Phylogenetic and functional trait-based community assembly within Pacific *Cyrtandra* (Gesneriaceae): evidence for clustering at multiple spatial scales

Melissa Johnson (mjohnso8@hawaii.edu)
USDA-ARS DKIUSPBARC https://orcid.org/0000-0003-1275-5374

Research Article

Keywords: Environmental filtering, functional traits, islands, neutral processes, niche partitioning

Posted Date: November 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1017906/v1

Abstract

Tropical rainforest communities are often characterized by a small number of species-rich genera that contribute disproportionately to the alpha diversity in these habitats. In the Pacific Basin there are nearly 200 species of *Cyrtandra*, most of which are white-flowered woody shrubs that are single-island endemics. Within these island communities, multiple *Cyrtandra* species are commonly observed to occur sympatrically in wet forest understories, forming swarms of what appear to be ecologically similar taxa. The aim of this study was to determine if communities of these plants are randomly assembled with respect to phylogenetic relatedness and traits that are ecologically relevant. Using a combination of ten functional traits and a well-resolved species phylogeny, I examined community assembly within 34 species of *Cyrtandra* across three Pacific archipelagoes. Coexisting species were generally found to be more closely related and more phenotypically similar than would be expected by chance. This pattern was observed at both broad (island communities) and fine (site communities) spatial scales. The retention of phylogenetic signal in floral traits and the strong influence of these traits on the observed degree of phylogenetic clustering may indicate that pollinators act as a biotic filter for closely related species of *Cyrtandra*. In contrast, the absence of phylogenetic signal in most leaf traits, coupled with the lower contribution of these traits to niche clustering, suggests that environmental filtering along this trait axis is minimal in the observed communities. This study supports the theory that plant communities are not randomly assembled, and instead, that niche-based processes structure biodiversity at broad and fine spatial scales in diverse congeneric species assemblages.

Introduction

A fundamental question in ecology and evolutionary biology is how particular species come together to form communities. Three major processes have been put forth to explain the structuring of species assemblages. Under the process of competition, species with shared niches are expected to compete for resources, such that one species will eventually exclude the other, or selective pressure will result in character displacement. A pattern of even spacing or over-dispersion in traits among coexisting species is often interpreted as evidence for competition's role in community assembly (Schluter 2000; Dayan & Simberloff 2005). In contrast, the abiotic environment can create a filter, such that species with similar ecological requirements are found in comparable environments (Weiher & Keddy 1999; Cornwell et al. 2006). Species may be ecologically similar due to shared evolutionary history, or the independent evolution of similar traits (i.e., trait convergence). These two processes thus result in opposing patterns, with competition resulting in niche partitioning and environmental filtering resulting in niche clustering. A third explanation for community assembly patterns involves neutral processes, under which species abundances result from a combination of dispersal, speciation, and stochastic variation in birth and death rates (i.e., ecological drift). Under neutral theory, the presence or absence of a given species in a community is dependent on chance events, not on the ability of the species to compete (Hubbell 2001). However, neutral processes can be difficult to detect as a combination of biotic interactions and environmental filtering may produce seemingly stochastic, or neutral, patterns (Purves & Pacala 2005).

Ultimately, patterns of species coexistence may depend on how environmental filtering, competition, and neutral processes interact over ecological and evolutionary time-scales (Webb et al. 2002).

Recent studies suggest that a combination of ecologically-relevant trait data and an understanding of the phylogenetic history of species may provide the most satisfactory approach for elucidating patterns of community assembly (Kraft et al. 2007; Kraft and Ackerly 2010; Sedio et al. 2012). Most studies that have employed these methods have focused on describing community assembly patterns within particularly diverse tropical forest regions such as the Amazon (Kraft et al. 2008; Lebrija-Trejos et al. 2010; Baraloto et al. 2012). While such studies have greatly advanced our understanding of community assembly patterns and processes, the high taxonomic diversity at this scale can rarely be assessed below the family or genus level given present day gaps in taxonomic knowledge. Furthermore, with few genic regions sequenced across many plant families and/or genera, phylogenetic relationships at this scale are often poorly resolved. This combination of issues has resulted in some uncertainty regarding the inferred patterns of community assembly in plants.

Fewer studies have sought to understand community assembly patterns within species-rich plant genera that host multiple sympatric species across their distributional range (but see *Banksia sensu* Merwin et al. 2012; *Psychotria sensu* Sedio et al. 2012; *Piper sensu* Salazar et al. 2016; *Inga sensu* Dexter et al. 2017). Such studies stand to provide unique insight into the problem of community assembly, in that congeneric species have had a relatively short period of time in which to diverge from their close relatives, compared to species belonging to different families or genera. Congeneric species may be able to coexist in each community by diverging in environmental niche preference, or by partitioning niche space via divergence in key functional traits. However, studies that have examined community structure within a single genus often suffer from the same limitations as large-scale studies of community assembly, with weakly supported phylogenies constructed from one or two genic regions (as in Merwin et al. 2012; Sedio et al. 2012; Salazar et al. 2016), and/or data for only one or a few functional traits (as in Merwin et al. 2012; Salazar et al. 2016).

Lastly, the traits selected to examine patterns of plant community assembly have traditionally been vegetative features that have some bearing on the physiological functioning of plants. In contrast, few studies have examined traits that are indirectly involved in competition for biotic resources (but see Sargent & Ackerly 2008; Alcantara et al. 2014; Wolowski et al. 2016). Given that pollinators are a significant evolutionary force in the diversification of flowering plants (Kay et al. 2005; Kay & Sargent 2009), the inclusion of floral traits in studies of plant community assembly is warranted.

In the present study, the influence of whole plant, vegetative, and floral functional traits on community assembly patterns within Pacific *Cyrtandra* was examined using a well-resolved species phylogeny. The genus *Cyrtandra* J.R. & G. Forster (Gesneriaceae) is a diverse group of plants, with ca. 800 species distributed across Southeast Asia and the Pacific (Atkins et al. 2013). Centers of diversity for the group include Borneo (ca. 200 spp.), the Philippines (ca. 150 spp.), and New Guinea (ca. 120 spp.). The remote volcanic islands of the Pacific are also exceptionally species-rich, with ca. 175 *Cyrtandra* species

distributed across an area that extends from the Solomon Islands, east to the Marquesas Islands, and north to the Hawaiian Islands (Atkins et al. 2013). Plants of species of *Cyrtandra* are morphologically diverse in habit (small trees, shrubs, or vines), flower color (pink, red, purple, yellow, green, white), and fruit type (indehiscent capsule or fleshy berry). However, plants of Pacific species of *Cyrtandra* are predominantly small trees or shrubs with white flowers and white fleshy fruits. Species are restricted to the understory of montane to lowland rainforests and occasionally mesic valleys, although several species occur near sea level.

Pacific *Cyrtandra* is an ideal study system for understanding patterns of species coexistence within a single genus, as anywhere from 2–11 sympatric species have been observed to occur within a single community (Gillett 1967; Wagner et al. 1990; M. Johnson, pers. obs.). The aims of this study were to address the following questions: 1) Are communities of Pacific *Cyrtandra* randomly assembled with respect to species relatedness? 2) Is there evidence of over-dispersion or clustering with respect to ecologically relevant traits? 3) Do trait-based patterns of community assembly reflect evolutionary relatedness?

Materials And Methods

Study Species And Sites

During the months of June to August 2013–2016, plants were sampled in their native habitat across three Pacific Island archipelagos that host high numbers of endemic *Cyrtandra* species: Fiji (42 spp.), Samoa (19 spp.), and the Hawaiian Islands (60 spp.). Islands within archipelagos were selected to encompass a range of substrate ages, land area, and isolation from source areas (Table S1). Sites within islands were selected to encompass the range of habitats (based on differences in elevation, climate, and vegetation type) occupied by Pacific *Cyrtandra* species, as well as a range of numbers of sympatric species (Table 1). Given the remote location and limited accessibility of many sites, it was not possible to set up permanent plots in which to explicitly test coexistence at varying spatial scales. Instead, plants were sampled along belt-like transects that extended between 0.3–0.9 km in length and 2 m in width. Transects followed trails through the forest understory (either pre-established or established during this study) or topographic features such as creeks, valleys, or ridgelines. GPS points were taken along each transect, and site localities were classified into specific habitat types according to those described in Mueller-Dombois and Fosberg (1998).

Table 1

Site information for sampled communities of *Cyrtandra* in the Pacific, listed by increasing elevation. Species in bold were collected from the specified site for the present study; species in black were included in analyses for the specified site due to previous observations of the species at the site; species in grey were previously observed for that site but were not included in the present study due to a lack of phylogenetic and/or functional trait data.

Study site	Island (Country)	Elevation (m)	Habitat type	Species
Lavena	Taveuni (FJ)	20–33	Lowland rainforest	C. gregoryi, C. tempestii
Komave	Viti Levu (FJ)	50–55	Lowland rainforest	C. involucrata, C. aloisiana
Waisali Village	Vanua Levu (FJ)	110–130	Lowland rainforest	C. longifructosa
Navua River	Viti Levu (FJ)	125–130	Lowland rainforest	C. milnei, C. vitiensis, C. hornei, *C. anthropophagorum, C. trichophylla*
Colo-i-Suva	Viti Levu (FJ)	150–185	Lowland rainforest	C. cephalophora, C. esothrix, C. vitiensis, C. pritchardii, C. tomentosa, *C. anthropophagorum, C. milnei*
Nambukelevu	Viti Levu (FJ)	165–180	Lowland rainforest	C. involucrata, C. leucantha, *C. muskarimba*
Mt. Korobaba	Viti Levu (FJ)	240–260	Lowland rainforest	C. esothrix, C. milnei, C. pritchardii, *C. tomentosa, C. anthropophagorum, C. vitiensis, C. cephalophora, C. trichophylla*
Mt. Voma	Viti Levu (FJ)	270–290	Lowland rainforest	C. anthropophagorum, C. vitiensis, *C. coleoides, C. milnei, C. hornei, C. multiseptata*
Waisali Forest Reserve	Vanua Levu (FJ)	335–355	Lowland rainforest	C. cephalophora, C. dolichocarpa, C. waisaliensis
Mt. Naitaradamu	Viti Levu (FJ)	400–445	Lowland rainforest	C. anthropophagorum, C. coleoides, C. milnei, C. multiseptata, C. vitiensis, *C. tomentosa, C. esothrix, C. involucrata, C. hornei, C. cephalophora, C. acutangula, C. trichophylla, C. montana*
Taga	Savaii (WS)	470–600	Lowland rainforest	C. pogonantha, C. richii
Mt. Koroyanitu	Viti Levu (FJ)	630–680	Montane mesic forest	C. hornei, *C. milnei, C. involucrata, C. vitiensis*
Matavanu Crater	Savaii (WS)	675–705	Montane rainforest	C. compressa, C. falcifolia, C. richii, *C. pogonantha, C. nudiflora*
A'opo	Savaii (WS)	765–875	Montane rainforest	C. compressa, C. richii, *C. aurantiicarpa, C. nudiflora*

Study site	Island (Country)	Elevation (m)	Habitat type	Species
Waiakea Forest Reserve	Hawaii Island (HI)	960–990	Montane rainforest	C. lysiosepala, C. paludosa, C. platyphylla, *C. giffardii*
Mt. Lomalagi	Viti Levu (FJ)	1020–1090	Cloud forest	C. hornei, C. multiseptata, C. prattii, C. vitiensis, *C. jugalis, C. victoria, C. leucantha*
Des Voeux Peak	Taveuni (FJ)	975–1150	Cloud forest	C. ciliata, C. hispida, C. leucantha, C. tuiwawai
Koke'e State Park	Kauai (HI)	1215–1260	Montane mesic forest	C. kauaiensis, C. longifolia, *C. paludosa*
Mt. Tomanivi	Viti Levu (FJ)	1000–1270	Cloud forest	C. chlorantha, C. victoriae, C. vitiensis, C. jugalis, *C. coleoides, C. esothrix, C. milnei, C. involucrata, C. multiseptata, C. prattii, C. occulta*

Treatments of the genus *Cyrtandra* in Fiji (Gillett 1967), Samoa (Gillett 1973), and the Hawaiian Islands (Wagner et al. 1990) were initially used to assign individuals to species. Identities were later confirmed using a combination of phylogenetic data and morphological comparisons with herbarium specimens. In total, 25 species were sampled from three islands in Fiji (Viti Levu, Vanua Levu, and Taveuni), four species were sampled from one island in Samoa (Savai□i), and five species were sampled from two of the Hawaiian Islands (Kaua□i and Hawai□i Island). Voucher specimens (deposited at RSA, SUVA, and PTBG) and silica-dried leaf material for phylogenetic analyses were collected from multiple individuals per species at each site. When possible, widespread species were sampled from multiple populations across the range of their known distribution, whereas species with smaller ranges were often sampled from a single population.

To determine community membership at each field site, distribution information was first compiled for each sampled species from herbarium specimens and from treatments of *Cyrtandra* in Fiji (Gillett 1967), Samoa (Gillett 1973) and Hawaii (Wagner et al. 1990). For each site that was sampled, I included: 1) all species that were collected from the site during my field surveys for the present study, and 2) all species previously collected at the site (according to herbarium specimens) but that were not seen during my field surveys. For this second group of species, I was able to acquire samples from nearby sites (see Table 1). In this way, I attempted to capture the full suite of species more accurately in each community, some of which may not have been detected during my field surveys since many species are present in low densities.

Functional Traits

For each species, 1–25 individuals were sampled for functional trait measurements depending on how common the species was at each site. A range of whole plant, leaf, and floral traits were selected to capture potential differences in abiotic resource use and biotic interactions that may affect species coexistence (Table 2). Protocols for measuring whole plant and leaf functional traits follow those of Cornelissen et al. (2003). The height (cm) of individual plants was measured from the base to the crown with an extendable meter stick. For leaf traits, two replicate leaves per individual were sampled at the third node from the crown. Leaves at this position were fully mature, yet not senescing. Leaves with herbivore damage were avoided. Petiole length, leaf length, and leaf width (at the widest part of the leaf) were measured in cm. The petiole was then removed from the leaf blade, and the leaves were immediately weighed with a portable scale in the field to acquire fresh leaf mass (g). Digital photographs were taken of each leaf with a ruler for scale to calculate leaf area in ImageJ (Abramoff et al. 2004). Leaf pubescence was also assessed in ImageJ by using the cell counter plug-in to estimate the number of trichomes in a cm^2 on the adaxial leaf surface. Leaves were then dried to a constant mass in a 60° oven to acquire dry leaf weight (g). Specific leaf area (SLA, $m^2\ kg^{-1}$) was calculated by dividing leaf area by fresh leaf mass, and leaf dry matter content (LDMC, $mg\ g^{-1}$) was calculated by dividing dry leaf mass by fresh leaf mass.

Table 2
Description of functional traits, abbreviations, units of measurement, and inferred ecological relevance.

Functional trait	Abbr.	Units	Ecological relevance
Maximum height	Height	cm	Competitive vigor, fecundity, light capture
Specific leaf area	SLA	$m^2\ kg^{-1}$	Photosynthetic rate, leaf lifespan
Leaf dry matter content	LDMC	$mg\ g^{-1}$	Photosynthetic rate, leaf lifespan, growth rate, water balance
Petiole length	Petiole.L	cm	Light capture efficiency
Leaf pubescence	Leaf.Pub	cm^2	Water balance, herbivore protection
Leaf size	Leaf.Sz	cm^2	Leaf cooling, light capture
Corolla tube length	Flwr.L	mm	Pollinator selection
Corolla tube width	Flwr.W	mm	Pollinator selection
Corolla lobe size	Lobe.Sz	mm^2	Pollinator attraction
Flower number	Flwr.No	#	Pollinator attraction

For floral traits, 1–2 flowers were measured per plant depending on the availability of open flowers. Given that *Cyrtandra* species are protandrous, only flowers in the female (ovulate) phase were used for measurements, as flowers attain their maximum size at this time. Calipers were used to measure the

length (mm) and width (at the mouth, in mm) of the corolla tube, as well as the length (mm) and width (at the widest part, in mm) of the posterior corolla lobe. To assess the size of floral displays, the maximum number of flowers per cymose inflorescence was counted. For each trait in which sub-sampling was performed, the replicate measurements for each plant were averaged to get a mean value for each individual. Four species from the island of Viti Levu in Fiji were not reproductive at the time of field surveys (*C. aloisiana*, *C. jugalis*, *C. pritchardii*, and *C. tomentosa*). For these species, values for flower number per inflorescence were taken from Gillett's (1967) taxonomic treatment, and flower length, width, and lobe size were estimated from herbarium specimens.

A principal components analysis (PCA) was conducted to reduce dimensionality of the trait data and evaluate the functional phenotype of each species. The relationship between species and functional traits was examined by fitting traits onto the ordination space using the function *envfit* in the vegan package (Oksanen et al. 2015) for the R statistical environment (R Development Core Team 2014). This multivariate correlation analysis partitions the linear component of each predictor on the final PCA axes.

Phylogeny Reconstruction

The time-calibrated phylogeny of Johnson et al. (2017) was used to infer relationships among Pacific *Cyrtandra* species. This phylogeny includes 109 *Cyrtandra* taxa sampled across three nuclear (ITS, ETS, Cyrt1) and two chloroplast loci (*rpl*32-*trn*L, *psb*A-*trn*H) (see Johnson et al. 2017 for additional details of phylogenetic methods). The ultrametric tree was pruned to include only the 34 species used in the present study using the *drop.tip* function in the R package ape (Paradis et al. 2004). For those species that included representatives from multiple islands, a single representative from the island sampled in this study was selected. Overall, the tree was well resolved with most major clades having support values ≥ 70 BS (maximum likelihood bootstrap support) and ≥ 0.95 PP (Bayesian posterior probability) (Fig. 1).

Phylogenetic Signal

Phylogenetic signal (the tendency for closely related species to resemble one another) was evaluated for each of the functional traits with the *multiphylosignal* function in the R package picante (Kembel et al. 2008), which uses generalized least squares to calculate Blomberg's K statistic (Blomberg et al. 2003). The species mean for each trait was used to calculate phylogenetic signal, except for height, in which the maximum for each species was used. Values of $K < 1$ indicate weak phylogenetic signal and suggest that closely related species are more different from one another than expected by chance (i.e., trait divergence). Values of $K \approx 1$ approach a Brownian motion model of trait evolution, where traits change by small random amounts and at a constant rate through time. Values of $K > 1$ indicate strong phylogenetic signal and suggest that closely related species are more similar than expected by chance (i.e., trait conservatism). Given that picante only tests for significant differences from zero, the *phylosig* function in the R package phytools (Revell 2012) was used to test for significant differences from one by conducting randomization tests.

Phylogenetic Community Structure

Phylogenetic structure was estimated at two spatial scales: 1) island communities (broad spatial scale), and 2) site communities within islands (fine spatial scale). To estimate community phylogenetic structure at both scales, the R package picante (Kembel et al. 2008) was used to calculate mean pairwise distance (MPD) and mean nearest taxon distance (MNTD; Webb 2000). MPD is a measure of tree-wide clustering versus over-dispersion, while MNTD is a measure of clustering versus over-dispersion at the branch tips. Trait statistics were calculated by using species presence-absence data for each community. The ultrametric phylogeny of 34 species was then converted into a distance matrix using the *cophenetic* function in the R stats package, which computes the pairwise distances between tips using branch lengths. Null models were then used to compare both the MPD and the MNTD metrics to expectations under neutral theory (Hubbell 2001). Null communities were generated using the independent swap null algorithm, in which the number of species in each community and the frequency of occurrence of each species across communities were held constant, while the species that coexist in each community were randomized (Gotelli and Entsminger 2003). Incorporating these assumptions into the null model has been shown to be effective in minimizing Type I error (Kembel and Hubbell 2006). Standardized effect sizes (SES) of MPD and MNTD were calculated across 9999 null communities. A negative SES value indicates clustering while a positive value indicates over-dispersion (Webb et al. 2002). The number of communities with negative and positive SES values was counted for both metrics. Statistical significance was determined by estimating P-values based on the proportion of simulated means that were more (clustered) or less (over-dispersed) extreme than the observed means (alpha = 0.05). Phylogenetic beta diversity (i.e., inter-community structure) between islands was also calculated using the MNTD metric with the *comdisnt* function in picante (Kembel et al. 2008).

Trait-based Community Structure

As with phylogenetic structure, community trait structure was estimated for islands (broad spatial scale) and sites within islands (fine spatial scale). The functional trait structure of both community types was also assessed with the MPD and MNTD metrics, with trait distance replacing phylogenetic distance. Analyses were conducted on both individual traits and on all 10 traits combined, using the mean value (or maximum for height) of each trait. Mean trait values were log10-transformed as needed and scaled to improve normality. Trait statistics were calculated by using species presence-absence data for each community. The SES of MPD and MNTD were calculated across 9999 null communities using the independent swap algorithm. Clustered and over-dispersed communities were determined as for phylogenetic community structure. Inter-island community trait structure was also calculated using the MNTD metric as above.

Results

Study Sites And Species

A total of 276 individuals were sampled from 34 species of *Cyrtandra* across 19 sites on six islands (Table 1). Sampled elevations ranged from 20–1270 m, and included four habitat types: lowland rainforest, montane rainforest, montane mesic forest, and cloud forest (*sensu* Mueller-Dombois & Fosberg 1998). The mean number of observed *Cyrtandra* species per site was 3 (min = 1; max = 5), and the mean number of individuals sampled per site was 15 (min = 3; max = 48).

Functional Traits

Across all 34 species examined, maximum plant height ranged from sprawling shrubs as low as 115 cm (e.g., *C. hispida*) to small trees as tall as 6 m (e.g., *C. richii*) (Table S2). Mean values for leaf traits varied across all species as follows (abbreviations as in Table 2): SLA 14–41 m^2 kg^{-1}, LDMC 79–224 mg g^{-1}, petiole length 2–14 cm, leaf size 29–776 cm^2, leaf pubescence 0–362 trichomes per cm^2. Mean values for floral traits varied across all species as follows: flower length 15–46 mm, flower width 5–14 mm, corolla lobe size 22–570 mm^2, and floral display size 1–15 flowers (Table S2). Within species, most traits exhibited low levels of variation (Table S2, Fig. 2A), although high intra-specific variation was observed in height and leaf size (Table S2). The PCA explained 71% of the variation across the first four axes. The first PCA axis was largely defined by corolla tube length and width followed by SLA and leaf size (26% of the variation; Fig. 2B, Table 3), the second PCA axis was defined by flower number, LDMC, leaf size and leaf pubescence (19% of the variation; Fig. 2B, Table 3), the third PCA axis was defined by petiole length, corolla lobe size, flower number and SLA (15% of the variation; Table 3), and the fourth PCA axis was defined by height, LDMC and leaf pubescence (11% of the variation; Table 3).

Table 3. Principal components analysis (PCA) loadings for 10 functional traits across the first four axes (71% of variation). The strongest contributors to each principal component are in bold (based on a cutoff of √(1/10)= +/- 0.316).

Functional trait	PC1	PC2	PC3	PC4
Corolla tube length	**0.511**	–	0.259	–
Corolla tube width	**0.412**	**-0.374**	-0.169	-0.249
Leaf size	**0.325**	**0.400**	-0.261	-0.120
Specific leaf area	**0.325**	0.259	**0.321**	0.161
Flower number	0.265	**0.432**	**-0.339**	–
Leaf dry matter content	-0.214	**-0.416**	-0.123	**-0.464**
Leaf pubescence	0.307	**-0.372**	-0.274	**0.355**
Petiole length	–	-0.210	**-0.528**	0.224
Corolla lobe size	0.247	-0.283	**0.499**	–
Maximum height	0.281	–	–	**-0.744**

Phylogenetic Signal

Estimates of phylogenetic signal in the ten functional traits resulted in a range of *K* values, from 0.34 for petiole length to 0.89 for flower width (Table 4). Five traits (SLA, LDMC, petiole length, leaf pubescence, and maximum height) had *K* values that were significantly different from one, suggesting absence of phylogenetic signal in these traits. In contrast, four traits (leaf size, flower length, flower width, and floral display) had *K* values significantly different from zero, indicating the presence of phylogenetic signal. Corolla lobe size was not significantly different from one or zero, although there was a trend towards this trait exhibiting phylogenetic signal ($K = 0.58$, $P = 0.09$).

Table 4
Estimates of phylogenetic signal in functional traits using Blomberg's *K* (Blomberg et al. 2003). Values of $K < 1$ indicate trait divergence, $K \approx 1$ indicates random trait evolution (Brownian motion), and values of $K > 1$ indicate trait conservatism. Significant differences from the null expectation are indicated by the following: '*' $p < 0.05$, '**' $p < 0.01$; NS indicates a result not significantly different from the null expectation.

Trait	Blomberg's *K*	Different from 0	Different from 1
Maximum height	0.45	0.233 NS	0.037 *
SLA	0.50	0.150 NS	0.046 *
LDMC	0.40	0.285 NS	0.013 *
Petiole length	0.34	0.682 NS	0.008 **
Leaf pubescence	0.36	0.603 NS	0.006 **
Leaf size	0.67	0.003 **	0.292 NS
Corolla tube length	0.63	0.003 **	0.217 NS
Corolla tube width	0.89	0.025 *	0.763 NS
Corolla lobe size	0.58	0.089 NS	0.148 NS
Flower number	0.63	0.006 **	0.236 NS

Phylogenetic Community Structure

Cyrtandra communities on all six islands were phylogenetically clustered; four were significantly more clustered than null communities under the MPD metric (Viti Levu, Vanua Levu, Kaua'i, and Hawai'i Island; mean $Z_{score} = -2.75$, $P = 0.007$), and/or the MNTD metric (Viti Levu, Vanua Levu, Savai□i, and Hawai□i Island; mean $Z_{score} = -2.53$, $P = 0.02$) (Table S3). The Fijian Island of Taveuni was the only island that did not exhibit significant clustering as estimated by both metrics (MPD: $Z_{score} = -1.22$, $P = 0.14$; MNTD: $Z_{score} = -1.11$, $P = 0.14$). Inter-island community structure inferred from the MNTD metric revealed several

patterns (Table S4). The smallest phylogenetic distances were between island communities within the same archipelago. Within the Fijian archipelago, Viti Levu and Vanua Levu were most similar to Taveuni (geologically the youngest, and also the smallest island of the three), while Taveuni was most similar to the neighboring island of Vanua Levu. Between archipelagos, phylogenetic distances were smallest between island communities that were separated by the shortest geographic distance (e.g., Savai□i, Samoa was most similar to Taveuni, Fiji; Kaua□i and Hawai□i Island were most similar to Viti Levu, Fiji).

Within islands, a total of 18 sites were examined for phylogenetic structure, with one site from Vanua Levu omitted due to the presence of only a single species at the site (*C. longifructosa*). Of the 18 sites, 15 were clustered and three were over-dispersed (Table S5). Only four sites were significantly clustered relative to null communities under the MPD metric (Komave, Waisali, Voma, and Koke□e; mean Z_{score} = −1.67, P = 0.03), while all other sites were not significantly different from the null expectation (mean Z_{score} = −0.74, P = 0.27). Similar results were obtained under the MNTD metric, with 16 clustered sites and two over-dispersed sites. Four communities were significantly clustered (Komave, Waisali, Matavanu, and Tomanivi; mean Z_{score} = −1.75, P = 0.03), while all others did not vary from the null expectation (mean Z_{score} = −0.79, P = 0.25).

Trait-based Community Structure

Analyses of island community structure based on the combination of all ten functional traits revealed that five islands were phenotypically clustered and one was over-dispersed (Hawai□i Island), although none of the communities were significantly different from the null expectation under the MPD metric (mean Z_{score} = −0.54, P = 0.32) or the MNTD metric (mean Z_{score} = −0.57, P = 0.31) (Table S6; Fig. 3). Island community structure based on individual traits suggested that four islands were significantly clustered for one or two traits under the MPD metric (Viti Levu, Vanua Levu, Savai□i, Kaua□i). Clustered traits included maximum height, SLA, flower length, corolla lobe size, and flower number. In contrast, two islands were not significantly different from null communities in any of the traits examined (Taveuni and Hawai□i Island, the youngest islands in each respective archipelago). Under the MNTD metric, two islands were significantly clustered for maximum height (Vanua Levu) or flower length (Kaua□i), while all others did not differ significantly from the null. Inter-island community structure based on functional trait distances corroborated the patterns seen in the phylogenetic inter-community analysis, with the single exception of Taveuni communities being most phenotypically similar to Viti Levu communities as opposed to Vanua Levu in the phylogenetic analysis (Table S7).

The community structure of sites within islands based on all functional traits combined revealed ten clustered sites and eight over-dispersed sites under the MPD metric (Table S8). Only three sites exhibited significant clustering (Colo-i-Suva, Korobaba, and Lavena; mean Z_{score} = −2.09, P = 0.03), while all other sites did not differ from null communities (MPD mean Z_{score} = 0.08, P = 0.52). Similar results were seen using the MNTD metric, with ten clustered sites and eight over-dispersed sites. Only one site was significantly clustered (Lavena, Z_{score} = −2.51, P = 0.03), while all other sites did not vary from the null

(MNTD mean Z_{score} = −0.08, P = 0.48). Analyses based on individual traits revealed six communities that were significantly clustered under the MPD metric (Komave, Waisali, Des Voeux, A□opo, Matavanu, and Koke□e), while all other sites did not differ significantly from null communities. The MNTD metric revealed similar results, with six communities being significantly clustered (Komave, Korayanitu, Waisali, Des Voeux, A□opo, and Koke□e), and all others not differing from the null expectation. Traits that exhibited significant clustering under one or both metrics included maximum height, SLA, LDMC, petiole length, flower length, and floral display.

Discussion

The present study examined patterns of community assembly within Pacific Island *Cyrtandra* across two spatial scales. Specifically, this study sought to determine whether communities of these plants, which are important components of the wet forest understory across islands of the Pacific Basin, are randomly assembled with respect to phylogenetic relatedness and traits that are ecologically relevant. Using a combination of ten functional traits and a well-resolved species phylogeny, I report evidence for non-random community assembly within Pacific *Cyrtandra*.

Phylogenetic and phenotypic clustering of Cyrtandra communities

Coexisting species of *Cyrtandra* were generally found to be more closely related than would be expected by chance. This pattern was observed at both broad (island communities) and fine (site communities) spatial scales. Of the six island communities examined, all were phylogenetically clustered (four significantly clustered) both at deeper nodes in the phylogeny (MPD metric) and at the tips (MNTD metric). Of the 18 site communities examined, 15 were phylogenetically clustered at deeper nodes (four significantly clustered), while 16 were clustered at the tips (four significantly clustered).

Coexisting species of *Cyrtandra* were also found to be more phenotypically similar than expected by chance. The overall functional phenotype of species that coexist on the same island was clustered in five of the six of the communities examined, although none significantly so. Examination of individual traits revealed significant clustering in whole plant (maximum height), leaf (SLA), and floral traits (flower length, corolla lobe size, flower number). The overall functional phenotype of species that coexist at the same site within islands was clustered in ten of the communities, although only three and one were significantly clustered under the MPD and MNTD metrics, respectively. Significant clustering was observed in whole plant (maximum height), leaf (SLA, LDMC, petiole length), and floral traits (flower length, flower number).

The general trend of phylogenetic and phenotypic clustering observed in communities of Pacific *Cyrtandra* is consistent with what is known about the evolutionary history of group. *Cyrtandra* is estimated to have emerged ca. 26 mya in Southeast Asia (Johnson et al. 2017), followed by widespread dispersal (likely by avian frugivores) across the Pacific. A rapid rate of diversification in Pacific *Cyrtandra* was recently reported (Roalson et al. 2016), with ca. 175 species evolving in the last 13 million years

(Johnson et al. 2017). Rapid and recent speciation across the Pacific Basin following rare long-distance dispersal events between islands, in combination with a preference for wet forest understories and pollination by generalist insects, may all contribute to the clustered phylogenetic and phenotypic structure observed in the present study.

Absence Of Trait Conservatism

Examination of phylogenetic signal revealed *K* values of less than one for all ten functional traits, suggesting that none of the traits examined are phylogenetically conserved among species. This result also suggests that closely related species resemble each other less than expected under a Brownian motion model of trait evolution. There was no evidence for phylogenetic signal in four of the five leaf traits examined (SLA, LDMC, petiole length, leaf pubescence), whereas three of the four floral traits (flower length, flower width, flower number) exhibited significant phylogenetic signal. Overall, this suggests that leaf traits tend to be more evolutionarily labile than floral traits in Pacific *Cyrtandra*.

Traits that exhibited significant phylogenetic signal displayed a pattern of clustering among coexisting species. At the broad spatial scale, traits that were clustered in most of the island communities included leaf size (83%), flower number (75%), and flower width (67%). At the fine spatial scale, traits that were clustered within most site communities included flower length (69%), maximum height (64%), leaf size (61%), and flower width (61%). The retention of phylogenetic signal in floral traits and the strong influence of these traits on the observed degree of phylogenetic clustering may indicate that pollinators act as a strong biotic filter for closely related species of *Cyrtandra*. In contrast, the absence of phylogenetic signal in most leaf traits, coupled with the lower contribution of these traits to niche clustering, may suggest that environmental filtering along this trait axis is minimal in the observed communities.

Potential Limitations

The biogeographic scale at which studies of community assembly are conducted have been shown to be important regarding inferred dispersal limitations (Hardy et al. 2012), appropriate sampling across regional species pools (Kraft et al. 2007), and the influence of environmental gradients (Kraft et al. 2008). Concerning dispersal limitation, the approach used in the present study assumed that all species could disperse everywhere. This approach was selected based on results from a recent study examining biogeographic patterns within Pacific *Cyrtandra* (Johnson et al., 2017), which suggested that there have been at least 19 long-distance dispersal events of *Cyrtandra* among Pacific archipelagos, including two dispersal events from Fiji to Samoa, and one from Fiji to Hawaii. At least 18 dispersal events were also inferred between islands within a single archipelago (Hawaiian Islands). This propensity for long-distance dispersal is likely the result of avian frugivory of the many-seeded berries. However, Johnson et al. (2017) also suggested that dispersal between geographically distant islands is less frequent than between islands that are in proximity. Thus, a potential caveat of the present study is that our results may over-

estimate dispersal potential, and limiting sampling to regional species pools (i.e., within archipelagos) may produce slightly different results.

The results presented here may also be influenced by the sampling intensity of regional species pools. Simulation-based analyses have revealed that studies including 30–60% of the regional species diversity have the greatest statistical power to detect phylogenetic community structure (Kraft et al. 2007). In the present study, sampling percentages were 60% of Fijian *Cyrtandra* species, 21% of Samoan species, and 8% of Hawaiian species. Thus, inferences of phylogenetic community structure in Samoan and Hawaiian lineages may not be robust to issues related to sampling intensity. Increased sampling efforts in these two regions that contain high numbers of endemic *Cyrtandra* species is therefore needed to improve resolution in these analyses.

Lastly, patterns of community assembly may be influenced by abiotic and biotic factors that change across climatic (Cavender-Bares et al. 2006), edaphic (Fine & Kembel 2011), and topographic (Kraft et al. 2008) gradients. To capture variation associated with habitats, the present study employed sampling strategies that were aimed at encompassing the full range of habitat types that Pacific *Cyrtandra* communities occupy. Specifically, communities varied in elevation (20–1270 m), substrate age (0.5–29 mya), and vegetation type (lowland wet forest, montane mesic forest, montane wet forest, cloud forest). Despite sampling across these broad gradients, there was no clear evidence of community assembly structure that was related to any habitat, although further studies with more explicit sampling schemes may reveal trait responses that are linked to environmental variables (e.g., light, soil moisture; see Sedio et al. 2012) and/or topographic features (elevation, slope; see Lasky et al. 2014).

Conclusions

This study suggests that Pacific *Cyrtandra* communities are not randomly assembled, and instead, that niche-based processes structure biodiversity at broad and fine spatial scales in diverse congeneric species assemblages. The observation of phylogenetic and phenotypic clustering within island and site communities suggests that trait-based patterns are reflective of evolutionary relatedness at both spatial scales. However, as with most recently published comparable studies, these results may be highly contingent on the choice of metrics used to assess community structure, the set of communities, and on the selection of functional traits. Studies that increase regional sampling in areas with high species diversity and incorporate estimates of abiotic variables associated with microhabitats would be needed to further address the underlying drivers of community assembly patterns described here.

Declarations

Funding: Rancho Santa Ana Botanic Garden; Garden Club of America Award in Tropical Botany; American Philosophical Society Lewis and Clark Fund for Exploration and Field Research; The Gesneriad Society Nelly D. Sleeth Scholarship Endowment Fund; The Gesneriad Society Elvin McDonald Fund; Society of Systematic Biologists Graduate Student Research Award; Sigma Xi Grants-in-Aid of Research; American

Society of Plant Taxonomists Graduate Student Research Grant; Claremont Graduate University; and The Smithsonian Institution.

Conflicts of interest/Competing interests: None

Ethics approval: Not applicable

Consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material:

The data used and/or analyzed for the current study are available from the author on reasonable request.

Code availability: The R code used for analyses in the current study are available from the author on reasonable request.

Author's contributions: MAJ conceived, designed, and executed this study and wrote the manuscript. No other person is entitled to authorship.

References

1. Abramoff MD et al (2004) Image processing with ImageJ. – Biophotonics Int 11:36–42
2. Alcantara S, Ree RH, Martins FR, Lohmann LG (2014) The effect of phylogeny, environment and morphology on communities of a lianescent clade (Bignonieae-Bignoniaceae) in Neotropical biomes. PLoS ONE 9:e90177
3. Atkins HJ, Bramley GLC, Clark JR (2013) The taxonomy of *Cyrtandra* (Gesneriaceae): current knowledge, future directions, in: Clark JR (Ed.), Proceedings of the World Gesneriad Research Conference 2010, Selbyana 31, 65–253, pp 157–165
4. Baraloto C, Hardy OJ, Paine CET, Dexter KG, Cruaud C, Dunning LT, Gonzalez M, Molino J, Sabatier D, Savolainen V, Chave J (2012) Using functional traits and phylogenetic trees to examine the assembly of tropical forest tree communities. J Ecol 100:690–701
5. Blomberg SP, Garland T, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57:717–745
6. Cavender-Bares J, Keen A, Miles B (2006) Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. Ecology 87:109–122
7. Clague DA (1996) The growth and subsidence of the Hawaiian-Emperor volcanic chain. In: Keast A, Miller SE (eds) The origin and evolution of Pacific Island biotas, New Guinea to eastern Polynesia: Patterns and processes. SPB Academic Publishing, Amsterdam, pp 35–50
8. Cornelissen JHC, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich DE, Reich PB, ter Steege H, Morgan HD, van der Heijden MGA, Pausas JG, Poorter H (2003) A handbook of protocols for

standardized and easy measurement of plant functional traits worldwide. Aust J Bot 51:335–380

9. Cornwell WK, Schwilk DW, Ackerly DD (2006) A trait-based test for habitat filtering: convex hull volume. J Ecol 87:1465–1471
10. Dayan T, Simberloff D (2005) Ecological and community-wide character displacement: the next generation. Ecol Letters 8:875–894
11. Dexter KG, Lavin M, Torke BM, Twyford AD, Kursar TA, Coley PD, Drake C, Hollands R, Pennington RT (2017) Dispersal assembly of rain forest tree communities across the Amazon Basin. PNAS 114:2645–2650
12. Fine PVA, Kembel SW (2011) Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. Ecography 34:553–656
13. Gillett GW (1967) The genus *Cyrtandra* in Fiji. Contr U S Natl Herb 37:107–159
14. Gillett GW (1973) The genus *Cyrtandra* (Gesneriaceae) in the South Pacific. University of California Publications in Botany, vol 66. University of California Press, Berkeley
15. Gotelli NJ, Entsminger GL (2003) Swap algorithms in null model analysis. Ecology 84:532–535
16. Hardy OJ, Couteron P, Munoz F, Ramesh BR, Pélissier R (2012) Phylogenetic turnover in tropical tree communities: impact of environmental filtering, biogeography and mesoclimatic niche conservatism. Global Ecol Biogeogr 21:1007–1016
17. Hubbell SP (2001) The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton
18. Johnson MA, Clark JR, Wagner WL, McDade LA (2017) A molecular phylogeny of the Pacific clade of *Cyrtandra* (Gesneriaceae) reveals a Fijian origin, recent diversification, and the importance of founder events. Mol Phyl Evol 116:30–48
19. Kay KM, Reeves PA, Olmstead RG, Schemske DW (2005) Rapid speciation and the evolution of hummingbird pollination in neotropical *Costus* subgenus *Costus* (Costaceae): evidence from nrDNA ITS and ETS sequences. Am J Bot 92:1899–1910
20. Kay KM, Sargent RD (2009) The role of animal pollination in plant speciation: integrating ecology, geography, and genetics. Ann Rev Ecol Evol Syst 40:637–656
21. Kembel SW, Hubbell SP (2006) The phylogenetic structure of a neotropical forest tree community. Ecology 87:S86–S99
22. Kembel SW, Ackerly DD, Blomberg SP, Cowan P, Helmus MR, Webb CO (2008) Picante: tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464
23. Kraft NJB, Cornwell W, Webb CO, Ackerly DD (2007) Trait evolution, community assembly, and the phylogenetic structure of ecological communities. Am Nat 170:271–283
24. Kraft NJB, Valencia R, Ackerly DD (2008) Functional traits and niche-based tree community assembly in an Amazonian forest. Science 322:580
25. Kraft NJB, Ackerly DD (2010) Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. Ecol Monogr 80:401–422

26. Lasky JR, Yang J, Zhang G, Cao M, Tang Y, Keitt TH (2014) The role of functional traits and individual variation in the co-occurrence of *Ficus* species. Ecology 95:978–990
27. Lebrija-Trejos E, Perez-Garcia EA, Meave JA, Bongers F, Poorter L (2010) Functional traits and environmental filtering drive community assembly in a species-rich tropical ecosystem. J Ecol 91:386–398
28. Merwin L, He T, Lamont BB (2012) Phylogenetic and phenotypic structure among *Banksia* communities in south-western Australia. J Biogeogr 39:397–407
29. Mueller-Dombois D, Fosberg FR (1998) Vegetation of the tropical Pacific Islands. Springer Verlag, New York
30. Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests MASS (2015) Vegan community ecology package: ordination methods, diversity analysis and other functions for community and vegetation ecologists. Version 2.3-1
31. Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290
32. Purves DW, Pacala SW (2005) In: Burslem D et al (eds) Ecological drift in niche-structured communities: neutral pattern does not imply neutral process. Biotic Interactions in the Tropics. Cambridge University Press, Cambridge, pp 107–138
33. Revell LJ (2012) Phytools: An R package for phylogenetic comparative biology (and other things). Meth Ecol Evol 3:217–223
34. R Development Core Team (2014) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
35. Roalson EH, Roberts WR (2016) Distinct processes drive diversification in different clades of Gesneriaceae. Syst Biol 65:662–684
36. Salazar D, Jaramillo MA, Marquis RJ (2016) Chemical similarity and local community assembly in the species rich tropical genus *Piper*. Ecology 97:3176–3183
37. Sargent RD, Ackerly DD (2008) Plant-pollinator interactions and the assembly of plant communities. Trends Ecol Evol 23:123–130
38. Schluter D (2000) Ecological character displacement in adaptive radiation. Am Nat 156:S4–S16
39. Sedio BE, Wright SJ, Dick CW (2012) Trait evolution and the coexistence of a species swarm in the tropical forest understory. J Ecol 100:1183–1193
40. Wagner WL, Herbst DR, Sohmer SH (1990) Gesneriaceae, *Cyrtandra*. In: Mill SW (ed) Manual of the Flowering Plants of Hawai'i, vol 1. University of Hawai'i Press, Honolulu, Hawai'i, pp 735–781
41. Webb CO (2000) Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. Am Nat 156:145–155
42. Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Ann Rev Ecol Syst 33:475–505

43. Weiher E, Keddy PA (1999) Ecological assembly rules: perspectives, advances, retreats. Cambridge University Press, Cambridge
44. Wolowski M, Carvalheiro LG, Freitas L (2017) Influence of plant-pollinator interactions on the assembly of plant and hummingbird communities. J Ecol 105:332–344

Figures

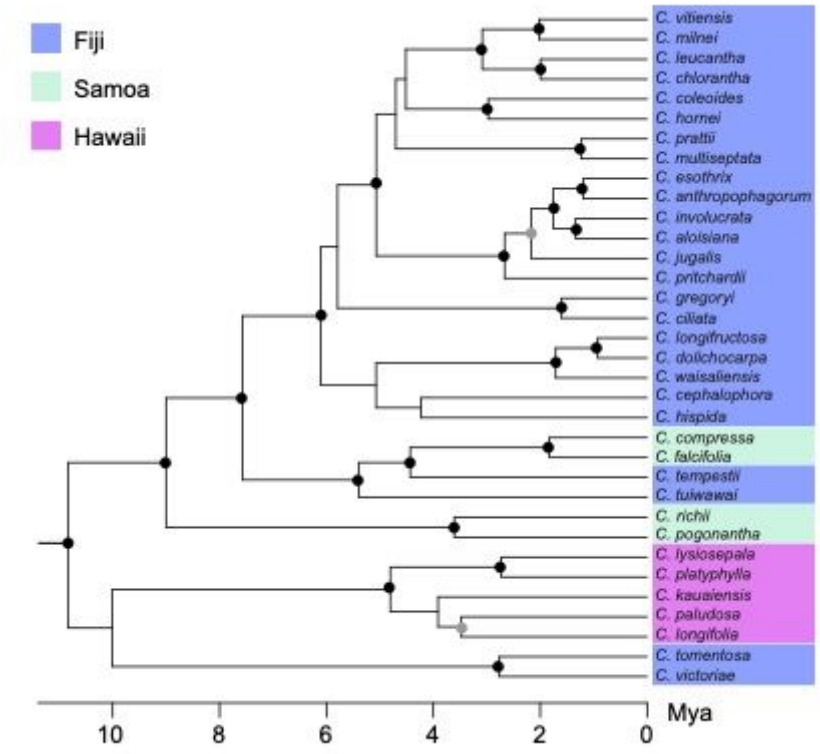

Figure 1

Species phylogeny of 34 Pacific Cyrtandra from Fiji, Samoa, and the Hawaiian Islands. Filled black circles indicate support values of ≥ 70 BS (maximum likelihood bootstrap support) and ≥ 0.95 PP (Bayesian posterior probability), while grey circles indicate support values of ≥ 70 BS or ≥ 0.95 PP.

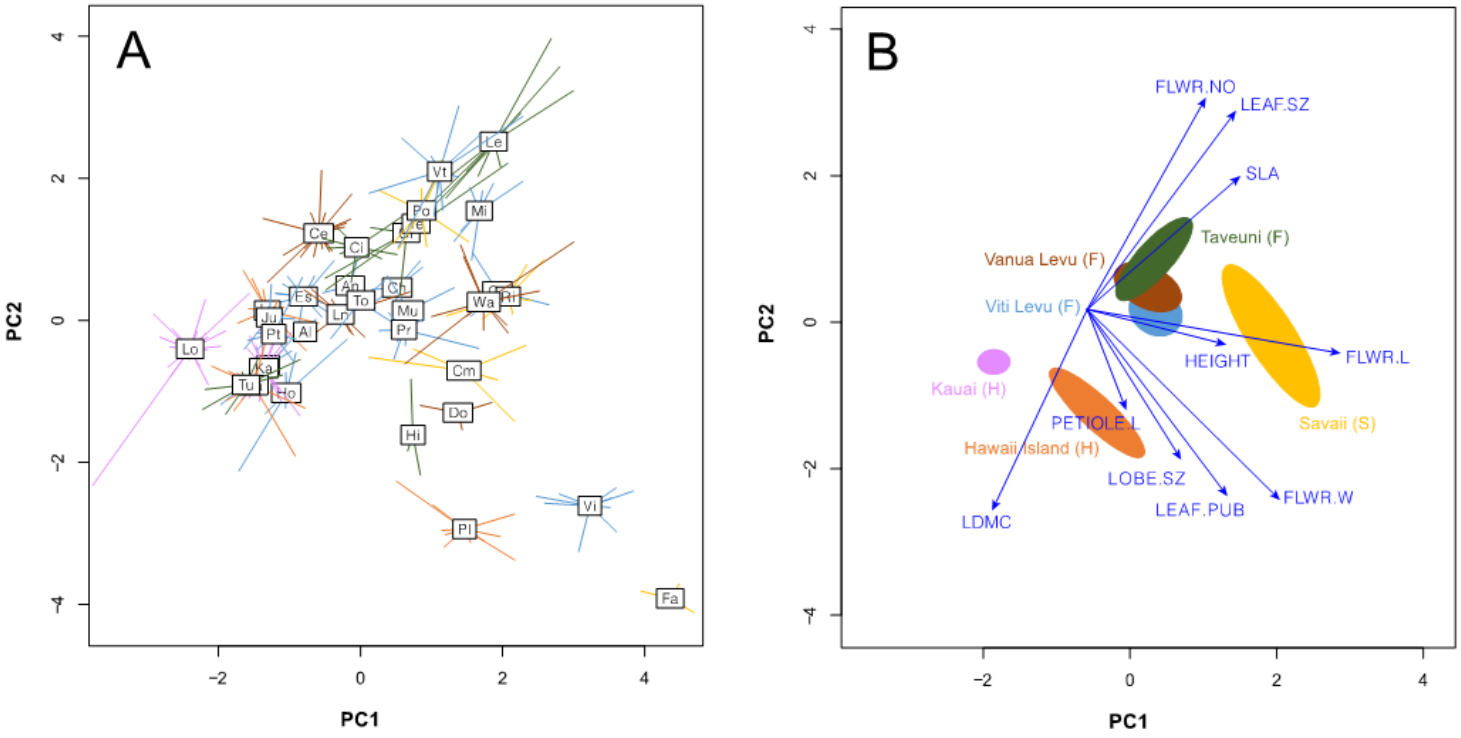

Figure 2

Results from a principal components analysis including 10 functional traits. The first two components (PC1 and PC2) represent 45% of the variation. The distributions of 34 Pacific Cyrtandra species (see species codes in Table S2) are shown in functional trait space (A) and each of the six sampled islands from three archipelagos (F = Fiji, S = Samoa, H = Hawaii) are represented in trait space with 95% confidence ellipses; arrows represent the contribution of each trait to functional phenotype (B).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- JohnsonSI.docx
- TableS2.xlsx