A molecular phylogeny of Southeast Asian Cyrtandra (Gesneriaceae) supports an emerging paradigm for Malesian plant biogeography

8 authors, including:

Jessica A Hannah
Medical University of South Carolina
4 PUBLICATIONS **18** CITATIONS

SEE PROFILE

Gemma Bramley
Royal Botanic Gardens, Kew
27 PUBLICATIONS **191** CITATIONS

SEE PROFILE

Melissa A. Johnson
DKI US Pacific Basin Agricultural Research Center USDA-ARS
23 PUBLICATIONS **145** CITATIONS

SEE PROFILE

Abdulrokhman Kartonegoro
Indonesian Institute of Sciences
76 PUBLICATIONS **55** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Streptocarpus genetics View project

Biodiversity View project

Title

A molecular phylogeny of Southeast Asian Cyrtandra (Gesneriaceae) supports an emerging paradigm for Malesian plant biogeography

Permalink

Journal

Authors

Atkins, Hannah J.
Bramley, Gemma L.C.
Johnson, Melissa A.
et al.

Publication Date

DOI

Supplemental Material

License

A molecular phylogeny of Southeast Asian *Cyrtandra* (Gesneriaceae) supports an emerging paradigm for Malesian plant biogeography

Hannah J. Atkins[1]*, Gemma L.C. Bramley[2], Melissa A. Johnson[3], Abdulrokhman Kartonegoro[4], Kanae Nishii[1,5], Goro Kokubugata[6], Michael Möller[1] and Mark Hughes[1]

[1] Royal Botanic Garden Edinburgh, 20a Inverleith Row, Edinburgh, EH3 5LR, Scotland, UK; [2] Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK; [3]USDA Agricultural Research Service, DKI US Pacific Basin Agricultural Research Center, 64 Nowelo St., Hilo, HI, USA; [4] Research Center for Biology, Indonesian Institute of Sciences (LIPI), Jl. Jakarta-Bogor KM. 46, Cibininong, West Java 16911, Indonesia; [5] Kanagawa University, 2946, Tsuchiya, Hiratsuka-shi, Kanagawa, Japan; [6] Department of Botany, National Museum of Nature and Science, Amakubo Tsukuba, Ibaraki 305-0005, Japan.
*Corresponding author: h.atkins@rbge.org.uk

Abstract

The islands of Southeast Asia comprise one of the most geologically and biogeographically complex areas in the world and are a centre of exceptional floristic diversity, harbouring 45,000 species of flowering plants. *Cyrtandra*, with over 800 species of herbs and shrubs, is the largest genus in the family Gesneriaceae and is one of the most emblematic and species-rich genera of the Malesian rainforest understorey. The high number of species and tendency to narrow endemism make *Cyrtandra* an ideal genus for examining biogeographic patterns. We sampled 128 *Cyrtandra* taxa from key localities across Southeast Asia to evaluate the geo-temporal patterns and evolutionary dynamics of this clade. One nuclear and four chloroplast regions were used for phylogenetic reconstruction, molecular dating, and ancestral range estimation. Results from the dating analysis suggest that the great diversity of *Cyrtandra* seen in the Malesian region results from a recent radiation, with most speciation taking place in the last five million years. Borneo was recovered as the most likely ancestral range of the genus, with the current distribution of species resulting from a west to east migration across Malesia that corresponds with island emergence and mountain building. Lastly, our investigation into the biogeographic history of the genus indicates high levels of floristic exchange between the islands on the Sunda shelf and the important role of the Philippines as a stepping stone to Wallacea and New Guinea. These patterns underlie much of the plant diversity in the region and form an emerging paradigm in Southeast Asian plant biogeography.

Highlights

- This paper provides the first well sampled phylogeny of *Cyrtandra* across Southeast Asia.

- One of the richest genera of the Asian rainforest understory, *Cyrtandra* appears to have originated in Borneo, and undergone west-to-east dispersal into the Pacific.

- Support was found for a mid-Miocene origin, with most extant diversity arising by speciation within the last 5 Ma.

- The Philippines appear to represent an important secondary source area and stepping stone to Wallacea, Taiwan and Japan, and New Guinea.

- *Cyrtandra* fits into an emerging paradigm for Southeast Asian plant geography, illustrating the role of geo-tectonic and climate processes in historical biogeographical patterns in the region.

Keywords: ancestral range estimation, biogeographic stochastic mapping, *Cyrtandra,* floristic exchange, island biogeography, molecular dating, recent divergence, Sahul shelf, Sunda shelf, Wallacea

Introduction

The islands of Southeast Asia comprise one of the most geologically and biogeographically complex areas in the world (Hall 2002, Lohman et al. 2011) and are the meeting and mixing point of floras and faunas of diverse origins (van Welzen et al. 2011, Richardson et al. 2012). The area is estimated to harbour approximately 45,000 species of vascular plants (Johns 1995) on more than 20,000 islands (Lohman et al. 2011). It contains the biodiversity hotspots of Sundaland, Wallacea and the Philippines (Brooks et al. 2006), and the mega-diverse island of New Guinea (Mittermeier et al. 2003, Takeuchi 2005, Hoover et al. 2017).

The Malesian region is a complex and intricate mosaic of islands of different origins with a dynamic history over the last 50 million years (Ma) (Hall 2002, 2012a,b). In the west of the region are the continental Sunda shelf islands of Sumatra, Java and Borneo, separated by shallow seas. In the centre are the numerous smaller terranes and oceanic islands that comprise the Philippines and Wallacea, and in the east is the Sahul shelf and the large island of New Guinea, which is itself of composite origin. Adding a layer of complexity to this is the changing climate of the past 50 ma (Morley 2012, 2018), most strikingly the fluctuating glacials and interglacials of the Pleistocene (Woodruff 2010, Morley 2012), which impacted sea levels and the extent of vegetation types (Woodruff 2010, Cannon 2012, Morley 2012).

Over the last decade, a number of dated molecular phylogenies (Thomas et al. 2012, Grudinski et al. 2014, Hughes et al. 2015, Williams et al. 2017) and meta-analyses (van Welzen et al. 2011, De Bruyn et al. 2014, Crayn et al. 2015) have supplemented our knowledge of how current patterns of diversity have been shaped by the geological and climatic history of Malesia. These studies have provided insights into where and when lineages diversified and revealed some remarkable cross-taxon biogeographic patterns (Lohman et al. 2011), including the predominance of west-to-east dispersal patterns (Su and Saunders 2009, Baker and Couvreur 2012, Richardson et al. 2012, Thomas et al. 2012, Grudinski et al. 2014, Richardson et al. 2014, Crayn et al. 2015) and the identification of Borneo as an ancestral area and centre of diversity. Our current understanding of the biogeographic history of Malesia, however, remains incomplete and there is a need for additional well sampled phylogenies of taxa with distributions across the region (Richardson et al. 2012, Webb and Ree 2012).

Cyrtandra is the largest genus in the Gesneriaceae, with over 800 species of herbs and shrubs (Atkins et al. 2013) and is a key component of the herbaceous layer of Malesian rainforest found from sea level to 3000m (Burtt 2001, Atkins et al. 2013). Centres of diversity for the genus in Southeast Asia are Borneo (~200 spp.), the Philippines (~150 spp.), and New Guinea (~120 spp.) (Atkins et al. 2013). Its high species diversity, large number of narrow endemic species, and wide distribution make it an ideal genus for examining biogeographic patterns (Atkins et al. 2001, Cronk et al. 2005, Clark et al. 2008, Johnson et al. 2017) and investigating the processes which underlie current patterns of biodiversity (Bramley et al. 2004a, Johnson et al. 2015, 2019). The genus is characterised by a combination of two fertile stamens and an indehiscent fruit, which varies from a tough-walled green or brown capsule in the west to a fleshy berry that ripens white or rarely orange in the east of its distribution, particularly in New Guinea and the Pacific (Fig. 1) (Clark et al. 2013, Johnson 2017, Atkins et al.

Figure 1. Range of fruit morphology in SE Asian and Pacific *Cyrtandra*. a *C. pendula* (Sumatra) SUBOE 2; b *C.* sp (Sumatra) SUBOE 6; c *C.* sp (Sumatra) SUBOE 9; d *C.* sp (New Guinea) Briggs MB838; e *C. pogonantha* (Samoa) Wood 16941 f *C. celebica* (Sulawesi) BAKK 12; g *C. polyneura* (Sulawesi) BAKK 18; h *C. pulleana* (New Guinea) Briggs MB845; I *C. richii* (Samoa) Wood 16935. Photos: a-c, f & g: Sadie Barber. e & i: Melissa Johnson. d & h: Marie Briggs.

2019). The flowers are often white but species with pink, red, purple, yellow, green, and orange flowers are also known (Fig. 2).

Earlier phylogenetic studies of Southeast Asian *Cyrtandra* have focused on particular localities with dense sampling from a small number of locations (Atkins et al. 2001, Bramley et al. 2004a). Larger scale studies have focused on *Cyrtandra* diversification across the Pacific (Cronk et al. 2005, Clark et al. 2008, 2009, Johnson et al. 2017), and these studies have signalled that the origin of the genus is within the Malesian region. Here, we sample *Cyrtandra* taxa from key localities across Southeast Asia to construct a well-resolved phylogenetic tree based on one nuclear and four chloroplast regions. We use this to estimate divergence times, ancestral ranges, and dispersal patterns in order to gain insights into the evolutionary history of *Cyrtandra.* We then consider whether the key patterns recovered for other taxa in Southeast Asia are also seen in *Cyrtandra,* one of the most species-rich and emblematic genera of the Malesian rainforest understorey.

Methods

Taxon sampling

We sampled a total of 192 accessions representing 128 *Cyrtandra* taxa (Supplementary Table S1), including samples that were representative of all the key regions in Southeast Asia (Fig. 3). Table 1 gives a summary of our sampling against current estimates of species numbers by island for the Southeast Asian region (Atkins et al. 2013). Five taxa from within Didymocarpinae (the same subtribe as *Cyrtandra*) were selected as outgroups (*Aeschynanthus roseoflorus, A. buxifolius, Agalmyla chalmersii, Didymocarpus antirrhinoides,* and *Loxostigma griffithii*). Outgroups were selected to reflect those in recent molecular dating analyses (Johnson et al. 2017, Ranasinghe 2017) to facilitate dating of the nodes using secondary calibration points. We applied names to as many of the samples as possible but many of the samples represent undescribed diversity. There is no single taxonomic treatment for *Cyrtandra* and therefore species concepts and identifications follow those used in regional treatments (e.g., Atkins and Cronk, 2001, Bramley et al. 2004b).

Molecular methods

DNA extraction methods and PCR conditions followed Nishii et al. (2019). Details of the primers used for each of the five regions (ITS, *mat*K, *trn*L-F, *psb*A-*trn*H and *rpl*32-*trn*L) are given in Table 2. Chloroplast sequence data (*mat*K, *trn*L-F, *psb*A-*trn*H and *rpl*32-*trn*L) from two of the outgroup collections, *Agalmyla chalmersii* and *Didymocarpus antirrhinoides,* was gifted to the project by Prof Gao Lian Ming, Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China.

Phylogenetic analyses

Maximum Parsimony (MP) analyses were initially conducted on individual regions to visually assess

Figure 2. Range of flower morphology in SE Asian *Cyrtandra*. a *Cyrtandra rantemarioensis* (Sulawesi) RBGE living collections 20000622; b. *Cyrtandra luteiflora* (Sulawesi) RBGE living collections 20021194; c. *Cyrtandra purpureofucata* (Sulawesi) Thomas & Ardi 09-88; d. *Cyrtandra serratifolia* (Sulawesi) RBGE living collections 20021210; e. *Cyrtandra celebica* (Sulawesi) BAKK 15; f. *Cyrtandra mollis* (Sulawesi) BAKK 42; g. *Cyrtandra cleopatrae* (Palawan, Philippines) RBGE living collections 19981745; h. *Cyrtandra bungahijau* (Yapen Island, New Guinea) RBGE living collections 20090826; i. *Cyrtandra peltata* (Sumatra) RBGE living collection 20161282; j. *Cyrtandra vittata* (Yapen Island, New Guinea) RBGE living collection 20090734. Photos: a & b: Steve Scott. c: Wisnu Ardi. d & g: Hannah Atkins. e, f & i: Sadie Barber. j: Lynsey Wilson

congruence, with areas of conflict determined by examining the placement of individual taxa on each gene tree. Relationships were considered incongruent if the placement of taxa varied among the individual gene trees and exhibited MP-BS values > 80%. MP analyses were carried out using PAUP v 4.0a163 (Swofford 2002) on unweighted and unordered characters. Alignment gaps were treated as missing data. A heuristic search was carried out using stepwise random addition of 10,000 replicates, with TBR and Multrees on. Statistical
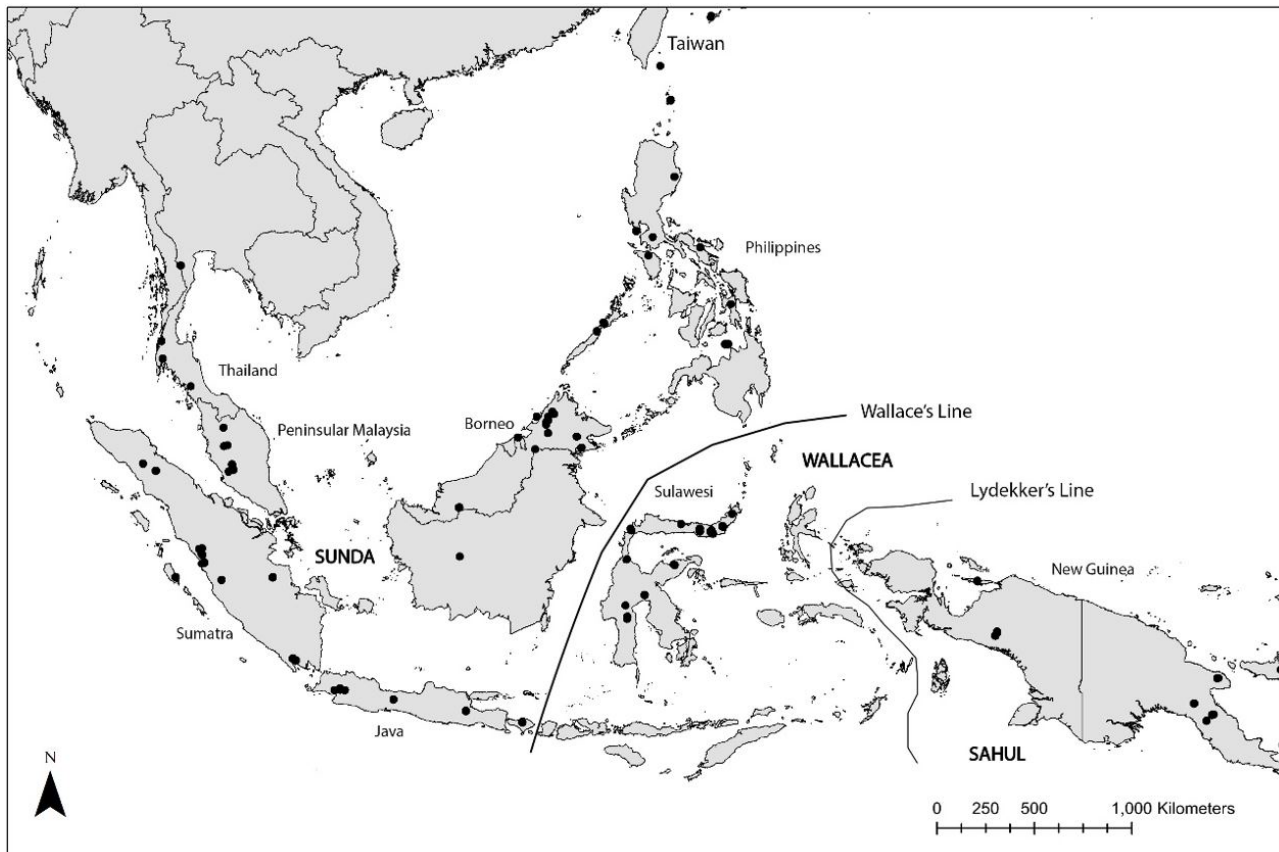

Figure 3. Map showing collection locations of southeast Asian *Cyrtandra* samples included in the present study. Pacific islands not shown on map.

Table 1. Current estimates of species numbers in *Cyrtandra* by area across Malesia, the number of species included in the present study, and the percentage of the total that this represents.

Geographic region (Southeast Asia only)	Total number of species (following Atkins et al. 2013)	Number of species sampled	% sampled (based on maximum numbers where ranges given in Atkins et al. 2013)
Thailand	6	2	33
Peninsular Malaysia	9	5	56
Sumatra	40-44	27	61
Java	19-32	11	34
Lesser Sunda Islands	3	1	33
Borneo	181-200	26	13
Taiwan and Japan	2	2	100
Philippines	105-150	17	11
Sulawesi	22-40	26	65
Moluccas	3	0	0
Australia	1	1	100
New Guinea	107-120	10	8

branch support was obtained from 10,000 heuristic bootstrap replicates each starting with a random addition tree, optimised with TBR on and Multrees off. For Bayesian Inference and Maximum Likelihood analyses, the data were divided into seven partitions (ITS spacers, 5.8S gene, *psbA-trnH*, *rpl*32-*trn*L, *trn*L-F, *matK* coding region, *matK* intron region) and analysed under the best-fit model of nucleotide evolution for each genic region selected using the AIC criterion as implemented in MrModeltest v 2.4 (Nylander 2004) (GTR+G for ITS spacers, *trn*L-F, *psbA-trnH* and *matK* intron region, GTR+I+G for *rpl*32-*trn*L and *matK* coding region and SYM+I for the ITS 5.8S gene). Bayesian inference (BI) phylogenetic analyses were carried out using Mr Bayes v 3.2.6 (Ronquist et al. 2012) on the partitioned dataset. Two runs with four chains each were implemented, run for 10,000,000 generations with a tree sampled every 1000th generation. The first 10% of sampled trees were discarded as burn-in and the remainder summarised as a maximum clade credibility tree and posterior probabilities (PP) extracted. Maximum Likelihood (ML) analyses were conducted with RAxML v 8 (Stamatakis 2014) via the CIPRES Gateway (Miller et al. 2010). The search for

the optimal ML tree was performed with the 'Let RAxML halt bootstrapping automatically' parameter selected. For the ML and BI analyses, tree topology and node support were examined in FigTree v. 1.4.3 (Rambaut 2007).

Divergence time estimates

A time-calibrated phylogeny was constructed on the partitioned five-gene dataset using an uncorrelated relaxed lognormal clock in the program BEAST v. 1.10.1 (Drummond et al. 2012, Rambaut et al. 2018). Secondary age calibrations were necessary as there are no unambiguous fossils in the Gesneriaceae family (Wiehler 1983, Clark et al. 2008). Five calibration points were taken from the family-wide phylogeny in Ranasinghe (2017) (Table 3) and were assigned a lognormal prior following Ho and Phillips (2009) and Schenk (2016) . Five separate runs were carried out, beginning with a random tree and run for 100 million generations under a Yule model of speciation, sampled every 1000 generations. Following Condamine et al. (2015) sensitivity analyses using the Birth-Death tree prior were also run and the results were not significantly changed by choice of tree prior. The results from the

Table 2. Details of primers used for PCR and sequencing of the five gene regions for *Cyrtandra*.

Region	Name	Direction	Primer sequences	References
ITS	ITS_5P	Forward	GGA AGG AGA AGT CGT AAC AAG	Möller & Cronk 1997
ITS	ITS_8P	Reverse	CAC GCT TCT CCA GAC TAC A	Möller & Cronk 1997
*trn*LF	trnLcG	Forward	GTG AAG ACT TCT AAA TTC AGA GAA AC	Nishii et al. 2019
*trn*LF	trnLf	Reverse	ATT TGA ACT GGT GAC ACG AG	Taberlet et al. 1991
*psb*A-*trn*H	psbAf	Forward	GTT ATG CAT GAA CGT AAT GCT C	Sang et al. 1997
*psb*A-*trn*H	trnHr	Reverse	CGC GCA TGG TGG ATT CAC AAA TC	Sang et al. 1997
*rpl*32-*trn*L	rpl32-F	Forward	CAG TTC CAA AAA AAC GTA CTT C	Shaw et al. 2007
*rpl*32-*trn*L	trnL(UAG)	Reverse	CTG CTT CCT AAG AGC AGC GT	Shaw et al. 2007
*mat*K	matK.206F	Forward	CCG GGT TAT GAC AAT AAA TCC AGT	Luna et al. 2019
*mat*K	matK.946R	Reverse	ATA AAT CCT TCT TGG ATG AAA CCA C	Luna et al. 2019
*mat*K	matK.cy2F	Forward	TGG CAA TGG CAT TTT TCG CT	Nishii et al. 2019
*mat*K	matK.1734R	Reverse	CCG TGC TTG CAT TTT TCA TTG C	Luna et al. 2019

Table 3. Details of the five secondary calibration points (node age, standard deviation, and prior distribution) from Ranasinghe (2017) used to generate the dated phylogeny of *Cyrtandra* in BEAST.

Calibration point	Node	Node Age (Ma)	Prior distribution	Standard deviation
1	Internal *Cyrtandra* node	6.42	LogNormal	2.0 (2.5, 10.97)
2	*Loxostigma* and *Cyrtandra* crown	18.03	LogNormal	2.0 (14.18, 21.85)
3	*Aeschynanthus* and *Loxostigma/ Cyrtandra* crown	18.79	LogNormal	2.0 (14.89, 22.71)
4	*Billolivia* (and *Aeschynanthus/ Loxostigma/ Cyrtandra* crown	20.15	LogNormal	2.0 (16.18, 24.28)
5	*Agalmyla* (and *Billolivia/ Aeschynanthus/ Loxostigma/ Cyrtandra* crown)	20.99	LogNormal	2.0 (17.04, 25.14)

analysis with the Yule model are presented here. Plots of the logged parameters for each run were visualised using Tracer v. 1.7.1 (Drummond and Rambaut 2007) to confirm convergence between runs by examining log likelihood plots and ensuring that Effective Sample Size (ESS) values were above 200. The trees from each run were combined in Logcombiner v 1.10.1 (Drummond et al. 2012) and support values and tree statistics were summarised onto a single maximum clade credibility (MCC) tree using the programme TreeAnnotator v. 1.10.1 (Drummond et al. 2012), visualised in FigTree v. 1.4.3 (Rambaut 2007).

Ancestral range estimation

The R package BioGeoBEARS (BioGeography with Bayesian Evolutionary Analysis in R Scripts) (Matzke 2013, 2014) was used to estimate ancestral ranges for *Cyrtandra* under three historical biogeography methods: DEC (Dispersal-Extinction-Cladogenesis; Ree and Smith, 2008), DIVA (Dispersal-Vicariance Analysis; Ronquist 1997), and BayArea (Bayesian inference of historical biogeography for discrete areas; Landis et al. 2013) models. Descriptions of each of these models and how BioGeoBEARS replicates their key assumptions are given in Matzke (2013). To allow for model comparison, all the models were implemented in a maximum likelihood framework. As *Cyrtandra* is distributed across a system of islands and shows high levels of narrow endemism, founder event speciation was likely to be a highly relevant biogeographic process (Cowie and Holland 2006, Matzke 2013, Roalson and Roberts 2016, Johnson et al. 2017), so we also explored the influence of founder event speciation in the analysis by including '+J' versions of the models. Due to concerns about the statistical methods in the package, both in terms of how the best fit model is selected and how the +J parameter operates (Ree and Sanmartín 2018), results from all of the six models will be discussed. Given that sampling density can also impact the results, a summary of our sampling rates against current species estimates is presented in Table 1.

The MCC tree was pruned to include only a single representative of each species except for those species that have multi-island distributions and were not monophyletic, such as *C. pendula*. In these cases, we included one representative from each area (following Johnson et al. 2017, 2019). For monophyletic species with multi-island distributions, such as *C. umbellifera* from Taiwan and the Philippines and *C. sandei* from Java and Sumatra, only one sample was included, and these were coded as present in each respective area in the analysis. Each taxon was assigned a distribution using 13 geographic regions based on a combination of geological information (Hall 2002, 2012), previous biogeographical studies (Atkins et al. 2001, van Welzen et al. 2011, Hughes et al. 2015) and current knowledge of species distributions and relationships in *Cyrtandra* (Atkins and Cronk 2001, Bramley and Cronk 2003, Johnson et al. 2017, Kartonegoro et al. 2018). The 13 regions used were: Hawaii, Marquesas and Society Islands, Japan and Taiwan, Australia, Thailand, Peninsular Malaysia, Sumatra, Borneo, Sulawesi, New

Guinea, Solomon Islands, Java and the Lesser Sunda Islands, and the Philippines.

The outgroup taxa were removed so as not to influence the root ancestral area. We set the maximum number of areas to two, as *Cyrtandra* is characterised by high levels of narrow endemism and only *C. pendula* is currently recognised as having a range size greater than two areas. The six models (DEC, DIVA-like, BayArea-like, and the '+J' variations of each) were compared for statistical fit using the Akaike Information Criterion (AIC) and a Likelihood Ratio Test (LRT).

Lastly, we used BioGeoBEARS to perform a Biogeographic Stochastic Mapping (BSM) analysis (Matzke 2016, Dupin et al. 2017). This generates simulated histories based on a given biogeographical model, the phylogeny, observed range data, and the calculated ancestral state probabilities at each node averaged over many realisations. The biogeographical events that are possible include within-area speciation, vicariance and dispersal events (range expansions and founder events). Event frequencies were estimated by taking the mean of event counts from 100 BSMs. We ran the BSM exercise on all the models to evaluate the impact that the choice of model has on the event counts.

Results

Phylogenetic relationships

In total, 940 new sequences were generated for this study and our final data matrix contained five gene regions and 5438 aligned base positions. Tree topologies of independent MP analyses of the ITS, *trn*L-f, *psb*A-*trn*H, *rpl*32, and *mat*K regions were congruent although there was far greater resolution in the ITS dataset than in any of the others individually. The tree based on the concatenated five-gene dataset (Fig. 4,5) largely followed the topology of the ITS tree, with increased support for the relationships between the major clades. There were no incongruences with greater than 80% MP bootstrap support. ML, MP, and BI analyses of the combined dataset resulted in trees with congruent topologies.

Divergence time estimates

In this study, divergence time estimates suggest that the genus *Cyrtandra* split from its closest relative, *Loxostigma,* c. 16 Ma (14.18-18.34, 95% HPD) during the early Miocene (Fig. 6). The crown age of *Cyrtandra* is estimated to be 13 Ma (10.84-15.56, 95% HPD). Summaries of estimated dates are shown in Table 4. Using mean ages, 109 of the 128 taxa included in the analysis split from their most recent ancestor in the last 5 ma (method following Madriñán et al. 2013; Richardson et al. 2014).

Ancestral range estimation

Significant improvement in the likelihood of the three standard models (DEC, DIVA-like, and BayArea-like) was seen when the founder event parameter (+J) was added (Table 5). Of the six models evaluated, the best fit model was BayArea-like +J.

Under all six models analysed, the island of Borneo was recovered as the most likely ancestral area for *Cyrtandra* (Fig. 7).

Biogeographic stochastic mapping

Under the best fit model, Borneo is resolved as a major source area with ~33% (11.5 events) of all dispersal events originating here (Fig. 8, Table 6). Dispersal from Borneo was most frequent to Sumatra (3.0 events) and the Philippines (2.7 events), followed by Peninsular Malaysia (2.0 events) and Sulawesi (1.5 events). In contrast, Borneo was the recipient of only 0.8% of dispersals from all other areas and no event counts from any single area above 0.1. The Philippines was the second largest source area with 21% of dispersal events originating here. The majority of dispersal events from the Philippines were to the island of Sulawesi (3.9 events), but there were also dispersals from the Philippines north to Taiwan and Japan (1.5 events) and east to New Guinea (0.9 events). The greatest source of dispersals into the Philippines was Borneo (2.7 events). Relatively high levels of dispersal were also recovered between Java, Sumatra, and Peninsular Malaysia (2.5 events from Sumatra to Peninsular Malaysia, 3.0 events from Java to Sumatra, and 1.2 events from Sumatra to Java). In marked contrast to Borneo, Sulawesi emerged as an island of high immigration (receiving 20% of all dispersals) and low emigration, with only 2% of dispersals originating here. New Guinea is resolved as the most likely source of dispersals to the Marquesas (0.9 events), Australia (1.0 event), and the Solomon Islands (1.0 event). The stochastic mapping exercise is more impacted by the choice of model than the ancestral range estimation as the six models rely on different biogeographic processes. In terms of dispersal patterns and potential routes through the region, however, Borneo is always in the top three sources of dispersal in all the models, along with the Philippines and Sumatra. Sulawesi is always

interpreted as being a poor source of dispersals under all models. The Philippines is the most important source of dispersals north to Taiwan and Japan under all models. It is the highest source of dispersals south to Sulawesi in all but the two worst fit models where it is the second highest after Borneo; and it is the highest source of dispersals east to New Guinea in all but the two worst fit models where Sumatra, Sulawesi, or Borneo are interpreted as the most important source. New Guinea is the most likely source of dispersals to Australia, the Marquesas/ Society Islands, and the Solomon Islands under all models.

Discussion

Phylogenetic patterns and relationships

There was no incongruence between the nuclear and chloroplast datasets in our large Malesian sample and hence no detectable evidence of hybridisation. This is in marked contrast to previous phylogenetic studies of *Cyrtandra,* where extensive hybridisation and/or incomplete lineage sorting has been reported, principally in Hawaii (Pillon et al. 2013, Johnson et al. 2019) but also in the recent radiation of the genus in the Pacific (Johnson et al. 2017). This also differs from the ecologically and biogeographically similar genus *Begonia,* where the high prevalence of hybrid events is considered to be an important factor driving genomic change and species evolution (Hughes et al. 2018). A recent study of reproductive isolation in four species of Hawaiian *Cyrtandra* showed that boundaries between sympatric *Cyrtandra* species are maintained predominantly through postzygotic barriers (Johnson et al. 2015). In Hawaii, all 60 species are the result of a single dispersal event to the archipelago < 5 ma (Cronk et al. 2005, Clark et al. 2008, 2009, Johnson et al. 2017), and species are remarkably similar in floral morphology and ecological preference, such that hybridisation is

Table 4. Estimated ages (Ma) of major nodes (crown and stem age of *Cyrtandra*) in the present study using BEAST, and comparable nodes in earlier studies. Ranges in parentheses represent the 95% highest posterior density (HPD).

Node	Present study	Johnson et al. 2017	Roalson and Roberts 2016
Cyrtandra stem	16.3 (14.28-18.43)	25.27 (16.9-35.02)	30.45 (14.79-37.54)
Cyrtandra crown	13.38 (11.05-15.79)	17.29 (12.54-22.15)	11.08 (3.25-21.78)

Table 5. Results of biogeographical model testing in BioGeoBEARS for *Cyrtandra*. Model parameters include anagenetic dispersal (d), extinction (e), and jump dispersal or founder events (j). The best fit model of BayAreaLike+J is highlighted in bold.

Model	LnL	AIC	AIC_wt	# parameters	d	e	J
DEC	-258.8	521.7	1.80E-21	2	0.0055	0.014	0
DEC+J	-216	438	0.0027	3	0.0012	1.00E-12	0.013
DIVALIKE	-252.4	508.7	1.20E-18	2	0.0058	1.00E-12	0
DIVALIKE+J	-218.3	442.6	0.0003	3	0.0014	1.00E-12	0.013
BAYAREALIKE	-280.4	564.8	8.00E-31	2	0.0059	0.092	0
BAYAREALIKE+J	**-210.1**	**426.2**	**1**	**3**	**0.0005**	**0.0043**	**0.013**

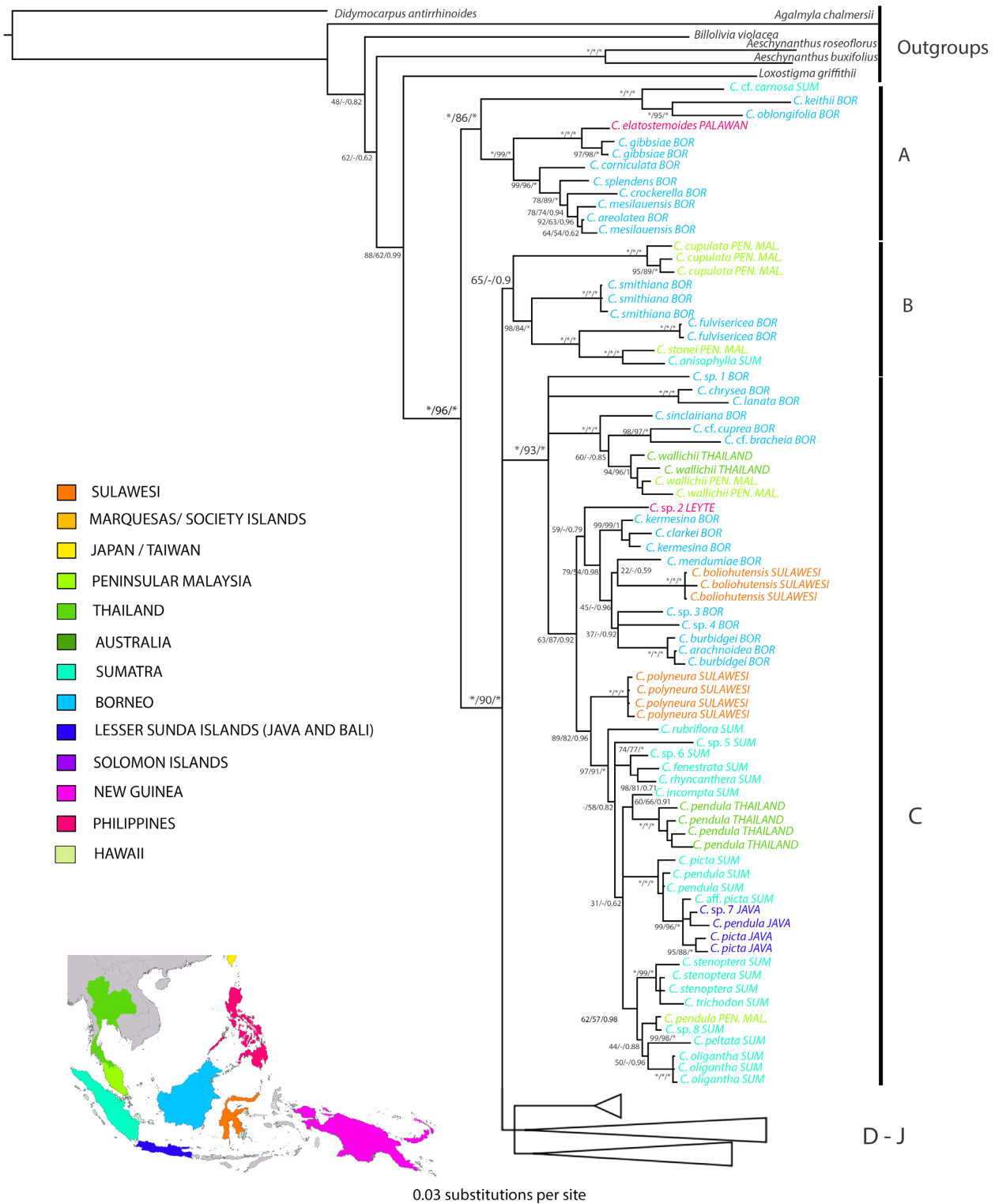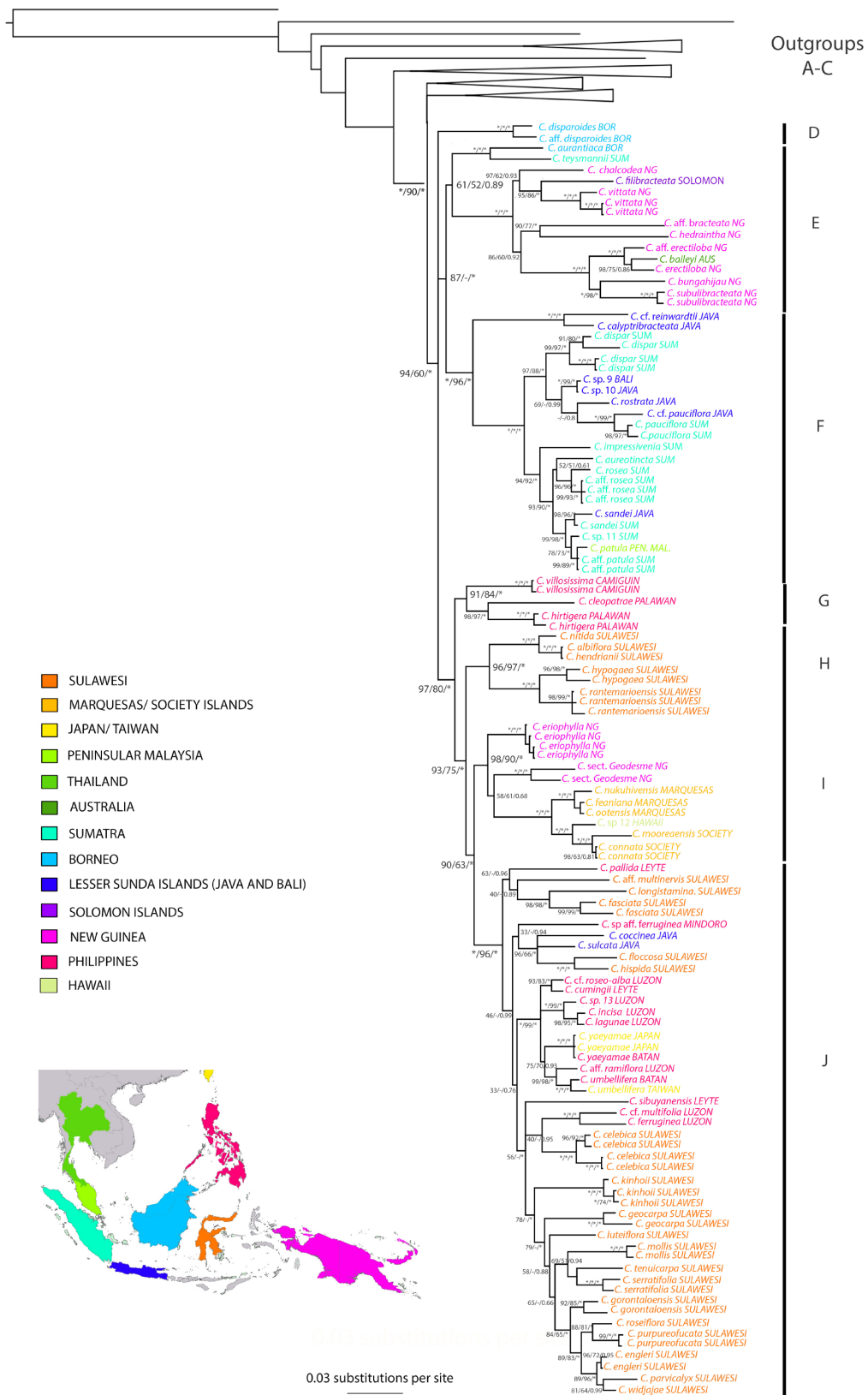LnL = Log Likelihood, AIC = Akaike Information Criterion, AIC wt = AIC weight

Figure 4. Bayesian inference tree of *Cyrtandra* based on the combined ITS, *psb*A-*trn*H, *rpl*32-*trn*L, *trn*L-F, and *mat*K regions and highlighting the three most basal clades (A, B and C). Node support is indicated as Maximum likelihood Bootstrap support (ML-BS), Maximum Parsimony Bootstrap support (MP-BS), and Bayesian Posterior Probabilities (BI-PP). Maximum values (100% BS or 1.0 PP) are indicated by *. No support (< 50% BS or 0.50 PP) is indicated by -. Species names are colour-coded by geographic region. Pacific islands not shown on map.
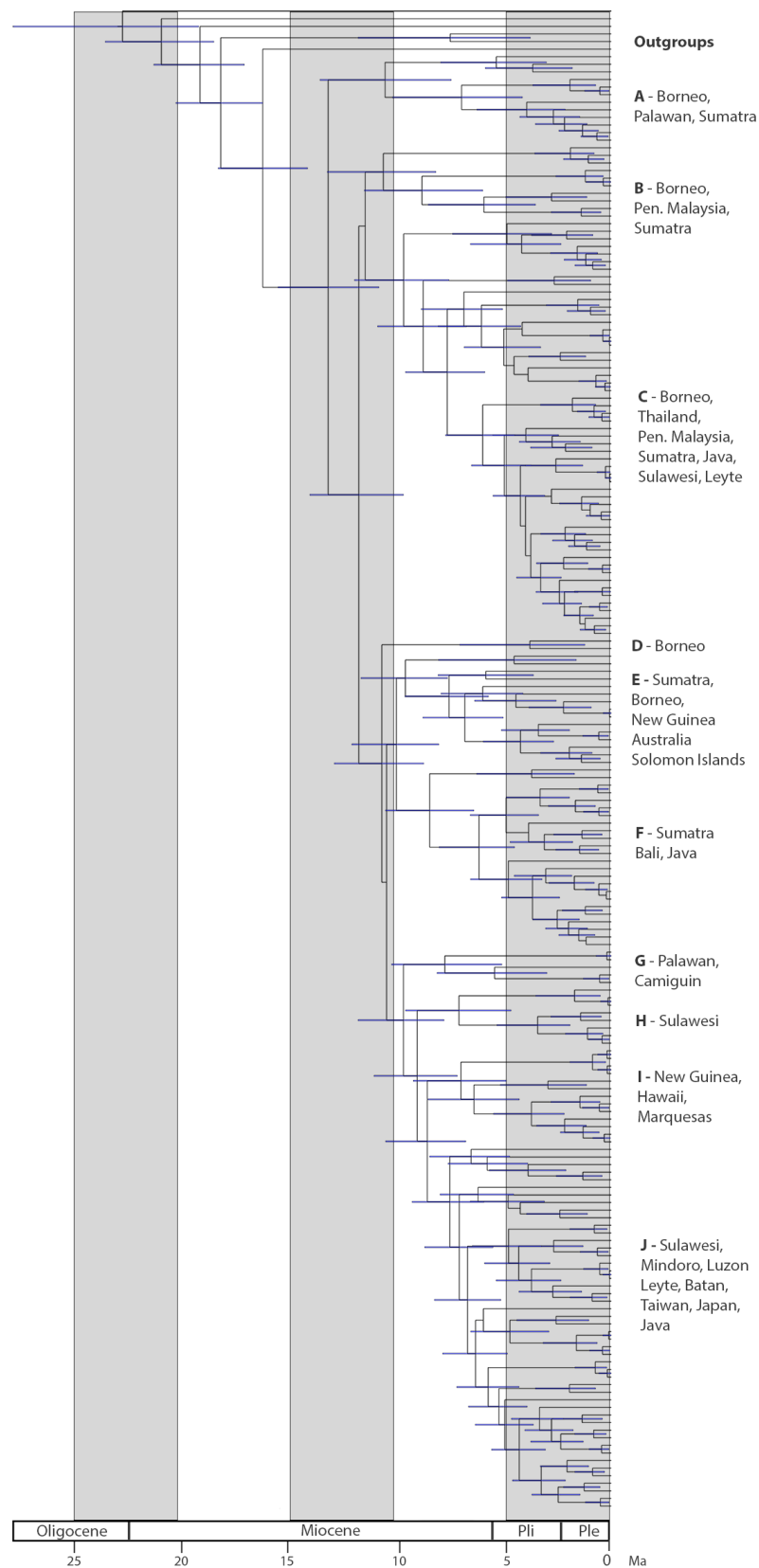
Figure 5. Bayesian inference tree of *Cyrtandra* based on the combined ITS, *psb*A-*trn*H, *rpl*32-*trn*L, *trn*L-F, and *mat*K regions, and highlighting clades D-J. Node support is indicated as Maximum Likelihood Bootstrap Support (ML-BS), Maximum Parsimony Bootstrap Support (MP-BS), and Bayesian Posterior Probabilities (BI-PP). Maximum values (100% BS or 1.0 PP) are indicated by *. No support (< 50% BS or 0.50 PP) is indicated by -. Species names are colour-coded by geographic region. Pacific islands not shown on map.

Figure 6. Maximum clade credibility tree of *Cyrtandra* based on a BEAST analysis of the combined ITS, *psb*A-*trn*H, *rpl*32-*trn*L, *trn*L-F, and *mat*K regions. Mean divergence time estimates are shown as millions of years ago (Ma), with the blue boxes showing the 95% highest posterior density (HPD). Pliocene is abbreviated to Pli and Pleistocene to Ple in the Time Scale below the tree.
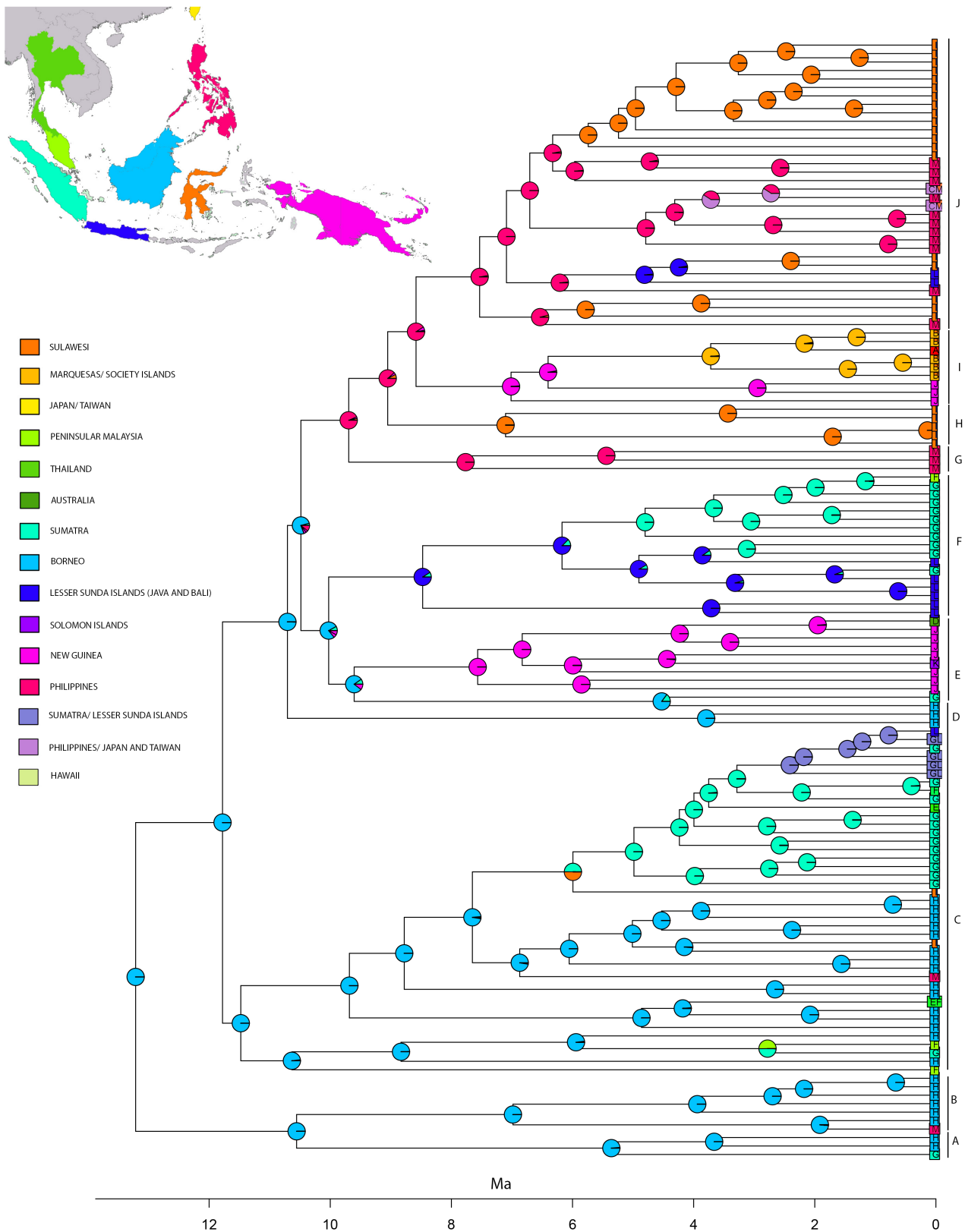
Figure 7. Ancestral range estimation for *Cyrtandra* based on the ultrametric tree produced in BEAST, and the best model determined by BioGeoBEARS (BayArea-like +J). Areas are colour-coded for the 13 geographic regions used in the analysis. Pacific islands not shown on map. Pie graphs at each node indicate the probability of a given area (or combined areas).
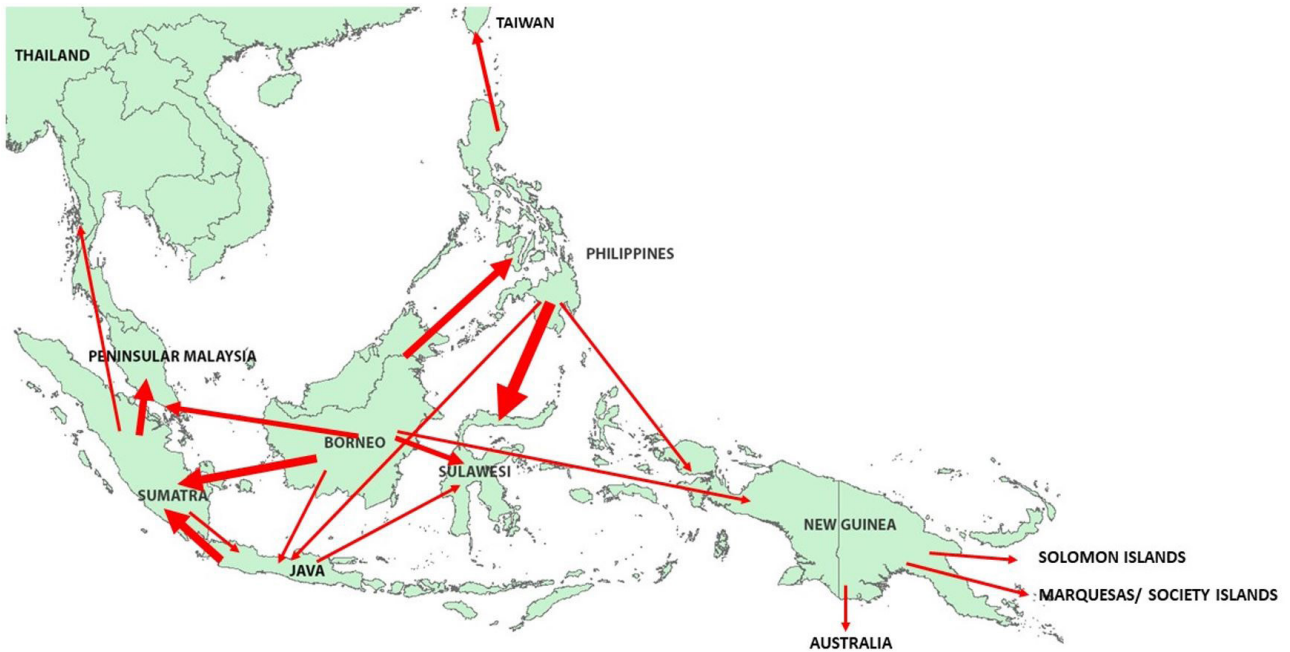
Figure 8. Summary of dispersal events in *Cyrtandra* estimated using a Biogeographic Stochastic Mapping (BSM) analysis in BioGeoBEARS based on the best fit model of BayArea+J (full results given in Table 6). The weight of each line indicates the number of predicted dispersal events (both founder and range expansion). All event counts of 0.9 and above are included.

Table 6. Summary of all dispersal counts for *Cyrtandra* averaged across 100 BSMs in BioGeoBEARS. Mapping was performed using parameters from the best-fit model of BayArea-like+J. Colour temperature indicates the frequency of events, with warmer colours indicating more common events (Red = >2.9, Orange = 2.0-2.9, Yellow = 0.9-1.9, Green = < 0.9). The ancestral states (i.e., where the lineage dispersed from) are given in the row, and the descendant states (where the lineage dispersed to) are given in the column.

FROM	TO	A Hawaii	B Marquesa/SI	C Japan/Taiwan	D Australia	E Thailand	F P. Malaysia	G Sumatra	H Borneo	I Sulawesi	J New Guinea	K Solomon	L Java/Bal	M Philippines	TOTALS	As %
A	Hawaii	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0.1	0.3
B	Marquesas/ Society Is	1.0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	2.8
C	Japan/ Taiwan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	Australia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	Thailand	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0.5	1.4
F	Pen. Malaysia	0	0	0	0	0.5	0	0.5	0	0	0	0	0	0	1.0	2.8
G	Sumatra	0	0	0	0	1.0	2.5	0	0.1	0.5	0.1	0	1.2	0	5.4	15.3
H	Borneo	0	0	0	0	0.5	2.0	3.0	0	1.5	0.9	0	0.9	2.7	11.5	32.7
I	Sulawesi	0	0	0	0	0	0	0.5	0	0	0	0	0	0.2	0.7	2.0
J	New Guinea	0	0.9	0	1	0	0	0	0.1	0.1	0	1	0.1	0.2	3.4	9.7
K	Solomon Islands	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	Java and Bali	0	0	0	0	0	0	3.0	0	1.0	0.1	0	0	0.1	4.2	11.9
M	Philippines	0	0	1.5	0	0	0	0	0.1	3.9	0.9	0	1	0	7.4	21.0
TOTALS		1	1	1.5	1	2	5	7	0.3	7	2	1	3.2	3.3	35.3	
As %		2.8	2.8	4.2	2.8	5.7	14	20	0.8	20	5.7	2.8	9.1	9.3		100

widespread. In contrast, sympatric Malesian *Cyrtandra* are more likely to be from distantly related lineages (Bramley et al. 2004a), and this is reflected by the higher level of floral divergence (Fig. 2) and apparent lack of hybridisation amongst sympatric species, all of which is congruent with pre-zygotic barriers playing a large role in maintaining species boundaries.

The majority of the 36 named taxa that were represented by more than one sample resolved as monophyletic with strong support, including two species with multi-island distributions, *C. umbellifera* from Taiwan and the northern Philippines (Clade J, Fig. 5) and *C. sandei* from Java and Sumatra (Clade F, Fig. 5). There were four cases where morphologically similar species pairs resolve as paraphyletic with respect to each other, such as the red-flowered *C. clarkei* and *C. kermesina* from Sabah (Clade B, Fig. 4). We might expect to see examples such as this in a recently evolved group where weak gene flow may be a barrier to regaining monophyly following speciation, as has been recorded elsewhere in Gesneriaceae (Hughes et al. 2005). The most striking examples of polyphyly are seen in the widespread and morphologically variable *C. pendula* and *C. picta,* distributed across a number of islands. Given patterns elsewhere in the genus, it seems likely that these species names do not represent natural groups but rather a phenetic assemblage which will need to be addressed through further collecting and revisionary work.

Cyrtandra species in New Guinea appear to have much wider distributions than are typically found elsewhere in Malesia (Atkins et al. 2019). This may indicate a shift in dispersal ability as so many of the New Guinea species have fleshy fruits. Alternatively, this may signal that these taxa represent very recent radiations that have not yet been subjected to range contractions or extinction.

Divergence times

The origin and early diversification of *Cyrtandra* in the region dates to the mid Miocene (crown age 13 Ma), and most of the current diversity in Southeast Asia is the result of speciation in the last 5 Ma (Fig. 6). These dates are similar to those reported for *Cyrtandra* in Roalson and Roberts (2016) and Johnson et al. (2017) and significantly younger than those reported in Clark et al. (2008, 2009). These last, much earlier, dates were based strictly on the geological ages of islands, an approach which has been shown to be problematic (Renner 2005, Heads 2011). The slightly younger stem age recovered in the present analysis is likely due to the inclusion of *Loxostigma,* an outgroup taxon that is more closely related to *Cyrtandra* than those used in earlier *Cyrtandra*-focused studies.

This pattern of origin in the mid Miocene and young species, mostly the result of speciation in the last 5 My, is reported in other Southeast Asian taxa such as *Begonia* (Thomas et al. 2012) and *Aglaia* (Grudinski et al. 2014), and it is highlighted by de Bruyn et al. (2014) in their meta-analysis of regional biodiversity. There are a number of geological factors which are likely to have been drivers of diversification

during this period. Approximately 23 Ma the Sunda and Sahul shelves moved closer together, creating land in the centre of the region for the first time (Hall 2002, 2012a, b). The subsequent rapid orogenesis on key islands such as Sulawesi and New Guinea (Hall 2002, Hall 2012a, b) also created new habitat. Finally, the climate and sea-level fluctuations of the Pleistocene resulted in cyclic vicariance with frequent habitat fragmentations and amalgamations (Voris 2000, Woodruff 2010, Cannon 2012, Morley 2012).

The mean diversification rate for *Cyrtandra* in Southeast Asia is 0.49 net speciation events per million years, significantly higher than the rate of 0.089 calculated by Magallon and Sanderson (2001) for angiosperms as a whole. The rates of Southeast Asian *Cyrtandra* diversification are comparable to that of the ecologically similar mega-diverse genus *Begonia* in the Neotropics (0.5) and in Asia (0.61) (Moonlight et al. 2015). They are slightly slower than in Pacific *Cyrtandra,* which has a rate of 0.68 (Roalson and Roberts 2016), and significantly slower than lineages of Hawaiian *Cyrtandra,* in which diversification rates as high as 3.5 are reported (Johnson et al. 2019). In the case of the Pacific radiation, Roalson and Roberts (2016) suggested that geography may have played a significant role in the rapid diversification of taxa, with the emergence of many island archipelagos in the last 5 Ma. Additionally, a transition to fleshy fruits may have aided long-distance dispersal by avian frugivores from source areas in Southeast Asia, followed by diversification in newly colonised island regions.

Ancestral range estimation

The island of Borneo emerges as the most likely ancestral area for the genus and for many of the early diverging clades of *Cyrtandra* (Fig. 7). Burtt (2001) speculated that Borneo represented the 'original heartland' of the genus based on its high species numbers (c. 200) and the abundant morphological diversity seen here, including richly developed anatomical characters such as sclereids and tracheoids, which 'decrease in all directions' from this centre. The combination of Borneo's large area, relatively stable geological history (Hall 2012, De Bruyn et al. 2014), and extensive areas of rainforest, even during glacial maxima (Cannon 2012), offer a compelling explanation for this lineage accumulation and *in-situ* diversification. The majority of samples from Borneo in this analysis are from NE Borneo. Increased sampling from elsewhere on the island, particularly the under-collected areas of Kalimantan, will help clarify whether Borneo's important role in the development of biogeographic patterns in the region, is largely due to the key role of NE Borneo's three highest mountains as significant rainforest refugia (as reported for birds; Sheldon 2016) or whether it is more generally attributable to the island as a whole.

With an estimated ancestral range on the Sunda Shelf, *Cyrtandra* is another example of the increasingly well-documented movement of taxa from the west to east of Southeast Asia, particularly in rainforest lineages (Su and Saunders 2009, Richardson et al. 2012, Thomas et al. 2012, Grudinski et al. 2014, Crayn et al.

2015). There are examples of dispersal in the opposite direction, such as in Proteaceae (Barker et al. 2007) and Myrtaceae (Sytsma et al. 2004), but there is a distinct asymmetry (Richardson et al. 2012, Crayn et al. 2015). The west-to-east dispersal appears to have been particularly prevalent from the mid-Miocene onwards as warmer and wetter conditions prevailed, rainforest expanded and extant land emerged east of Wallace's Line (Richardson et al. 2012, Grudinski et al. 2014, Crayn et al. 2015).

The earliest example of a Sahul–Sunda long distance dispersal in this study is within Clade E at 9.6 Ma (7.64-11.68 Ma, 95% HPD) from Sunda to Sahul. This is comfortably within the time-frame of Sahul–Sunda disjunction events compiled by Crayn et al. (2015). Many of the *Cyrtandra* taxa east of Wallace's Line are characterised by fleshy berries as opposed to the predominantly dry indehiscent capsules of the Sunda shelf taxa (Fig. 1; Burtt 2001, Johnson et al. 2017). This change in fruit morphology is possibly associated with a transition in dispersal mode from small mammal dispersal to bird dispersal (Gillett 1967, Burtt 2001). A zoochorous dispersal mode is presented by Crayn et al. (2015) as being the most prevalent for Sunda–Sahul dispersals, with 90% of ancestral species possessing zoochorous propagules, and it seems likely that the transition to fleshier berries has facilitated dispersal of *Cyrtandra* across the region. As the majority of the species in the Sahul clade (Clade E), including *C. vittata* and *C. bungahijau* from New Guinea, *C. baileyi* from Australia, and *C. subulibractea* from the Solomon Islands, have fleshy fruits (Gillett 1975, Atkins et al. 2019), it seems likely that it is the ancestral state for this clade.

Biogeographic patterns

Borneo and the islands of the Sunda shelf

Borneo is the source of the highest number of dispersal events for Malesian *Cyrtandra*, with dispersals to the Philippines, Sulawesi, Sumatra, Peninsular Malaysia, Java, and New Guinea originating here (Fig. 8). This is consistent with De Bruyn et al.'s (2014) characterisation of the island as an evolutionary hotspot defined not only in terms of high species numbers and *in situ* diversification but also subsequent emigration. High dispersal levels between the islands on the Sunda shelf reflect the shared geological history of these continental islands, which would have formed continuous land during at least some of the glacial maxima (Voris 2000, Hall 2012). At these times there would also have been more extensive areas of rainforest (Cannon et al. 2009, 2014, Cannon 2012), facilitating exchange and dispersal in a wet forest genus like *Cyrtandra.* There are only two samples from the Sunda shelf that fall within the large, predominantly Wallacean, clade (Clade J, Fig 5), and these are both from Central Java, confirming Java's position as anomalous, with links both to the Sunda shelf and Wallacea, as reported by Van Welzen et al. (2011) in their phytogeographic study of the region.

Philippines

The Philippines represent an important secondary source area for *Cyrtandra,* as well as a stepping-stone for dispersal to some of the more distant regions such as Taiwan and Japan and New Guinea (Fig. 8, Table 6). Dispersal events from the Philippines were recovered south to Sulawesi, north to Taiwan and southern Japan, east to New Guinea, and even south-west to Java. The very different position of the Philippine islands 10 Ma, when *Cyrtandra* was diversifying, with southern Philippine islands such as Mindanao located much closer to the equator (Hall 2002, Hughes et al. 2015), offers an explanation for the key role of the Philippines as a route through the region. The Philippines is an area of high species diversity for the genus, with c. 150 species already recorded and new species still being described (Olivar et al. in press). Species from these islands are morphologically very diverse and there are a number of species with fleshy fruits in the Philippines, notably *C. hirtigera* from Palawan and *C. fragilis* from Negros and Mindanao, which is congruent with the high vagility of lineages in the Philippine clades. For *Cyrtandra*, the earliest diverging branch of Philippine taxa are from Palawan and Camiguin (Clade G), dating to just before 10 Ma, suggesting that the Philippines were colonised relatively early in the diversification of the genus and that both short and longer distance dispersal from Borneo played a part in the colonisation of the archipelago. Our results provide some support for the theory that Palawan, or some part of it, could have been above sea level significantly earlier than the start of the Pliocene c. 5 Ma, as proposed for the Palawan Ark Hypothesis (Blackburn et al. 2010, Siler et al. 2012). These results would also require Camiguin to be above sea level earlier than the estimated 2 Ma maximum age for this island (Steppan et al. 2003), although incomplete sampling, particularly from nearby islands in the archipelago, could also explain the discrepancies in dates.

Sulawesi

Sulawesi is an area of significant immigration, being the recipient of 20% of all dispersals, the joint highest in our analysis (Table 6), with dispersals from Java, the Philippines and Borneo. In contrast, dispersal events from Sulawesi to surrounding islands were very infrequent (0.5 events to Sumatra and 0.2 events to the Philippines, Table 6, Fig. 8). It is notable that although fleshy fruited *Cyrtandra* species, such as the unusual epiphytic *C. purpurea,* occur on Sulawesi, the majority of species, and all of the most common species, on the island, such as *C. hypogaea, C. polyneura* and *C. kinhoii,* are characterised by the drier, tough-walled fruits typical of the Sunda shelf. This low level of emigration and the relative insignificance of Sulawesi in terms of a dispersal route across the region is, however, also seen in other groups such as *Begonia* (Thomas et al. 2012). Sulawesi is encircled by biogeographic boundaries, including Wallace's and Huxley's line to the west, and Weber's and Lydekker's to the east, suggesting that there are real barriers to dispersal in this area. However, immigration on to the

island, particularly from the Philippines and Borneo across the western lines, has clearly occurred more than once in *Cyrtandra*. Sulawesi's highly dynamic geological history (Hall et al. 2012a, Stelbrink et al. 2012) and the resultant increase in habitat diversity and available niche space for colonising species, offers a possible explanation for the high level of immigration and establishment on the island. Dispersal back across Wallace's line from Sulawesi and successful establishment on the Sunda shelf islands appears to have been difficult for *Cyrtandra,* possibly due to niche pre-emption in the older, more established forests of those islands. Increased sampling of *Cyrtandra* from the islands east of Sulawesi, particularly from the Moluccas and New Guinea, will help illuminate whether there is a barrier in this direction or whether this is an artefact of sampling.

New Guinea and the Pacific

New Guinea is resolved as the most likely source of dispersals to Australia, the Solomon Islands, and French Polynesia for *Cyrtandra* (Fig. 8, Table 6). West-to-east dispersal patterns have been well documented from New Guinea into the eastern Pacific (Keast 1996). *Cyrtandra* is notable for being the only Malesian Gesneriaceae genus with a distribution that extends significantly into the Pacific (Hilliard and Burtt 2002). The large Malesian genera *Aeschynanthus* and *Agalmyla* have wind-dispersed seeds and, despite being highly diverse in New Guinea, do not extend beyond the Solomon Islands and the Louisade Archipelago (Hilliard and Burtt 2002), respectively. Johnson et al. (2017) reported strong asymmetry in the direction of founder events in their study of *Cyrtandra* in the Pacific, with the majority of dispersals occurring from a west-to-east direction, which they postulated to be the result of bird dispersal. The routes to Australia and the Solomon Islands found here were reported by Johnson et al. (2017) and were suggested by Gillett (1975) based on his extensive knowledge of the *Cyrtandra* of New Guinea and the Pacific. He reported strong morphological affinities between the *Cyrtandra* of New Guinea and the Bismarck Archipelago and Solomon Islands, and an attenuation of morphological diversity with increasing distance from New Guinea into the Pacific (Gillett 1975). The longest-distance dispersal event in this study is from New Guinea (Clade I, from Mt Jaya in the far western part of the main cordillera) to the Marquesas/Society Islands. However, this result is most likely an artefact of the relatively low level of sampling from the South Pacific in this study. In the study by Johnson et al. (2017), which included higher density sampling of Pacific taxa, the Marquesan lineage is the result of a single dispersal event from Samoa, while the Society Islands taxa are the result of two dispersal events, one from Fiji and one from Samoa.

Conclusions

Using a robust and well sampled phylogenetic tree to study evolutionary relationships and biogeographical processes in Southeast Asian *Cyrtandra*, we found evidence in support of (i) Borneo as the most likely ancestral area for the genus, (ii) west-to-east dispersal across the region and into the Pacific, (iii) the Philippines as an important secondary source area and stepping stone to Wallacea, Taiwan and Japan, and New Guinea, and (iv) a mid-Miocene origin for the genus with most of the extant diversity being the result of speciation in the last 5 Ma. These patterns are increasingly well-documented and are beginning to form an emerging paradigm for Southeast Asian plant biogeography. The present study has provided further insight into the fundamental questions of when and where plant diversification took place in Southeast Asia and the role of geo-tectonic and climatic processes in shaping the floristic composition of the area and setting the stage for significant species diversifications. Further work is needed to understand niche evolution and the genomic basis of adaptation to unravel how the massive species richness of *Cyrtandra* evolved and how it is maintained.

Frontiers of Biogeography 2020, 12.1, e44814   15

Supplementary Materials

The following materials are available as part of the online article from https://escholarship.org/uc/fb

Table S1. Taxon list for samples in the current study.

References

Atkins, H. & Cronk, Q.C.B. (2001) The genus *Cyrtandra* (Gesneriaceae) in Palawan, Philippines, Edinburgh Journal of Botany 58, 443–458.

Atkins, H., Preston, J., & Cronk, Q.C.B. (2001) A molecular test of Huxley's line: *Cyrtandra* (Gesneriaceae) in Borneo and the Philippines. Biological Journal of the Linnean Society, 72, 143–159.

Atkins, H.J., Bramley, G.L.C. & Clark, J.R. (2013) Current knowledge and future directions in the taxonomy of *Cyrtandra* (Gesneriaceae), with a new estimate of species number. Selbyana, 31, 157–165.

Atkins, H.J., Heatubun, C.D., Galloway, L. & Bramley, G.L.C. (2019) Two new species, *Cyrtandra bungahijau* and *C. vittata*, and notes on *Cyrtandra* (Gesneriaceae) from Yapen Island, Indonesia. Kew Bulletin, 74, 29. doi.org/10.1007/S12225-019-9817-2

Baker, W.J. & Couvreur, T.L. (2012) Biogeography and distribution patterns of Southeast Asian palms. In: Biotic evolution and environmental change in southeast Asia. (ed. by D. Gower, K. Johnson, J. Richardson, B. Rosen, L.Ruber, S. Williams), pp. 164–190. Systematics Association Special Volume Series. Cambridge University Press, UK.

Barker, N.P., Weston, P.H., Rutschmann, F. & Sauquet, H. (2007) Molecular dating of the "Gondwanan" plant family Proteaceae is only partially congruent with the timing of the break-up of Gondwana. Journal of Biogeography, 34, 2012–2027.

Blackburn, D.C., Bickford, D.P., Diesmos, A.C., Iskandar, D.T. & Brown, R.M. (2010) An ancient origin for the enigmatic flat-headed frogs (Bombinatoridae: Barbourula) from the Islands of Southeast Asia. PLoS ONE, 5, doi. org/10.1371/journal.pone.0012090

Bramley, G.L.C. & Cronk, Q.C.B. (2003) The *Cyrtandra* (Gesneriaceae) species of Mount Kerinci, Sumatra. Harvard Papers in Botany 7, 407-421.

Bramley, G.L.C., Pennington, R.T., Zakaria, R., Tjitrosoedirdjo, S.S. & Cronk, Q.C.B. (2004a) Assembly of tropical diversity on a local scale: *Cyrtandra* (Gesneriaceae) on Mount Kerinci, Sumatra. Biological Journal of the Linnean Society, 81, 49-62.

Bramley, G.L.C., Weber, A. & Cronk, Q.C.B. (2004b) The genus *Cyrtandra* (Gesneriaceae) in Peninsular Malaysia and Singapore. Edinburgh Journal of Botany 60, 331–360.

Brooks, A.T.M., Mittermeier, R.A., da Fonseca, G.A.B., Gerlach, J., Hoffmann, M., Lamoreux, J.F., Mittermeier C.G., Pilgrim, J.D. & Rodrigues, A.S.L. (2006) Global biodiversity conservation priorities. Science, 313, 58–61.

Burtt, B.L. (2001) A survey of the genus *Cyrtandra* (Gesneriaceae). Phytomorphology, Golden Jubilee Issue, 393–404.

Cannon, C.H. (2012) Quarternary dynamics of Sundaland forests. In: Biotic evolution and environmental change in southeast Asia. (ed. by D. Gower, K. Johnson, J. Richardson, B. Rosen, L.Ruber, S. Williams), pp. 115-137. Systematics Association Special Volume Series. Cambridge University Press, UK.

Cannon, C.H., Morley, R.J. & Bush, A.B.G. (2009) The current refugial rainforests of Sundaland are unrepresentative of their biogeographic past and highly vulnerable to disturbance. Proceedings of the National Academy of Sciences USA, 106, 11188–11193.

Cannon, C.H., Hijmans, R.J., Slik, J.W.F., van Welzen, P.C., Saw, L.G., Piessens, T., & Raes, N. (2014) Historical distribution of Sundaland's Dipterocarp rainforests at Quaternary glacial maxima. Proceedings of the National Academy of Sciences USA, 111, 16790–16795.

Clark, J.R., Wagner, W.L. & Roalson, E.H. (2009) Patterns of diversification and ancestral range reconstruction in the southeast Asian-Pacific

angiosperm lineage *Cyrtandra* (Gesneriaceae). Molecular Phylogenetics and Evolution, 53, 982–94.

Clark, J.R., Ree, R.H., Alfaro, M.E., King, M.G., Wagner, W.L. & Roalson, E.H. (2008) A comparative study in ancestral range reconstruction methods: retracing the uncertain histories of insular lineages. Systematic Biology, 57, 693–707.

Condamine, F.L. Nagalingum, N.S., Marshall, C.R. & Morlon, H. (2015) Origin and diversification of living cycads: a cautionary tale on the impact of the branching process prior in Bayesian molecular dating. BMC Evolutionary Biology 15, 65 doi: 10.1186/s12862-015-0347-8

Cowie, R.H. & Holland, B. (2006) Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. Journal of Biogeography, 33, 193–198.

Crayn, D.M., Costion, C. & Harrington, M.G. (2015) The Sahul-Sunda floristic exchange: Dated molecular phylogenies document Cenozoic intercontinental dispersal dynamics. Journal of Biogeography, 42, 11–24.

Cronk, Q.C.B., Kiehn, M., Wagner, W.L. & Smith, J.F. (2005) Evolution of *Cyrtandra* (Gesneriaceae) in the Pacific Ocean: the origin of a supertramp clade. American Journal of Botany, 92, 1017–1024.

De Bruyn, M., Stelbrink, B., Morley, R.J., Hall, R., Carvalho, G.R., Cannon, C.H. & Von Rintelen, T. (2014) Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. Systematic Biology, 63, 879–901.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology, 7, 214.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution, 29, 1969–1973.

Dupin, J., Matzke, N.J., Särkinen, T, Knapp, S., Olmstead, R.G., Bohs, L. & Smith, S.D. (2017) Bayesian estimation of the global biogeographical history of the Solanaceae. Journal of Biogeography, 44, 887–899.

Gillett, G.W. (1967) The genus *Cyrtandra* in Fiji. Contributions to the United States Herbarium, 37, 107–158.

Gillett, G.W. (1975) *Cyrtandra* (Gesneriaceae) in the Bismarck Archipelago and Solomon Islands. Kew Bulletin, 30, 371–412.

Grudinski, M., Wanntorp, L., Pannell, C.M. & Muellner-Riehl, A.N. (2014) West to east dispersal in a widespread animal-dispersed woody angiosperm genus (*Aglaia*, Meliaceae) across the Indo-Australian Archipelago. Journal of Biogeography, 41, 1149–1159.

Hall, R. (2002) Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: Computer-based reconstructions, model and animations. Journal of Asian Earth Sciences, 20, 353–431.

Hall, R. (2012a) Late Jurassic–Cenozoic reconstructions of the Indonesian region and the Indian Ocean. Tectonophysics, 570, 1–41.

Hall, R. (2012b) Sundaland and Wallacea: geology, plate tectonics and palaeogeography. In: Biotic evolution and environmental change in southeast Asia. (ed. by D. Gower, K. Johnson, J. Richardson, B. Rosen, L. Ruber, S. Williams), pp. 32–78. Systematics Association Special Volume Series. Cambridge University Press, UK.

Heads, M. (2011) Old taxa on young islands: a critique of the use of island age to date island-endemic clades and calibrate phylogenies. Systematic Biology, 60, 204–218.

Hilliard, O.M. & Burtt, B.L. (2002) The genus *Agalmyla* (Gesneriaceae-Cyrtandroideae) Edinburgh Journal of Botany, 59, 1-210.

Ho, S.Y.W. & Phillips, M.J. (2009) Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. Systematic Biology, 58, 367-380.

Hoover, J.D., Kumar, S., James, S.A., Leisz, S.J. & Laituri, M. (2017) Modeling hotspots of plant diversity in New Guinea. Tropical Ecology, 58, 623–640.

Hughes, M., Möller, M., Bellstedt, D.U., Edwards, T.J. & De Villiers, M. (2005) Refugia, dispersal and divergence in a forest archipelago: a study of *Streptocarpus* in eastern South Africa. Molecular Ecology, 14, 4415-4426.

Hughes, M., Rubite, R.R., Blanc, P., Chung, K.-F. & Peng, C.-I. (2015) The Miocene to Pleistocene colonization of the Philippine archipelago by *Begonia* sect. Baryandra (Begoniaceae) American Journal of Botany, 102, 695–706.

Hughes, M., Peng, C.I., Lin, C.W., Rubite, R.R., Blanc, P. & Chung, K.F. (2018) Chloroplast and nuclear DNA exchanges among *Begonia* sect. Baryandra species (Begoniaceae) from Palawan Island, Philippines, and descriptions of five new species. PLoS ONE, 13, 1–29.

Johns, R. (1995) Malesia - an introduction. Curtis's Botanical Magazine, 12, 52-62.

Johnson, M., Price, D.K., Price, J. & Stacy, E.A. (2015) Postzygotic barriers isolate sympatric species of *Cyrtandra* (Gesneriaceae) in Hawaiian montane forest. American Journal of Botany, 102, 1870–1882.

Johnson, M.A., Clark, J.R., Wagner, W.L. & McDade, L.A. (2017) A molecular phylogeny of the Pacific clade of *Cyrtandra* (Gesneriaceae) reveals a Fijian origin, recent diversification, and the importance of founder events. Molecular Phylogenetics and Evolution, 116, 30–48.

Johnson, M.A., Pillon, Y., Sakishima, T., Price, D.K. & Stacy, E.A. (2019) Multiple colonizations, hybridization and uneven diversification in *Cyrtandra* (Gesneriaceae) lineages on Hawai'i Island. Journal of Biogeography, 46, 1178-1196.

Kartonegoro, A., Bone, R.E. & Atkins, H.J. (2018) Eleven new species of *Cyrtandra* (Gesneriaceae) from Sulawesi, Indonesia. Edinburgh Journal of Botany, 75, 173-204.

Keast, A. (1996) Pacific biogeography: patterns and processes. In: The origin and evolution of Pacific island biotas, New Guinea to eastern Polynesia: patterns and processes (ed. by A. Keast & S. E. Miller), pp. 477–512. Amsterdam. SPB Academic Publishing.

Landis, M.J., Matzke, N.J., Moore, B.R. & Huelsenback, J.P. (2013) Bayesian analysis of biogeography when the number of areas is large. Systematic Biology, 62, 789-804.

Lohman, D.J., De Bruyn, M., Page, T., von Rintelen, K., Hall, R., Ng, Peter, K.L. & von Rintelen, T. (2011) Biogeography of the Indo-Australian archipelago. Annual Review of Ecology, Evolution and Systematics, 42, 205–226.

Luna, J.A., Richardson, J.E., Nishii, K., Clark, J.L. & Möller, M. (2019) The family placement of *Cyrtandromoea.* Systematic Botany, 44, 616-630.

Madriñán, S., Cortés, A.J. & Richardson, J.E. (2013) Páramo is the world's fastest evolving and coolest biodiversity hotspot. Frontiers in Genetics, 4, 1–7.

Magallon, S. & Sanderson, M J. (2001) Absolute diversification rates in Angiosperms. Evolution, 9, 1762–1780.

Matzke, N.J. (2013) Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Frontiers of Biogeography, 5, 242–248.

Matzke, N.J. (2014) Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. Systematic Biology, 63, 951–970.

Matzke, N.J. (2016) Stochastic mapping under biogeographical models. Retrieved from http://phylo.wikidot.com/biogeobears#stochastic_mapping

Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE) 14 Nov 2010. pp. 1–8. New Orleans.

Mittermeier, R.A., Mittermeier, C.G., Brooks, T.M., Pilgrim, J.D., Konstant, W.R., da Fonseca, G.A.B. & Kormos, C. (2003) Wilderness and biodiversity conservation. Proceedings of the National Academy of Sciences USA, 100, 10309–10313.

Möller, M. & Cronk, Q.C.B. (1997) Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. American Journal of Botany, 84, 956–965.

Moonlight, P., Richardson, J.E., Tebbitt, M.C., Thomas, D.C., Hollands, R., Peng, C.-I., & Hughes, M. (2015) Continental-scale diversification patterns in a megadiverse genus: the biogeography of Neotropical *Begonia*. Journal of Biogeography, 42, 1137–1149.

Morley, R. J. (2012) A review of the Cenozoic palaeoclimate history of Southeast Asia. In: Biotic evolution and environmental change in southeast Asia. (ed. by D. Gower, K. Johnson, J. Richardson, B. Rosen, L. Ruber, S. Williams), pp. 79-114. Systematics Association Special Volume Series. Cambridge University Press, UK.

Morley, R. J. (2018) Assembly and division of the South and South-East Asian flora in relation to tectonics and climate change. Journal of Tropical Ecology, 34, 209–234.

Muellner, A.N., Pannell, C.M., Coleman, A. & Chase, M.W. (2008) The origin and evolution of Indomalesian, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales) Journal of Biogeography, 35, 1769–1789.

    18

Nauheimer, L., Boyce, P.C. & Renner, S.S. (2012) Giant taro and its relatives: a phylogeny of the large genus *Alocasia* (Araceae) sheds light on Miocene floristic exchange in the Malesian region. Molecular Phylogenetics and Evolution, 63, 43–51.

Nishii, K., Kokubugata, G., Möller, M. & Atkins, H.J. (2019) Notes on *Cyrtandra* (Gesneriaceae) from Japan, Taiwan and Batan Island (Philippines) Edinburgh Journal of Botany, 76, 333-344.

Nylander, J.A.A. (2004) MrModeltest v2. Uppsala: Evolutionary Biology Centre, Uppsala University.

Olivar, J.E., Atkins, H., Hauenschild F. & Muellner-Riehl, A. (in press) *Cyrtandra argentii*, a new species of *Cyrtandra* (Gesneriaceae) from the Philippines and a review of the *C. villosissima* group. European Journal of Taxonomy.

Pillon, Y., Johansen, J.B., Sakishima, T., Roalson, E.H., Price, D.K. & Stacy, E.A. (2013) Gene discordance in phylogenomics of recent plant radiations, an example from Hawaiian *Cyrtandra* (Gesneriaceae). Molecular Phylogenetics and Evolution, 69, 293–298.

Rambaut, A. (2007) FigTree. Online. Available: http://tree.bio.ed.ac.uk/software/figtree

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology, 67, 901-904.

Ranasinghe, S.W. (2017) Molecular species delimitation, taxonomy and biogeography of Sri Lankan Gesneriaceae. PhD thesis. University of Edinburgh.

Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction and cladogenesis. Systematic Biology, 57, 4-14.

Ree, R.H. & Sanmartin, I. (2018) Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. Journal of Biogeography, 45, 741-749.

Renner, S.S. (2005) Relaxed molecular clocks for dating historical plant dispersal events. Trends in Plant Science, 10, 550–558.

Richardson, J.E., Costion, C.M. & Muellner, A.N. (2012) The Malesian floristic interchange: plant migration patterns across Wallace's Line. In: Biotic evolution and environmental change in southeast Asia. (ed. by D. Gower, K. Johnson, J. Richardson, B. Rosen, L.Ruber, S. Williams), pp. 138-163. Systematics Association Special Volume Series. Cambridge University Press, UK.

Richardson, J.E., Bakar, A.M., Tosh, J., Armstrong, K., Smedmark, J., Anderberg, A.A., Slik, F. & Wilkie, P. (2014) The influence of tectonics, sea-level changes and dispersal on migration and diversification of Isonandreae (Sapotaceae). Botanical Journal of the Linnean Society, 174, 130-140.

Roalson, E.H. & Roberts, W.R. (2016) Diversification in Gesneriaceae. Distinct processes drive diversification in different clades of Gesneriaceae. Systematic Biology, 65,662-684.

Ronquist, F. (1997) Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Systematic Biology, 46, 195-203.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L. Suchard, M.A. & Huelsenbeck, J. P. (2012) Software for Systematics and Evolution MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology, 61, 539–542.

Sang, T., Crawford, D.J. & Stuessy, T.F. (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of Paeonia (Paeoniaceae). American Journal of Botany, 84, 1120–1136.

Schenk, J.J. (2016) Consequences of secondary calibrations on divergence time estimates. PLoS ONE, 11. doi.org/10.1371/journal.pone.0148228

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany, 94, 275–288.

Sheldon, F.H. (2016) On the biogeography of Sabah's rainforest birds. Sabah Society Journal, 33, 31-43.

Siler, C.D., Oaks, J.R., Welton, L.J., Linkem, C.W., Swab, J.C., Diesmos, A.C. & Rissler, L. (2012) Did geckos ride the Palawan raft to the Philippines? Journal of Biogeography, 39, 1217–1234.

Stamatakis, A. (2014) RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30, 1312–1313.

Steppan, J., Zawadzki C. & Heaney L.R. (2003) Molecular phylogeny of the endemic Philippine rodent *Apomys* (Muridae) and the dynamics

of diversification in an oceanic archipelago. Biological Journal of the Linnean Society, 80, 699–715.

Stelbrink, B., Albrecht, C., Hall, R. & von Rintelen, T.. (2012) The biogeography of Sulawesi revisited: is there evidence for a vicariant origin of taxa on Wallace's 'anomalous island'? Evolution, 66, 2252-2271.

Su, Y.C. & Saunders, R.M. (2009) Evolutionary divergence times in the Annonaceae: evidence of a late Miocene origin of *Pseuduvaria* in Sundaland with subsequent diversification in New Guinea. BMC Evolutionary Biology, 9, 153.

Swofford, D. (2002) PAUP - Phylogenetic analysis using parsimony (*and other methods). Sunderland, MA: Sinauer Associates.

Sytsma, K.J., Litt, A., Zjhra, M.L., Pires, J.C., Nepokroeff, M. & Conti, E. (2004) Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the southern hemisphere. International Journal of Plant Sciences, 165, S85–S105.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primer for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology, 17, 1105–1109.

Takeuchi, W.N. (2005) Introduction to the flora of Papua. In: The ecology of Papua.(ed. by Marshall, A.J. & Beehler, B.M.), pp. 269-302. Periplus Editions, Singapore.

Thomas, D. C., Hughes, M., Phutthai, T., Ardi, W.H., Rajbhandary, S., Rubite, R. & Richardson, J.E. (2012) West to east dispersal and subsequent rapid diversification of the mega-diverse genus *Begonia* (Begoniaceae) in the Malesian archipelago. Journal of Biogeography, 39, 98–113.

van Welzen, P.C., Parnell, J.N. & Slik, J.W.F. (2011) Wallace's Line and plant distributions: two or three phytogeographical areas and where to group Java? Biological Journal of the Linnean Society, 103, 531–545.

Voris, H. (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. Journal of Biogeography, 27, 1153–1167.

Webb, C.O. & Ree, R. (2012) Historical biogeography inference in Malesia. In: Biotic evolution and environmental change in southeast Asia.(ed. D. Gower, K. Johnson, J. Richardson, B. Rosen, L.Ruber, S. Williams), pp. 191-215. Systematics Association Special Volume Series. Cambridge University Press, UK.

Wiehler, H. (1983) A synopsis of neotropical Gesneriaceae. Selbyana, 6, 1–219.

Williams, E.W., Gardner, E.M., Harris, R., Chaveerach, A., Pereira, J.T. & Zerega, N.J.C. (2017) Out of Borneo: biogeography, phylogeny and divergence date estimates of *Artocarpus* (Moraceae). Annals of Botany, 119, 611–627.

Woodruff, D.S. (2010) Biogeography and conservation in Southeast Asia: how 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugial-phase biodiversity. Biodiversity and Conservation, 19, 919–941.