. wagneri Lorence & Perlman | C0014 | Perlman 17675; PTBG | Hawai'i, Hawai'i | GQ475165 | GQ475077 | GQ475115 |
| C. wagneri Lorence & Perlman | C0015 | Lorence 8907; PTBG | Hawai'i, Hawai'i | GQ475166 | GQ475078 | GQ475116 |
| C. wainihaensis H. Lév. | C0021 | Clark 549; SEL | Hawai'i, Kaua'i | EU919937 | EU919876 | EU919996 |
| C. wawrae C.B. Clarke | C0022 | Clark 550; SEL | Hawai'i, Kaua'i | EU919938 | EU919877 | EU919997 |

Specimens organized alphabetically by species. ID numbers are J. R. Clark's DNA extraction numbers and are here used for reference. GenBank accession numbers are included for all taxa for each of the three genic regions analyzed in the current study. *C.* = *Cyrtandra*; *A.* = *Aeschynanthus*. ITS = internal transcribed spacer regions 1 and 2, including the 5.8S subunit; ETS = external transcribed spacer region; *psbA-trnH* = chloroplast sequence data.
*Cyrtandra umbellifera* ITS sequence retrieved from GenBank.
* Unsequenced regions.

gel-dried leaf material was used for total genomic DNA extraction. Purified cycle sequence products were 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 further 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. (2008). 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 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 despite the presence of missing data.

Aligned sequences were analyzed using maximum likelihood (ML) and Bayesian inference (BI) methods. ML analyses were performed using PAUP* 4.0b10 (Swofford, 2002) implementing heuristic searches with TBR branch swapping and initial starting trees 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, sampled every 10,000 generations. The first 20% of trees were excluded as burn-in. Posterior probabilities were compared between two identical runs to insure that stationarity had been 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 was conducted 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 ($\geqslant$70% bootstrap support) among the various topologies were in conflict. The three genic regions were thus combined and analyzed.

### 2.3. Estimation of phylogeny divergence times

We used the r8s program (v. 1.7.1; Sanderson, 2004) to estimate a chronogram for *Cyrtandra* based on the combined ITS-ETS-*psbA-trnH* 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 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 (Clark et al., 2008) including 35 million years before the present (MYBP) for the Indonesian grade/Pacific split, six MYBP for the origin of the Marquesas Islands, and 5.1 MYBP for the Hawaiian Islands.

### 2.4. Ancestral range analysis

We analyzed the chronogram from the SPRS analysis using the dispersal–extinction–cladogenesis (DEC) model (Ree et al., 2005; Ree and Smith, 2008a) as implemented in Lagrange version 2 (Ree and Smith, 2008b). 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., 2008). Polytomies on the chronogram were arbitrarily resolved and minimal branch lengths (10e−4) assigned to new branches using Mesquite 1.12 (Maddison and Maddison, 2006). 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, we restricted ancestral areas to no more than two areas per node. This restriction allows DEC to best approximate the possibility of dispersal-mediated allopatry, a scenario that is considered probable in insular systems. An inverse-scaled distance matrix was constructed to account for distance among areas in the DEC analysis (see Supplementary data for the absolute, scaled, and inverse-scaled distance matrices).

### 2.5. Taxonomic assignments

Morphological assessments of species were made using herbarium specimens, in situ observations, and notes on species characters from the historical literature, where appropriate. We compared the molecular phylogeny to three major classification schemes: (1) For the South Pacific, we consulted the only

treatment for the region (Gillett, 1973). Although Gillett did not propose sectional rankings, he did suggest major species groupings that can be compared to the current phylogenetic hypothesis. (2) Fosberg and Sachet (1981) proposed two major lineages for Marquesan species. (3) The Hawaiian species have been previously divided into six sections (Wagner et al., 1990). Principal characters including inflorescence bract persistence and calyx symmetry and persistence after anthesis have been noted as diagnostic for each of these lineages.

## 3. Results

### 3.1. 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. Uncorrected *p*-distance for the combined dataset was 11% between the two outgroup taxa. Distances ranged from 14% to 23% divergence between outgroup and ingroup taxa, and between 0% and 17% within the ingroup. 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; our 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 found 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 to 55 bp. Based on these two difficult DNA regions, we conducted preliminary analysis on *psbA-trnH* to interpret the effects 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 ⩾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.

### 3.2. Phylogeny

Combined analysis of ITS, ETS and *psbA-trnH* genic regions resulted in one most likely tree (TreeBase accession S2453, M4665; −ln *L* = 9782.6046; Fig. 1). Identical analysis was conducted on ITS + ETS with *psbA-trnH* removed (M4666; −ln *L* = 8046.1170; Fig. 2). These topologies varied little, having virtually identical taxon placement and most resolved relationships exhibiting similar support values (Figs. 1 and 2). Where node support differed, slightly higher values were normally recovered in the complete combined dataset analysis. Node 7 was supported (70% BS and 99% PP) only in the complete combined analysis and node 22 within the Hawaiian clade exhibited 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 (Roalson and Friar, 2004).

Resolution across the complete combined dataset ML phylogeny (Fig. 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.

A grade of southeast Asian clades with a nested, monophyletic Pacific clade, is reconstructed (Fig. 1). The grade includes a clade of Javan, Sulawesian, Taiwanese and Philippines taxa (clade 23; 89% BS; >99% PP) immediately sister to the Pacific clade (93% BS; >99% PP). Our results differ from the relationships inferred using parsimony 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 their study (including an identical sample used in our study, *C. ferruginea*). In our analysis, the Pacific clade and the Javan–Sulawesian–Taiwanese–Philippines clade (clade 3) is sister to a distinct Javan clade (clade 24) that is in turn sister to a Javan–Bornean clade (clade 25).

Several geographic areas are polyphyletic within the Pacific clade (clade 4; Fig. 1) including Samoa with two well-supported clades (clades 9 and 13), the latter having Society Islands, Tongan and Micronesian taxa nested within it (clade 13). Clade 13 is also noteworthy in that *C. samoensis* is paraphyletic; two samples from U'polu Island (Samoa) are sister to the two Micronesian taxa (clade 16) and together this clade is sister to a clade including the Ofu Island (Samoa) and Tongan *C. samoensis* samples (clade 17). Fiji is also paraphyletic with two separate lineages represented (clades 12 and 18). 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 21) and an O'ahu clade (clade 22). A third clade exists (clade 20) that is not supported and is made up of taxa from principally the island of Hawai'i and the Maui Nui complex.

### 3.3. Divergence times and ancestral range inheritance

The root age of the chronogram is estimated at 48 MYBP (Fig. 3). This does not statistically differ from the root age estimate of 42 MYBP in the previous study (Clark et al., 2008). 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 9 (11.6 ± 4.5 MYBP), Samoan clade 13 (12.0 ± 4.3 MYBP), Fijian clade 12 (11.4 ± 4.3 MYBP) and Fijian clade 18 (8.9 ± 4.4 MYBP). Node 4 (the Pacific clade) is a polytomy of Hawai'i (clade 20), the South Pacific (clade 5) and the Fiji clade 18, all of which are estimated to have diverged 21.7 ± 4.7 MYBP. Divergence event confidence intervals in more terminal clades overlap substantially owing to short branches in the phylogeny and some degree of phylogenetic uncertainty.

### 3.4. DEC ancestral range analysis

Ancestral range reconstructions in the DEC analysis are consistent with inferences from previous analyses (Clark et al., 2008). For node 2, the area is inferred to be Java–Fiji, Java or Java–Samoa (Table 2; Fig. 4). For daughter lineages node 3 and node 24, both are reconstructed as Java, although not exclusively at node 3. The split between southeast Asia and the Pacific occurs at node 3; the daughter lineage node 23 is exclusively reconstructed also as Java and later spawns Sulawesian, Taiwanese and Philippine lineages. The other sister lineage, node 4, the Pacific lineage, is not conclusively reconstructed and is inferred to be either Fiji, Java, Samoa or Java–Fiji.

Within the Pacific, the Hawaiian clade (node 19) is inferred to have originated by a divergence event within Fiji or a broader Fiji–Samoa lineage followed by dispersal to and divergence within Hawai'i. Likewise, the South Pacific lineage originates in Fiji or Fiji–Samoa (node 5) and persists for some time as a greater Fiji and/or Samoa lineage (node 6 and node 7). Major divergences originate from this Fiji–Samoa lineage including the Marquesas (node 8), a

Q = Marquesas
S = Samoa
F = Fiji
I = Society Islands
M = Micronesia
O = Tonga
H = Hawaii
J = Java
U = Sulawesi
P = Philippines
T = Taiwan
B = Borneo



—— 0.005 substitutions/site
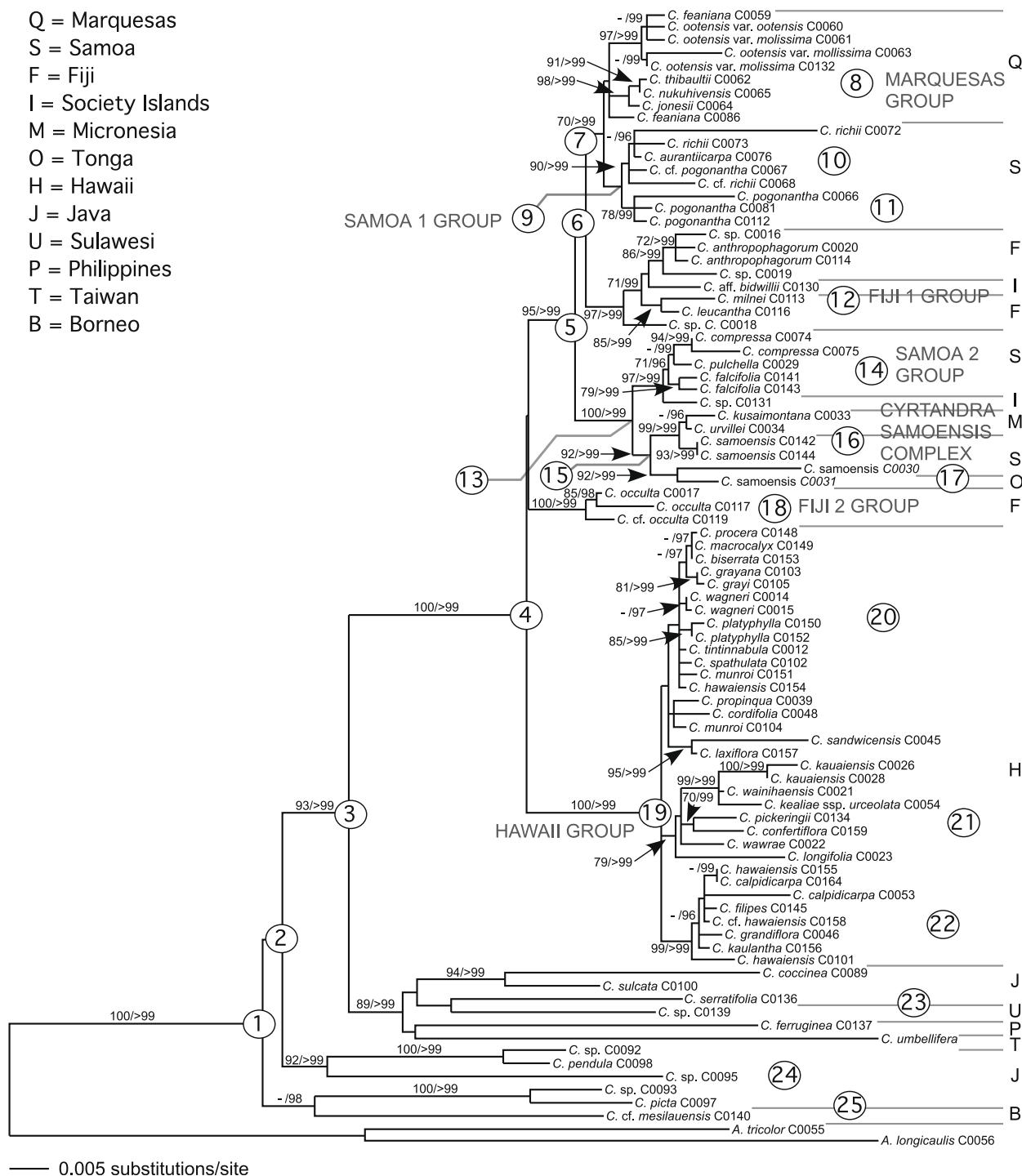
**Fig. 1.** Maximum likelihood phylogram (−ln L = 9782.6046; single ML tree). Analysis of ITS, ETS and *psbA-trnH* regions; K80 + G substitution model; letters to the right indicate geographic regions; numbers along branches indicate branch support (bootstrap support ⩾70%/Bayesian posterior probabilities ⩾95%); circled numbers by selected nodes (1–25), and SMALL CAPITALS, are for reference in the text.

Samoan lineage (node 9), and one Fijian lineage (node 12). The other major Samoan lineage (node 13) is reconstructed as independently derived from Fiji or Fiji–Samoa and includes Society Islands, Tongan and Micronesian lineages.

### 3.5. Recognizing major groupings

Using the resolved clades in our phylogeny, terminal groups can be compared based on morphological characteristics common

within each. We 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 (Fig. 1; in the text that follows, SMALL CAPITALS are used to indicate when a morphological group is being discussed; "clades" referenced here are the same as the "nodes" referenced in the previous section). These include a South Pacific clade consisting of six major groups: (1) MARQUESAS (clade 8), (2) SAMOA 1 (clade 9), (3) FIJI 1 (clade 12), (4) SAMOA 2 (clade

Q = Marquesas
S = Samoa
F = Fiji
I = Society Islands
M = Micronesia
O = Tonga
H = Hawaii
J = Java
U = Sulawesi
P = Philippines
T = Taiwan
B = Borneo
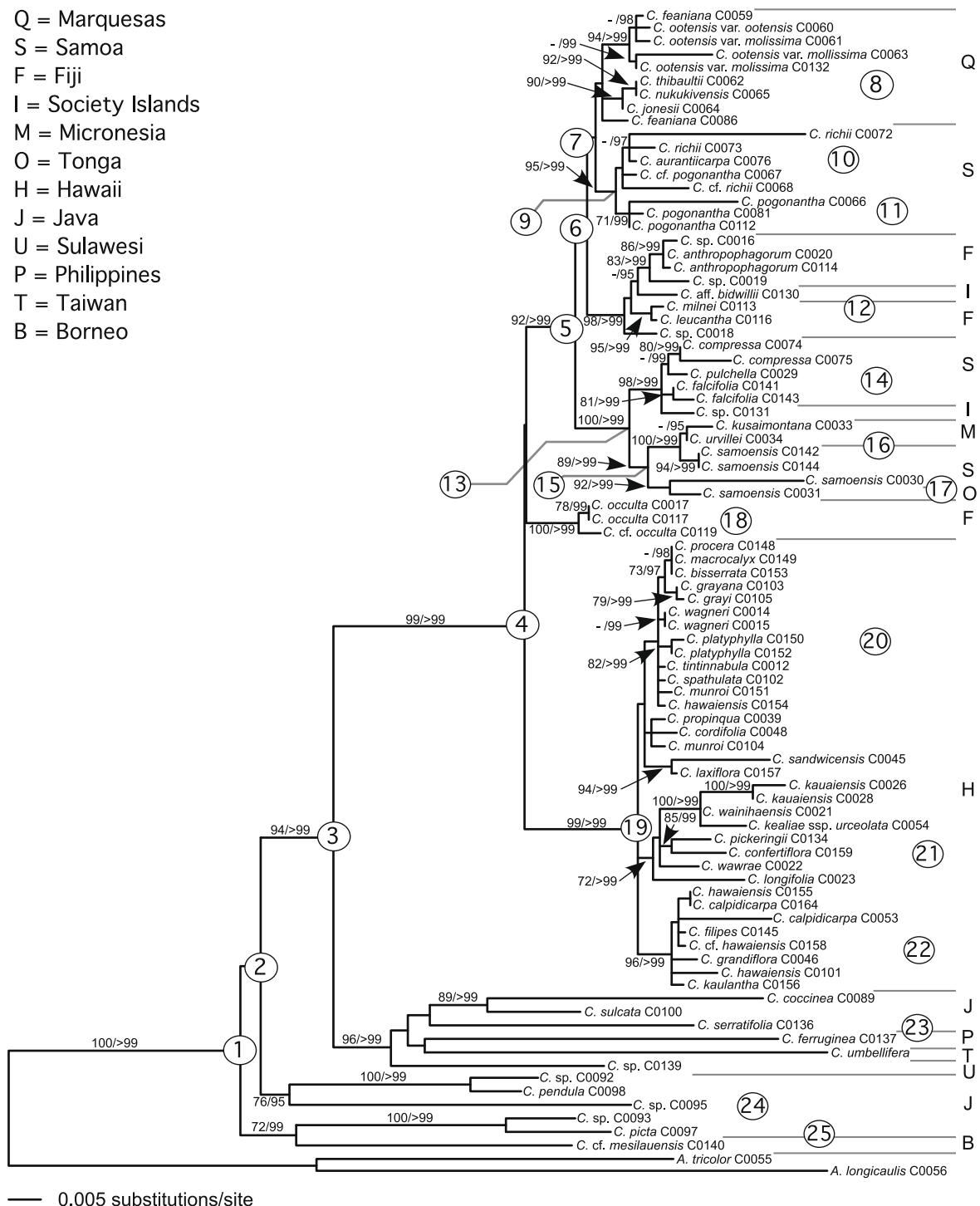


**Fig. 2.** Maximum likelihood phylogram (−ln L = 8046.1170; single ML tree); analysis of ITS and ETS; K80 + G substitution model; symbols like those in Fig. 1.

14), (5) CYRTANDRA SAMOENSIS COMPLEX (clade 15), and (6) FIJI 2 (clade 19), and a seventh distinct group, the HAWAII group (clade 20). The western 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 partially 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-shrub, fleshy-stemmed habit with universally white, fleshy fruit unify the MARQUESAS group. Two subgroups within this group are clearly delineated by calyx characters: one including *C. feaniana* and *C. ootensis* and varieties, has calyces divided nearly to the base; the other includes *C. thibaultii*, *C. nukuhivensis*, and *C. jonesii* and is distinguished in having calyces divided ¾ the way to the base. The sister lineage to the MARQUESAS group, SAMOA 1 group, is differentiated in having a markedly woody shrub or small tree habit and distinctive orange fruit. SAMOA 1 group species are further segregated in having either calyces divided asymmetrically, usually into an upper and lower beak, and inflorescence bracts deciduous (in *C. ri-*
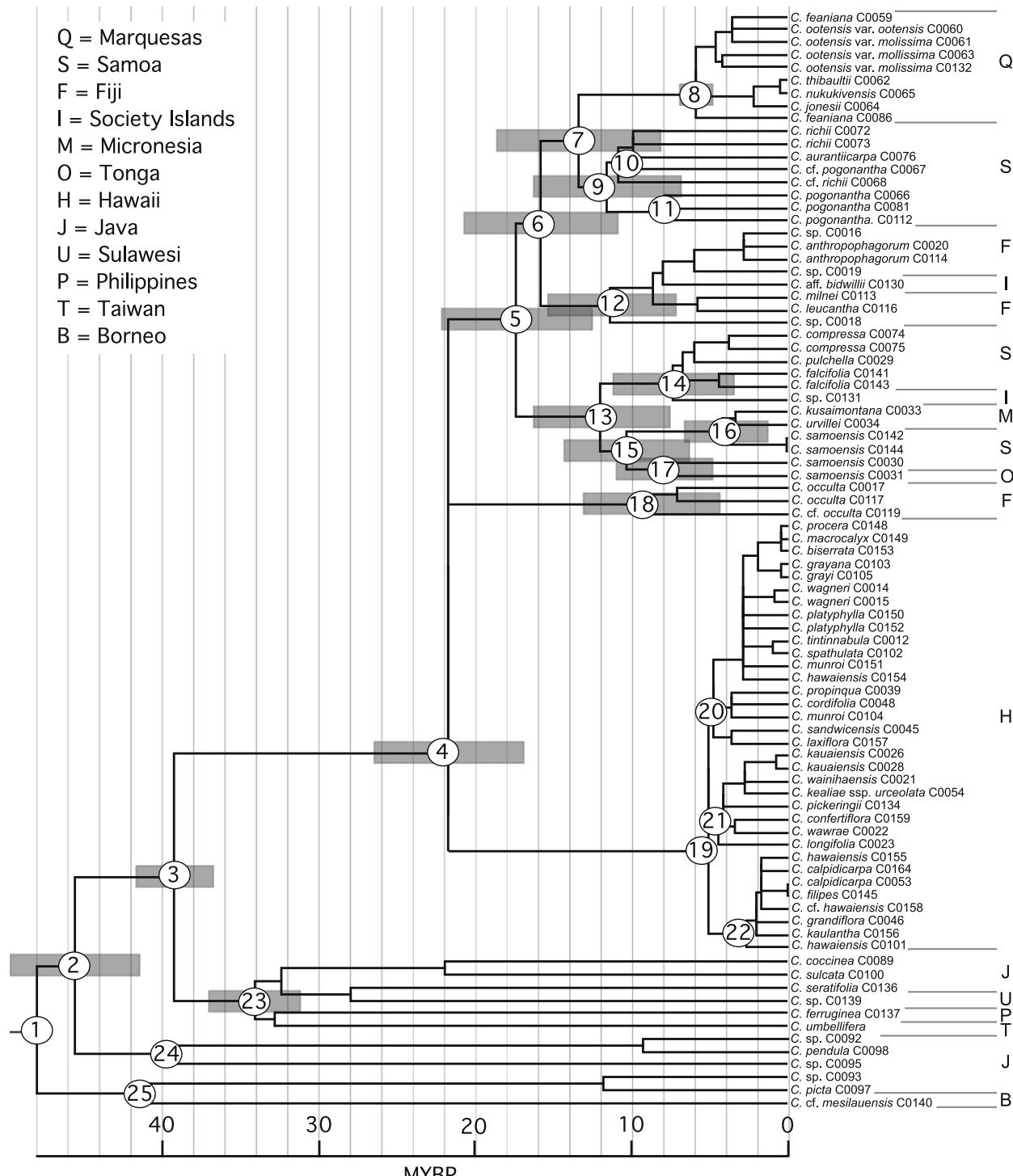
**Fig. 3.** Chronogram based on penalized likelihood analysis of the ML tree calibrated using island ages referred to in the text; numbers at the bottom are ages in millions of years before the present (MYBP); gray bars indicate standard deviations around selected nodes; numbers by selected nodes (1–25) are for reference and are referred to in the text.

*chii* and related species) or with five distinct lobes and a fused involucre bract forming a capitate inflorescence (in *C. pogonantha*).

The clade immediately sister to the combined MARQUESAS group and SAMOA 1 group is the FIJI 1 group. Among species in this group, *C. anthropophagorum* is characterized in having a somewhat woody shrub habit, deciduous inflorescence bracts, and symmetrical calyces with lobes as long as the tube. *Cyrtandra bidwillii* from the Society Islands shares similar characteristics with *C. anthropophagorum* including deciduous inflorescence bracts and symmetrical or

nearly symmetrical calyces, although corollas are noticeably larger in *C. bidwillii*. *Cyrtandra milnei* and *C. leucantha* are included in this group but differ morphologically from the aforementioned species in having a capitate inflorescence and markedly pilose or lanate leaves and young stems. *Cyrtandra milnei* and *C. leucantha* are also less woody than others in the FIJI 1 group.

The next major clade includes two groups identified in our rankings, the SAMOA 2 group and the *CYRTANDRA SAMOENSIS COMPLEX*. Species in the SAMOA 2 group are quite distinctive from other species of

**Table 2**
DEC ancestral range reconstructions at numbered nodes of the chronogram.

| Nodes | Areas | ln L | Relative probability |
|---|---|---|---|
| Node 1 [2\|25] | [JF\|J] | −103 | 0.3706 |
| | [J\|BJ] | −103.6 | 0.2052 |
| | [J\|J] | −103.8 | 0.1738 |
| | [JS\|J] | −104.3 | 0.0973 |
| Node 2 [3\|24] | [J\|J] | −102.8 | 0.4353 |
| | [JF\|J] | −103 | 0.3681 |
| | [JS\|J] | −104.4 | 0.0953 |
| Node 3 [4\|23] | [F\|J] | −102.9 | 0.4245 |
| | [J\|J] | −103.6 | 0.2006 |
| | [S\|J] | −104.3 | 0.1047 |
| | [JF\|J] | −104.6 | 0.0745 |
| Node 4 [–\|18] | [F\|F] | −102.5 | 0.6140 |
| | [FS\|F] | −103.5 | 0.2192 |
| Node – [5\|19] | [F\|F] | −102.4 | 0.6767 |
| | [FS\|S] | −104.2 | 0.1120 |
| | [FS\|F] | −104.4 | 0.0945 |
| Node 5 [6\|13] | [FS\|S] | −102.1 | 0.9018 |
| Node 6 [7\|12] | [S\|F] | −102.3 | 0.7631 |
| | [FS\|F] | −104 | 0.1386 |
| Node 7 [8\|9] | [S\|S] | −102.2 | 0.8604 |
| Node 9 [10\|11] | [S\|S] | −102 | 0.9967 |
| Node 13 [14\|15] | [S\|S] | −102.2 | 0.7955 |
| | [SI\|S] | −103.9 | 0.1557 |
| Node 15 [16\|17] | [S\|S] | −102.2 | 0.8612 |
| | [S\|SO] | −104 | 0.1360 |
| Node 19 [–\|22] | [H\|FH] | −102.4 | 0.6894 |
| | [H\|HS] | −103.8 | 0.1715 |
| Node – [20\|21] | [H\|H] | −102.1 | 0.8872 |

The "nodes" column refers to a node and its two daughter lineages as numbered in Fig. 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 25; these are reconstructed as Java–Fiji and Java, respectively (first among three additional likely reconstructions). The "–" are nodes that collapse in the chronogram and have been arbitrarily resolved for DEC analysis (see text). The −ln L and relative probabilities are listed for each likely reconstruction.

*Cyrtandra* in having large (>5 cm) campanulate corollas. Samoa 2 group species also exhibit a markedly woody shrub or small tree habit, similar to species in the Samoa 1 group. All species sampled are from Samoa with the exception of an unidentified specimen from the Society Islands. The *Cyrtandra samoensis* complex includes *C. samoensis*, the closely related *C. urvillei* and *C. kusaimontana,* both from Micronesia. *Cyrtandra samoensis* complex species share a common sub-shrub or shrub habit with fleshy to slightly woody stems. Flowers are born in spreading axillary clusters with numerous flowers; calyx lobes are distinct and have five symmetrical lobes.

The last group we recognize in the South Pacific clade is the Fiji 2 group containing *C. occulta* and another very similar species. This group shares many features with *C. milnei* and *C. leucantha* in the Fiji 1 group but differs in having inflorescence bracts completely fused into a cup-shaped involucre, most similar to *C. pogonantha* in the Samoa 1 group.

The Hawaii group, and clades therein, are more difficult to define morphologically. Hawaiian lineages can be divided into three major subgroups based roughly on geographical areas (Fig. 5): a Kaua'i subgroup (clade 21), an O'ahu subgroup (clade 22), and a broad O'ahu–Maui Nui–Hawai'i subgroup (clade 20). Hawaiian groupings will be addressed further in the discussion.

## 4. Discussion

### 4.1. Phylogeny of Cyrtandra

The phylogenetic hypothesis presented here, using the most comprehensive sampling of Pacific species to date, is in line with previous hypotheses on relationships in *Cyrtandra* (Atkins et al., 2001 [30 taxa; 26 species]; Cronk et al., 2005 [36 taxa; 36 species]; Clark et al., 2008 [61 taxa; 57 species]). Principally, a large, monophyletic Pacific lineage found east of the Philippines and extending throughout the Pacific Islands, is nested within a "western grade" of species from southeast Asia. Most major clades in the phylogeny are well supported by both bootstrap and Bayesian posterior probabilities.

We recovered a far greater stratification in relationships among South Pacific and Hawaiian species than in previous studies. In particular, distinct clades representing major geographic areas throughout the South Pacific are reconstructed with strong support. Of areas that were appreciably sampled, the Marquesas Islands and Hawai'i appear to be monophyletic whereas Fiji and Samoa are polyphyletic and each include at least two separate clades.

One relationship of note that remains unresolved is the placement of the Fijian *C. occulta* clade (clade 18). In the combined dataset analysis, this clade is reconstructed sister to the remaining South Pacific, which 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 18 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 in an attempt to improve the resolution of this relationship (Clark et al., 2008). To date, regions tested have been uninformative at this phylogenetic depth or recalcitrant to characterization.

### 4.2. Divergence dates

The Gesneriaceae is one of the oldest families of the Lamiales (Oxelman et al., 1999; Olmstead et al., 2000), and is estimated to have diverged around 71–74 MYBP (Wikström et al., 2001). Crown group ages are estimated to be nearly as old (71 MYBP; Bremer et al., 2004) with *Cyrtandra* being one of the more recently derived genera in the family (Mayer et al., 2003). Dating of the split between *Cyrtandra* and 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, our results do provide ages for the terminal-most split between Borneo/Java, thought to be some of the oldest 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.* Age estimates here are also in line with parallel diversifications in the New World subfamily Gesnerioideae, tribe Gloxineae (20–30 MYBP), a slightly less species-rich, recently derived lineage in the Gesneriaceae (Roalson et al., 2008).

Not all too unexpectedly, lineage divergence within the Pacific clade corresponds with the geologic origin of Pacific islands. Although the split between the last southeast Asian lineage (the Javan–Sulawesi–Philippines–Taiwan clade) and the South Pacific occurred around 40 MYBP (clade 3), the crown group of Pacific lineages did not diverge until ~22 MYBP. *Cyrtandra* dispersal and diversification in the Pacific apparently did not take place until well after a relatively dense overstory formed on islands such as Fiji (first hypothesized by Gillett (1967)).

### 4.3. Cyrtandra biogeography

Our data indicate that Fiji and surrounding areas have played a central role in the Pacific expansion of *Cyrtandra*. The area has long been recognized as a major biogeographic interface centered between southeast Asian/Malesian and Pacific/Polynesian bioregions (Hedley, 1899; Takhtajan, 1986; Stoddart, 1992). A general pattern of west to east stepping stone dispersal into the Pacific is
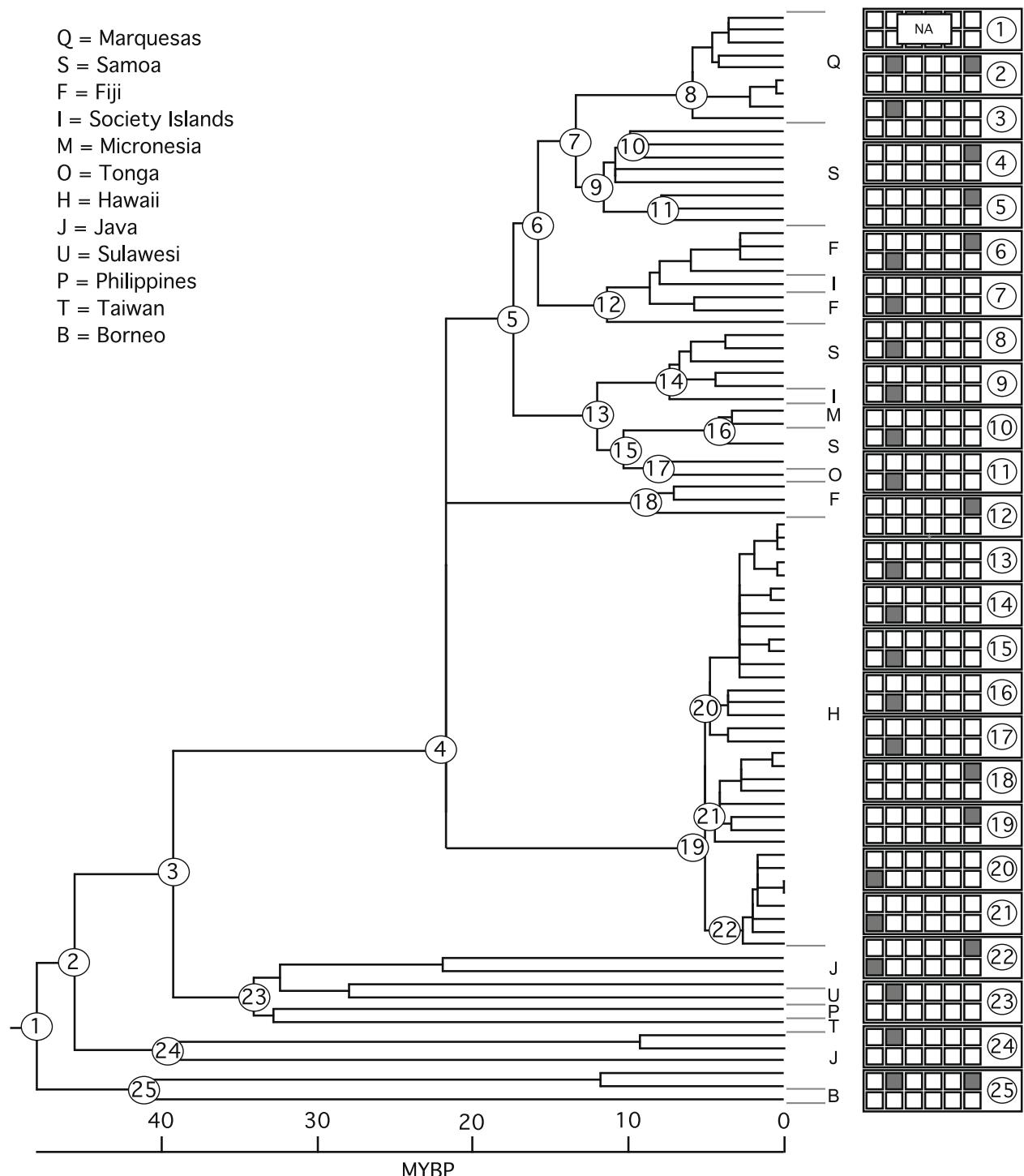
**Fig. 4.** Simplified chronogram with areas inferred using DEC. Labeled nodes refer to taxonomic groups referred to in the Results. Schematics to the right indicate areas inferred at each numbered node, from left to right within the schematic, top = B,J,U,P,T,F; bottom = H,S,O,M,I,Q; NA = node not inferred. In instances where more than one likely reconstruction (within 2 − ln L) exists, only the most likely reconstruction is shown. See Table 2 for the complete set of reconstructions.

seen in many taxa, both plant and animal, from more mainland southeast Asia, through Fiji, and into the Pacific (Stoddart, 1992). Takhtajan (1969, 1986) recognized this area, including Fiji, the Santa Cruz Islands, Vanuatu, Niue, Samoa and Tonga, as the "Fijian Region". For *Cyrtandra*, the Fijian Region appears to have been a staging ground for the genus' Pacific range expansion.

Fiji is a geologically old island chain (∼35 MYBP; Evenhuis and Bickel, 2005) and in close proximity to areas associated with the southeast Asian grade of species of *Cyrtandra* making a Fiji-first hypothesis more plausible. The alternative hypothesis, Hawaii-first, is suspect based on the archipelagos remote locality and relatively recent age of its extant high islands. Geological data indicate that a major gap of several million years existed between the formation of Kauai (5.1 MYBP) and any previous islands that were appreciably above sea level (Price and Clague, 2002). This large window of time where no high islands existed precludes the persistence of upland terrestrial lineages on the Hawaiian Islands during this time.
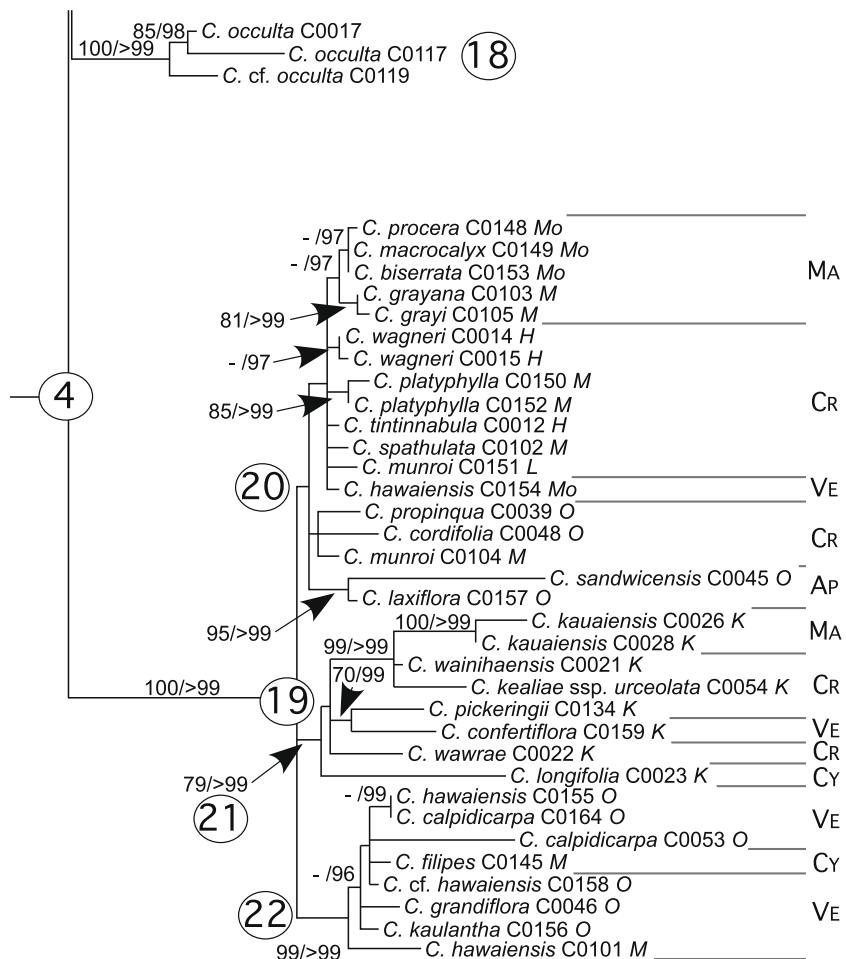
**Fig. 5.** Detail of the Hawaiian clade of the combined maximum likelihood phylogram (Fig. 1). Letters immediately following species names and identification numbers are abbreviations of islands (Mo = Molokai, M = Maui, H = Hawai'i, L = Lana'i, O = O'ahu, K = Kaua'i). Letters in large and small capitals to the right indicate sectional placement based on Wagner et al. (1990; Ma = *Macrosepalae;* Cr = *Crotonocalyces;* Ve = *Verticillatae;* Ap = *Apertae;* Cy = *Cylindrocalyces*).

## 4.4. Biological significance

To our knowledge, *Cyrtandra* represents the first Pacific plant lineage identified as originating in the Fijian Region. Over the course of 20 million years, lineages were dispersed from the Fijian Region to the far-flung corners of the Pacific, including west to Micronesia, northeast to Hawai'i, and east to the Society and Marquesas Islands. Similar Pacific radiations have been proposed for plant lineages originating in other areas (e.g., Australia for *Santalum* L., Harbaugh and Baldwin, 2007; New Zealand for *Metrosideros* subg. *Metrosideros* Banks ex Gaertn., Wright et al., 2000). In *Cyrtandra*, it appears that the whole of Pacific species originated from a Fijian Region center of origin once the barrier into the Pacific was bridged. This supports the idea that a fundamental change in the biology of *Cyrtandra* occurred that allowed for increased vagility in the Pacific in comparison to the more continental-restricted lineages of southeast Asia (Cronk et al., 2005).

## 4.5. Notes on taxonomy and phylogenetic affinities

The two distinct clades within the Marquesas group correspond with differences in calyx structure identified by Fosberg and Sachet (1981). The Marquesas group, being on the periphery of the Pacific range for *Cyrtandra*, has apparently evolved recently and from a single introduction. Morphologically, this group is remarkably dif-

ferent from its sister clade, the Samoa 1 group. Within this latter group, two distinct clades are supported (*C. richii* and *C. pogonantha*) that were not previously hypothesized to share a recent common ancestor.

Among species of the Fiji 1 group, a lone specimen sister to the remaining group, *C.* sp. C0018 from Koro Island, Fiji, along with *C. bidwillii* from the Society Islands and *C. anthropophagorum* from Fiji all have solitary to relatively few flowers born in branching cymes along woody stems or in the axils of leaves. Nested within this clade are the decidedly different *C. milnei* and *C. leucantha* characterized by bracteate, capitate inflorescences. Gillett (1967) placed *C. anthropophagorum* in his "group 3" (1967; note that Gillett's "groups" are not analogous to ours) and *C. milnei* and *C. leucantha* in his "group 1". A.C. Smith in his treatment of *Cyrtandra* for The Flora of Fiji (1991) noted that Gillett's Fiji groups were artificial but were useful in dividing the species for identification.

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. The striking, large campanulate corollas shared by all three species hint at the underlying relationships, however. The Samoa 2 group extends from Samoa east into French Polynesia where other species with unusually large corollas are found. Although not sampled in the present analysis, these species may be of a common evolutionary origin.

The *Cyrtandra samoensis* complex was first recognized by Gillett (1973). He considered *C. samoensis* to be a single species only

tentatively, remarking that a lack of satisfactory characters exist with which to separate this species further. He also noted a close taxonomic affinity between *C. samoensis* and *C. urvillei* from Micronesia (included in this study) as well as several other species across a broad range in the Pacific. Gillett went as far as to say that these species might warrant synonymy. If synonymized, *C. samoensis sensu lato* would represent the greatest ranging single species in the genus *Cyrtandra*. The phylogeny presented here supports the paraphyly of *C. samoensis*, thus potentially warranting synonymy of allied species as Gillett noted. 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 Fɪᴊɪ 2 group containing *C. occulta* shares morphology with a number of separate groups in the Pacific including *C. pogonantha* from Samoa, *C. leucantha* and *C. milnei* from Fiji, and section *Verticillatae* of Hawaiian *Cyrtandra* (Wagner et al., 1990). In particular, the capitate inforescences often accompanied by large, foliate bracts, are partially diagnostic for each of these species. The distribution of this character among a variety of species and clades within the Pacific lineage suggests a possible ancestral condition.

We have sampled five of the six recognized sections of Hawaiian *Cyrtandra* (*sensu* Wagner et al., 1990). Our phylogenetic hypothesis supports three major clades that partially correspond with these sections (Fig. 5). However, supported Hawaiian clades also include species classified in disparate sections. Hybridization between species is one possible explanation of this phenomenon.

### 4.6. 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) 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 several well-supported clades are grouped not by sections and morphological traits but principally by area supports this hypothesis. Clark et al. (2008) noted that hybridization, if occurring in *Cyrtandra*, could be affecting resolution at tip lineages (i.e., at the species level). Hybridization-based hypotheses will need to be examined in more detail through population-level lineage sampling to explicitly test the influences of hybridization and/or other factors leading to common morphologies in disparate lineages in Hawaiʻi and elsewhere.

### 5. Conclusions and future directions

This study has contributed marked resolution to a general understanding of ancestral range evolution and morphological diversification 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, principally Fiji and Samoa, as the staging ground of this lineage, which served as a center of origin for other lineages of *Cyrtandra* in the Pacific. The relationship between Pacific species and more mainland/Malesian species remains largely unresolved, however. Further work is needed both in the Fijian Region (including the Solomon Islands, Fiji, Samoa and Vanuatu) to collect more *Cyrtandra* from this diverse area, and also from the under-sampled Borneo, Papua New Guinea, and Philippines regions. These areas hold hundreds of species, some of which may represent intermediary lineages between the Malesian and Pacific groups. Additionally, the center of origin hypothesis and the apparent shift in vagility of Pacific species needs to be explored in detail. Methods are currently being developed (e.g., Ree, 2005) that may be useful in detecting the evolution of potential key innovations in *Cyrtandra* that may have allowed Pacific species in this genus to disperse throughout the Pacific.

Distinct morphological lineages can be recognized in *Cyrtandra* using a phylogenetic-based approach. Whereas some clades such as the Mᴀʀǫᴜᴇsᴀs group were predicted through morphology alone, other clades including the Fɪᴊɪ 1 group and the Cʏʀᴛᴀɴᴅʀᴀ sᴀᴍᴏᴇɴsɪs ᴄᴏᴍᴘʟᴇx have nested within them unexpected, distinct clades. Ultimately, monophyletic clades identified in this study will have the greatest utility in species-level and population-level studies. These novel monophyletic groupings will provide context to explore additional characters, chiefly in habit, vegetative morphology, and microcharacters. Far more detailed analyses of morphological traits are needed, both in using herbarium material and also through in situ observations.

Hawaiʻi is the best known of all areas defined in this study and represents an excellent proving ground for application of molecular systematics approaches to evaluating taxonomic groupings. Population-level markers and analyses are needed to better understand population dynamics including hybridization and its influence on the evolution of these lineages. In addition, a revised synopsis 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 taxonomic knowledge in Hawaiian *Cyrtandra* and may provide a model for future study and revisions within this genus.

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