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Assembly of tropical plant diversity on a local scale: *Cyrtandra* (Gesneriaceae) on Mount Kerinci, Sumatra

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At a regional scale, the high species numbers (gamma diversity) of tropical forests have been explained by either a gradual accumulation of species through time (museum hypothesis) or, by contrast, rapid recent speciation in large genera. However, the origins of local rain forest diversity (alpha diversity) have been given little attention. *Cyrtandra* (Gesneriaceae), an understorey genus in the highly species-rich Indo-Malayan rain forest, has considerable capacity for producing local endemics, making it particularly suitable for studying diversity on a local scale. We sampled *Cyrtandra* species from one community on Mount Kerinci, Sumatra, and phylogenetic analyses of ITS sequences suggest that this community is an assembly of three distinct phyletic lineages: (1) a group of herbaceous or subshrub plants of Bornean affinity, (2) one member of a group of widespread shrubs forming *Cyrtandra* section *Dissimiles* and (3) a second group of shrubs. The evolutionary origin of this community is therefore not a result of rapid and recent speciation: it is assembled from species resulting from a gradual accumulation of diversity through time (museum hypothesis), although one lineage shows evidence of more recent, continuing speciation than the other two. The community includes two distantly related, apparently endemic species, but there is no evidence for a local adaptive radiation. The protection of representative species from each lineage would allow the conservation of genetic diversity.

ADDITIONAL KEYWORDS: alpha diversity – conservation – endemics – gamma diversity – Malesian flora – phytogeography – speciation – understorey genera.

INTRODUCTION

Tropical forest biodiversity has traditionally been considered from a regional perspective (e.g. Moritz *et al*., 2000; Richardson *et al*., 2001) or local ecological viewpoint (e.g. Newbery, Prins & Brown, 1998). At an evolutionary scale, Stebbins (1974) has suggested that the generation of modern forest diversity (gamma diversity), defined here in terms of numbers of species, is the result of a gradual accumulation of species through time with low extinction rates (the museum hypothesis). This hypothesis has been challenged by data showing that rapid recent speciation in large species-rich genera may be responsible for much biodiversity, as observed by Richardson *et al*. (2001) in the neotropical tree genus *Inga* (Leguminosae).

Few studies have considered the evolutionary origin of local diversity (alpha diversity), i.e. the phyletic origin of communities of related species, for instance members of the same genus. The recent speciation demonstrated by Richardson *et al*. (2001) suggests that community diversity of a genus may be generated recently. By contrast, the 'museum hypothesis' (Stebbins, 1974) would suggest that community diversity in a particular genus has accumulated gradually over time – speciation is more likely to have been ancient.

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In addition to this dimension of time, there is a second issue of the number of distant lineages that have contributed to a local community. In the first situation, in which the community has resulted from recent speciation, it is perhaps more likely to have a single origin – *in situ* radiation of a single lineage has occurred, and all species are more or less closely related. By contrast, in the second situation, in which the community diversity has accumulated gradually over time, with perhaps more ancient speciation, we suggest the community is perhaps more likely to be assembled by the migration of species from different distinct, and ancient phyletic lineages.

In order to test these ideas in the Indo-Malayan rain forest, we focused on the species-rich genus *Cyrtandra* (Gesneriaceae), which contains probably over 600 species.

The Indo-Malayan region is the second largest global expanse of rain forest (Whitmore, 1998), its largest forest areas being in the Malay Peninsula, Sumatra and Borneo (Sundaland). Sundaland is the second 'hottest' global hotspot for plants, with estimates of 15 000 endemic plant species, and 5% of the total global plant species (Myers *et al.*, 2000). In addition, it is one of the three most significant areas for all biodiversity, appearing in the top ten hotspots for all five factors tested by Myers *et al.* (2000) (numbers of endemic plants; numbers of endemic vertebrates; endemic plants/area ratio; endemic vertebrates/area ratio; remaining primary vegetation as a percentage of the original extent).

Sumatra is a large island forming part of Sundaland (Fig. 1). It has an area of 476 000 km^2 and probably 10 000 species of higher plants (Whitten *et al.*, 1997). Politically it forms part of Indonesia, a country second only to Brazil in the amount of rain forest it possesses (Whitmore, 1998). The west coast is dominated by the Barisan Mountains, uplifted as a result of thrust associated with the collision of the Indian plate with Asia about 70 Mya (Whitten *et al.*, 1997). A significant part of this range falls within the boundaries of Kerinci–Seblat National Park. This is the largest national park in Sumatra, covering approximately 1.4 million hectares, and perhaps the most undiminished of Sumatra's representative ecosystems. At its heart is Mount Kerinci, an active volcano that at *c.* 3805 m is the second highest peak in Indonesia. Kerinci is a young volcano; it forms the youngest peak in a volca-

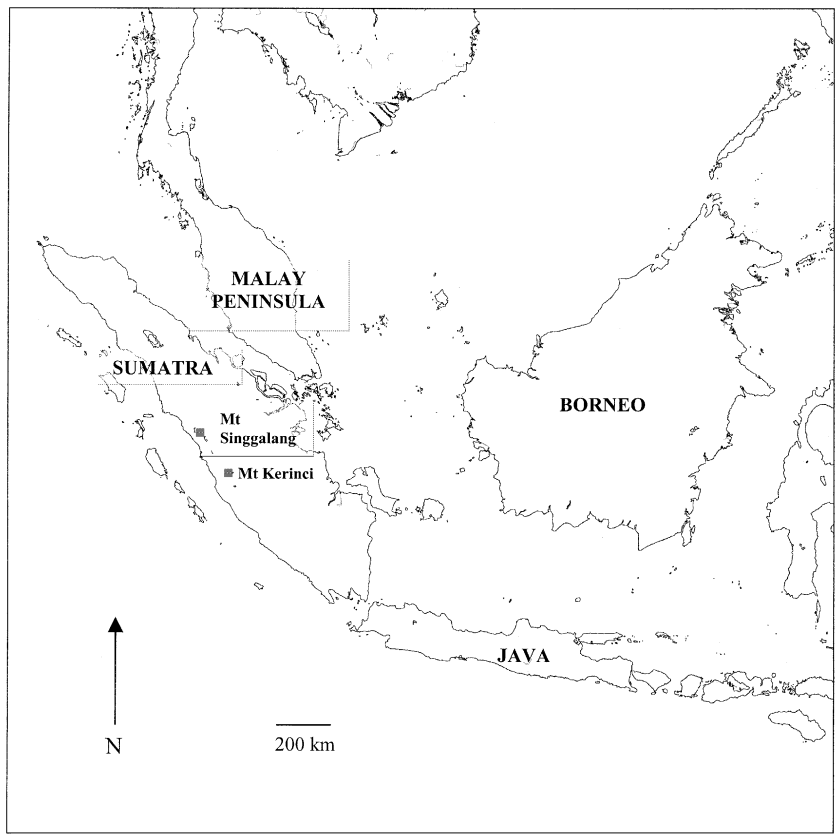

Figure 1. Map of Sundaland (Borneo, Java, Malay Peninsula and Sumatra) showing the location of Mount Kerinci and Mount Singgalang on Sumatra.

nic formation breaking through the Barisan range in an E–W direction (Jacobs, 1958). The last major period of mountain building in the range occurred about three Mya (Whitten *et al*., 1997), suggesting that the still active Kerinci has a late Pliocene origin. Emissions of volcanic gases and ash mean that the vegetation ceases at an altitude of about 3400 m. Mount Kerinci forms an easily definable geographical area with primary rain forest remaining, making it an appropriate location for study.

Cyrtandra (Gesneriaceae), with over 600 species, is a highly suitable genus for the study of tropical forest plant community diversity. It occurs as an important understorey element in primary rain forest, with habit ranging from epiphytes, herbs and shrubs, and occasionally small trees. It is distributed from the Nicobar Islands in the west, and southern Thailand in the north, throughout Malesia to the Philippines, Taiwan, southern Ryukyu Islands, south-east to Queensland and the Loyalty Islands and east to the high islands of the Pacific to the Hawaiian Islands. One of its most remarkable features is its capacity for producing local endemics, making it particularly suitable to study local species richness. Furthermore, one of its centres of species diversity is Sundaland (Burtt, 2001). Fieldwork carried out by Radhiah and Cronk in 2000 on Mount Kerinci and other locations in West Sumatra has led to a taxonomic revision of the *Cyrtandra* species of Mount Kerinci (Bramley & Cronk, 2003). This study recognized 13 species of *Cyrtandra* on Mount Kerinci, two of which are apparently endemic to Mount Kerinci, four to Kerinci and the immediate surrounding area and four that occur more widely in West Sumatra. An additional two species occur also in West Sumatra and are also reported in Java. Only one species, *C. anisophylla* C.B.Clarke, appears to be widespread in Sumatra. Herbarium collection data suggest that altitudinal zonation of these species of *Cyrtandra* on Mount Kerinci is weak and that they form part of one broad community (Fig. 2).

A number of studies have shown the presence or absence and type of foliar sclereids to vary significantly in *Cyrtandra*, and that they have the potential to be important taxonomic characters (Bokhari & Burtt, 1970; Burtt & Bokhari, 1973; Atkins & Cronk, 2001). Leaves from Sumatran *Cyrtandra* species were therefore examined for sclereids, in order to assess the relevance of this character to this sample.

Cyrtandra and other genera in the Gesneriaceae have been the subjects of systematic and biogeographical studies using the internal transcribed spacer (ITS) region of 18–26S nuclear ribosomal DNA (Atkins, Preston & Cronk, 2001 [*Cyrtandra*]; Möller & Cronk, 1997 [*Saintpaulia*, *Streptocarpus*]; Denduangboripant, Mendum & Cronk, 2001 [*Aeschynanthus*]). These studies have shown that ITS is particularly suitable for species-level phylogenetics in this family. Using an ITS phylogeny of *Cyrtandra* species from Mount Kerinci and West Sumatra, and species representing other areas within the distribution, we test the following two hypotheses:

1. the *Cyrtandra* community of Mount Kerinci will have a single origin with rapid recent radiation, appear in one clade in the phylogenetic tree and be the result of recent speciation;
2. the Kerinci *Cyrtandra* community will have multi-

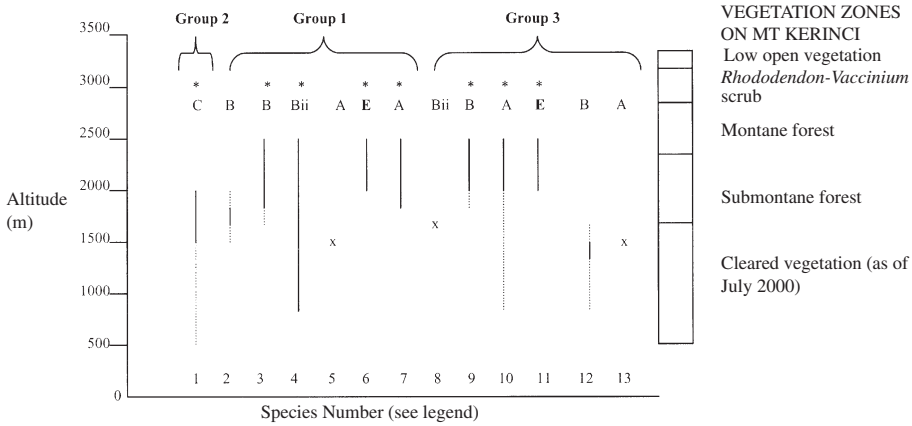

Figure 2. Altitudinal ranges of species. *= included in molecular analysis; x = only one altitude recorded/one specimen only. Distributions: E = endemic to Mount Kerinci; A = Mount Kerinci and immediate surrounding area; B = Mount Kerinci, extending to West Sumatra; Bii = Mount Kerinci, extending to West Sumatra (with records in Java); C = all Sumatra. Unbroken lines designate the altitudinal range of each species on Mount Kerinci; dotted lines are distribution-wide altitudinal ranges for each species. 1 = *C. anisophylla*; 2 = *C. fenestrata*; 3 = *C. rhyncanthera*; 4 = *C. longepetiolata*; 5 = *C. membranacea*; 6 = *C. trichodon*; 7 = *C. stenoptera*; 8 = *C. ampla*; 9 = *C. impressivenia*; 10 = *C. rosea*; 11 = *C. aureotincta*; 12 = *C. patentiserrata*; 13 = *C. flabelligera*.

ple origins, appear in various clades in the phylogenetic tree and be due to more ancient speciation in many phylogenetic lineages over time.

MATERIAL AND METHODS

Outgroup and ingroup taxa

Aeschynanthus pulcher (Blume) G.Don was selected as the outgroup following Atkins *et al*. (2001): from sequencing at RBGE (M. Möller & Q. C. B. Cronk, unpubl. data) *Aeschynanthus* appears to be one of the most closely related genera to *Cyrtandra*. The ingroup contained ITS sequences for 38 *Cyrtandra* accessions (for accession details see Table 1). Ten sequences representing seven of the 13 *Cyrtandra* species known to occur on Mount Kerinci (Bramley & Cronk, 2003) were included in the analysis. Sequences for the 21 non-Sumatran *Cyrtandra* accessions (collections other than those made by Radhiah and Cronk) were obtained from work previously carried out at RBGE (Atkins *et al*., 2001; J. Preston, unpubl. data). Non-Sumatran *Cyrtandra* were included to elucidate the relationships between Sumatra and other geographical areas within the overall distribution of the genus.

DNA extraction, PCR and sequencing protocol

DNA was extracted from silica dried leaf material collected by Radhiah and Cronk. The protocol was a modified CTAB procedure as followed by Möller & Cronk (1997).

The complete ITS region was amplified using the Polymerase Chain Reaction (PCR) with the primers 'ITS 5P' (forward) (modified from White *et al*., 1990) GGAAGGAGAAGTCGTAACAAG and 'ITS 8P' (reverse) (Möller & Cronk, 1997) CACGCTTCTCCA GACTACA. The PCR reaction mixture contained 16.25 μL sterile distilled water; 2.5 μL of 10× BIOTAQ Reaction Buffer (10×: 160 mM $(NH4)_2SO_4$, 670 mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20), 2.5 μL of a 2 mM master mix of BIOLINE dNTPs, 1.25 μL of BIOTAQ, 50 mM $MgCl_2$, 0.125 μL BIOTAQ DNA Polymerase (all products from BIOLINE); 0.75 μL of each primer (TAG) and 1-μL aliquots of genomic DNA. The PCR program follows Möller & Cronk (1997). Following successful amplification, the PCR product was purified using the QIAquick PCR purification kit (Qiagen Ltd).

Sequencing primers were identical to those used for PCR (ITS 5P, ITS 8P) but, in addition, to ensure that both ITS1 and ITS2 regions were sequenced in both forward and reverse directions, the internal primers 'ITS 3P' (modified from White *et al*., 1990) GCATC GATGAAGAACGTAGC and 'ITS 2G' (modified from Möller & Cronk, 1997) GTGACACCCAGGCAGACGT were used. All primers were obtained from TAG, Copenhagen. The annealing sites of the primers 3P and 2G are located at the beginning and end, respectively, of the conserved 5.8S region.

Purified PCR products were sequenced using a Thermosequenase II dye terminator kit (Amersham Pharmacia Biotech) according to the manufacturer's recommendations. Sequencing products were analysed on an ABI 377 Prism Automatic DNA Sequencer (Perkin Elmer, Applied Biosystems Division).

Analysis of sequence data

Sequences were imported into Sequence Navigator (Version 1.0.1, Perkin Elmer) and aligned manually. Alignment of the 39 ITS sequences analysed resulted in a 559-bp-long data matrix. Sequence characteristics were calculated using PAUP Version 4.08b (Swofford, 2001) except for the transition/transversion ratio, which was determined using MacClade Version 3.07 (Maddison & Maddison, 1997). (Table 2).

Phylogenetic trees were generated using PAUP Version 4.08b (Swofford, 2001) and MrBayes (version 2.01; Huelsenbeck & Ronquist, 2001). Maximum parsimony (MP) analyses involved a heuristic search strategy with 10 000 random stepwise addition sequence replicates and tree bisection reconnection (TBR) branch swapping with the option 'collapse branches if minimum length is zero' selected. Further searches (with options MULTREES and steepest descent on) using as starting trees those stored in the memory from the initial search were carried out for tree optimization, but no additional trees were found. Only combined ITS1 and ITS2 data were subjected to analyses. Individual gap characters were treated as missing data and gaps were coded as additional characters according to the simple method of Simmons & Ochoterena (2000). To investigate the effects of the additional gap characters, an analysis was carried out without them. Ambiguous regions that allowed alternative alignment interpretations were excluded (bp 260–6, 267–90, 483–9). An analysis including these regions was carried out in order to test their effects. A successive reweighting analysis was carried out using the rescaled consistency index for each character in order to select a phylogram from the unweighted analysis identical in topology to the reweighted tree for display.

Bootstrap values (Felsenstein, 1985) were calculated from a 1000 replicate analysis using a heuristic search strategy with simple addition of the taxa, MULTREES option on and TBR branch swapping. Decay indices (Bremer, 1988) were determined by running the program AutoDecay (Eriksson, 1998) in conjunction with PAUP version 4.08b (Swofford, 2001).

Table 1. Details of accessions

Taxon	Origin (distribution)	Collector(s)	Collector number
Aeschynanthus pulcher (Blume) G.Don	Gunung Salak, Java (Malay Peninsula)	Argent	19882557 (RBGE accession no.)
Cyrtandra sp. (Lantuyang)	Oriental Province, Mindoro	Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	29035
C. sp. (Naga)	Camarine Sur, Naga Province, Luzon	Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	29130
C. sp. (Isabella)	Isabella Province, Barangay San Jose, Luzon	Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	29009
C. cumingii C.B.Clarke	Oriental Province, Mindoro (Philippines)	Mendum, Argent, Pennington, Wilkie, Reynoso, Gaerlan	29034
C. ferruginea Merrill	Camarine Sur, Naga Province, Luzon	Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	29182
C. sp. (Halcon 1)	Oriental Province, Mindoro	Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	29053
C. sp. (Halcon 2)	Oriental Province, Mindoro	Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	29054
C. baileyi F.Muell.	Queensland, Australia	Cronk & Percy	T118
C. monticola K.Schum.	Lae, Morobe Province, New Guinea	Takeuchi	6002
C. umbellifera Merrill	Taiwan	Wen-Pen Leu	1388
C. tohiveaensis G.W.Gillett	Society Islands	Cronk & Percy	T28
C. sandei de Vriese	Limau Manis Research Forest, West Sumatra [LMRF] (Java)	Radhiah & Cronk	53
C. picta Blume	LMRF, West Sumatra (Java)	Radhiah & Cronk	54
C. sandei de Vriese	LMRF, West Sumatra (Java)	Radhiah & Cronk	55
C. sp. (LMRF1)	LMRF, West Sumatra	Radhiah & Cronk	57
C. sp. (LMRF2)	LMRF, West Sumatra	Radhiah & Cronk	58
C. peltata Jack	Lembah Anai, West Sumatra	Radhiah & Cronk	71
C. pendula Blume	Lembah Anai, West Sumatra (Java, Malay peninsula)	Radhiah & Cronk	74
C. longepetiolata de Vriese	Mount Kerinci (Sumatra,?Java)	Radhiah & Cronk	108
C. anisophylla C.B.Clarke	Mount Kerinci (Sumatra)	Radhiah & Cronk	109
C. stenoptera Bramley & Cronk (a)	Mount Kerinci (and surrounding area)	Radhiah & Cronk	110
C. rhyncanthera C.B.Clarke (b)	Mount Kerinci (West Sumatra)	Radhiah & Cronk	111
C. rhyncanthera C.B.Clarke (a)	Mount Kerinci (West Sumatra)	Radhiah & Cronk	112
C. stenoptera Bramley & Cronk (b)	Mount Kerinci (and surrounding area)	Radhiah & Cronk	113
C. rosea Ridl.	Mount Kerinci	Radhiah & Cronk	115
C. impressivenia C.B.Clarke	Mount Kerinci (West Sumatra)	Radhiah & Cronk	116
C. aureotincta Bramley & Cronk	Mount Kerinci	Radhiah & Cronk	122
C. trichodon Ridl.	Mount Kerinci	Radhiah & Cronk	124
C. mesilauensis B.L.Burtt	Mesilau Park, Sabah (Borneo)	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM7
C. kermesina B.L.Burtt	Mesilau Park, Sabah (Borneo)	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM8
C. corniculata B.L. Burtt	Mesilau Park, Sabah (Borneo)	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM9
C. gibbsiae S.Moore	Mesilau Park, Sabah (Sarawak)	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM11
C. multibracteata C.B.Clarke	Mount Kinabalu, Sabah (Sarawak)	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM16
C. clarkei Stapf	Mount Kinabalu, Sabah (Borneo)	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM19
C. chrysea C.B.Clarke	Mount Kinabalu, Sabah (Brunei)	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM20
C. burbidgei C.B.Clarke	Crocker Range, Sabah (Borneo)	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM22
C. aurantiaca B.L.Burtt	Mount Kinabalu, Sabah	Cronk, Burtt, Hilliard, Mendum, de Wilde	CBHM23
C. smithiana B.L.Burtt	Mount Kinabalu, Sabah	Cronk, Burtt, Hilliard, Mendum, Gunsalam	CBHM25

Table 2. Sequence characteristics

Parameter	ITS1	ITS2	ITS1 and ITS2
Length range (total) (bp)	219–242	229–264	449–506
Length mean (total) (bp)	225	243	473
Length range (ingroup) (bp)	219–242	229–264	449–506
Length mean (ingroup) (bp)	225	243	473
Length range (outgroup) (bp)	228	249	477
Aligned length (bp)	266	293	559
G+C content range (%)	53.3–62.1	52.6–60.6	52.9–60.6
G+C content mean (%)	58.1	58	58
Number of excluded sites (%)	2.6	10.6	6.8
Sequence divergence (ingroup) (%)*	0–16.8	15.1–25.1	0–18.9
Sequence divergence (in/outgroup) (%)*	11.5–17.7	0–22.6	13.7–20.1
Number of indels (ingroup)*	27	42	69
Number of indels (total)*	30	44	74
Size of indels (ingroup)*	1.0–7.0	1.0–11.0	1.0–11.0
Size of indels (total)*	1.0–7.0	1.0–11.0	1.0–11.0
Number of sites after exclusion*	259	262	521
Number of variable sites*	130	136	266
Number of constant sites (%)*	49.8	48.1	48.9
Number of informative sites (%)*	27.8	27.9	27.8
Number of autapomorphic sites (%)*	22.4	24.1	23.2
Transitions (min.–max.)*	210–216	179–186	389–402
Transversions (min.–max.)*	81–87	114–121	248–261
Mean no. transitions/mean no. transversions*	2.54	1.55	1.55

*Based on alignment excluding ambiguous sequence sites.

Parameters and assumptions used in the maximum likelihood (ML) searches were selected using the programs Modeltest (Posada & Crandall, 1998) and based on the hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC). The model selected was TrN (Tamura & Nei, 1993) with a gamma distribution. ML heuristic search parameters were simple addition sequence of taxa with TBR branch swapping, MULTREES and COLLAPSE. An ML analysis was performed using Bayesian methods and a general time reversible (GTR) model with a gamma distribution in MrBayes. For this analysis four simultaneous Monte Carlo Markov Chains (MCMC) were run for 1 000 000 generations, saving a tree every 100 generations.

Examination of leaves for the presence of foliar sclereids

Sections of dried leaf were soaked in 5% KOH for 1–5 h, rinsed, then transferred to formalin-acetic acid-alcohol, 5 : 5 : 90 (FAA) for a minimum of 30 min. Transverse sections 15 μm thick were taken using a freezing microtome. The sections were bleached, stained with 2% safranin in absolute alcohol and destained with absolute alcohol. They were then mounted with euparal and examined under a light microscope.

RESULTS

Sequence characteristics

The average lengths of ITS1 and ITS2 were 225 and 243 bp, respectively (Table 2). Alignment of all taxa required the insertion of 74 gaps of 1–11 bp length, 30 in ITS1 and 44 in ITS2, of which 15 in each were potentially phylogenetically informative. The lengths of aligned ITS1 and ITS2 regions were 266 and 293 bp, respectively. Owing to alignment ambiguities (where alternative alignments were possible) 38 sites were excluded (seven sites in ITS1 and 31 sites in ITS2). Of the remaining 521 unambiguously aligned sites, 48.9% were constant, 27.8% were phylogenetically informative and 23.2% were autapomorphic (Table 2).

Within the ingroup, sequence divergence (Table 3) of unambiguously alignable positions of ITS1 ranged from 0 to 16.8%, and from 11.5 to 17.7% between the ingroup and the outgroup. ITS2 was more variable, with 0–22.6% divergence within the ingroup, and 15.1–25.1% between the ingroup and the outgroup.

Table 3. Sequence divergence between groups

Group	Sequence divergence (%)		
	ITS1 and ITS2	ITS1	ITS2
Ingroup/outgroup	13.7–20.1	11.5–17.7	15.1–25.1
Ingroup	0–18.9	0–16.8	0–22.6
Sumatran species	0–11.3	0–13.5	0–11.4
Kerinci species	0.2–11.3	0–13.5	0.45–11.0
Between clades containing Sumatran material (groups 1,2 & 3)	8.7–11.3	7.6–13.5	6.4–11.0
Between Bornean and Sumatran material	3.0–14	3.6–13.5	1.4–15.9
Bornean species	1.8–13.0	1.8–12.5	1.8–14.8

Pairwise comparisons of individual taxa across both spacer regions revealed 0–18.9% sequence divergence within the ingroup, and 13.7–20.1% divergence between the ingroup and outgroup analysed. Atkins *et al*. (2001) found a maximum sequence divergence of 15.7% within an ingroup of different *Cyrtandra* species.

Among the Sumatran *Cyrtandra*, combined ITS1 and ITS2 sequences showed 0–11.3% divergence (Table 3); *Cyrtandra* from Mount Kerinci showed a similar divergence of 0.23–11.3%. The most divergence between Sumatran collections occurred between two Kerinci species (*C. impressivenia* C.B.Clarke and *C. stenoptera* Bramley & Cronk). Maximum sequence divergence was between *C. ferruginea* Merrill (from the Philippines) and *C. chrysea* C.B.Clarke (from Borneo). The lowest sequence divergence between different species was 0.23%, between *C. stenoptera* and *C. trichodon* Ridl. (from Sumatra).

Phylogenetic patterns

Parsimony analysis of unambiguously aligned ITS sequences yielded five most parsimonious trees of 650 steps when the gaps were added to the data matrix (consistency index [CI] = 0.611; retention index [RI] = 0.678). Reweighting yielded a single most parsimonious tree. Figure 3 is a phylogram, showing branch lengths, of one of the five most parsimonious trees, chosen to be identical in topology to the single most parsimonious reweighted tree: nodes that collapsed on the strict consensus tree are indicated. Analysis including the ambiguously alignable regions (bp 260–6, 267–90, 483–9) resulted in more trees of a longer length (15 of 711 steps) with an identical consensus topology. Analysis without the gap matrix produced ten most parsimonious trees of 596 steps; the strict consensus topology was identical.

Sumatran *Cyrtandra* are present in three different groups on the strict consensus tree (Fig. 4). Group one, which includes a Philippine species (*C.* sp. '*Lantuyang*') and is nested within a larger clade of Bornean species, is poorly supported (Bootstrap value [BS] < 50, Decay Index [DI] +1) but the whole clade, within which it is nested, is well supported (BS 85, DI +4). Group two is the Sumatran *C. anisophylla* C.B.Clarke, which forms a pair with the Bornean species *C. multibracteata* C.B.Clarke (BS 84, DI +4). Group three is purely Sumatran and particularly well supported (BS 96, DI +7).

The *Cyrtandra* from areas outside Sundaland (the Philippines, Australia, New Guinea, the Pacific and Taiwan) form a separate clade to *Cyrtandra* from Sundaland, with the exception of *C.* sp. '*Lantuyang*', from Mindoro, the Philippines.

The ML tree (Fig. 5) is very similar to the MP tree except for the position of *C. smithiana* B.L.Burtt and *C. aurantiaca* B.L.Burtt. The Bayesian analysis also gave a similar tree; the Bayesian majority rule consensus percentages are plotted on the ML tree in Figure 5. The majority of nodes supported in the MP analysis are also supported in the Bayesian analysis.

Estimated ages of lineages

To assess whether substitution rates in ITS are clock-like, a likelihood-ratio test (Felsenstein, 1981) that is twice the difference in log likelihood of branch lengths between a tree that is constrained by a molecular clock and a tree that is unconstrained was carried out. The log likelihoods (3832.2 vs. 3766.7) were significantly different, and therefore the null hypothesis, that the data were constrained by clock-like change, was rejected with $P < 0.005$. In a case such as this, where the molecular clock is rejected, algorithms that accommodate ancestor–descendant rate variation such as non-parametric rate smoothing (NPRS) (Sanderson, 1997) and penalized likelihood (Sanderson, 2002) are the preferred means of estimating ages for cladogram nodes. However, both require the calibration of at least

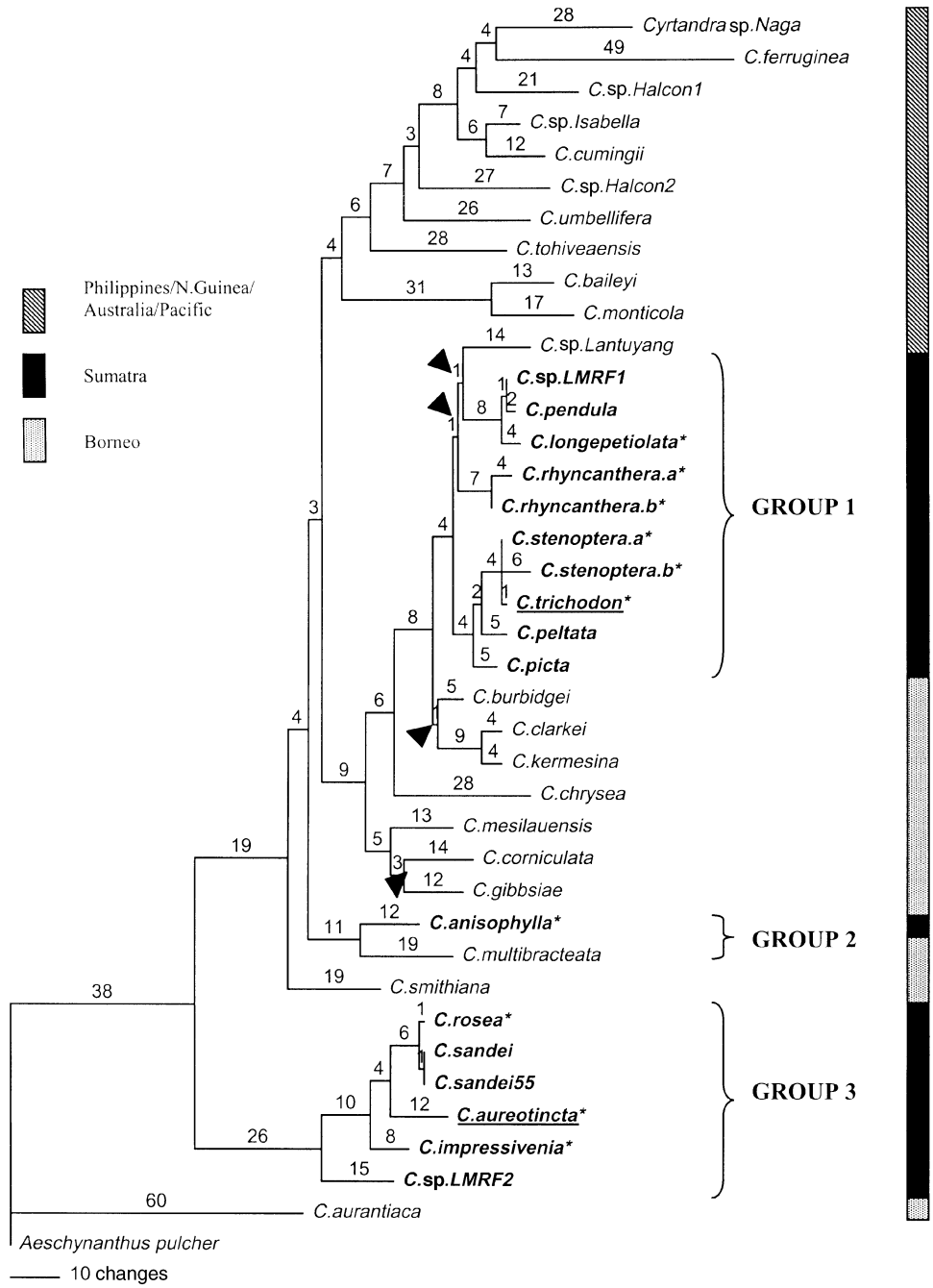

Figure 3. Phylogram, one of five most parsimonious trees identical in topology to the single most parsimonious reweighted tree. Numbers indicate branch lengths. Triangles indicate nodes that collapse in the strict consensus tree. Species from Sumatra are in bold type; asterisks mark species from Mount Kerinci; species thought to be endemic to Mount Kerinci are underlined. Bars show the geographical distribution of the species.

one node on the tree using independent evidence such as fossils or geological events. Such evidence is not available for *Cyrtandra*, so these methods were not used.

Given the rejection of the molecular clock, the following approximate approach was taken. The generation time of taxa is the major determinant of the rate of accumulation of neutral mutations. We therefore suggest that a typical substitution rate for ITS in taxa with similar life histories to *Cyrtandra* would be an approximate substitution rate for *Cyrtandra*. Such a typical rate is 5×10^{-9} substitutions per site per year,

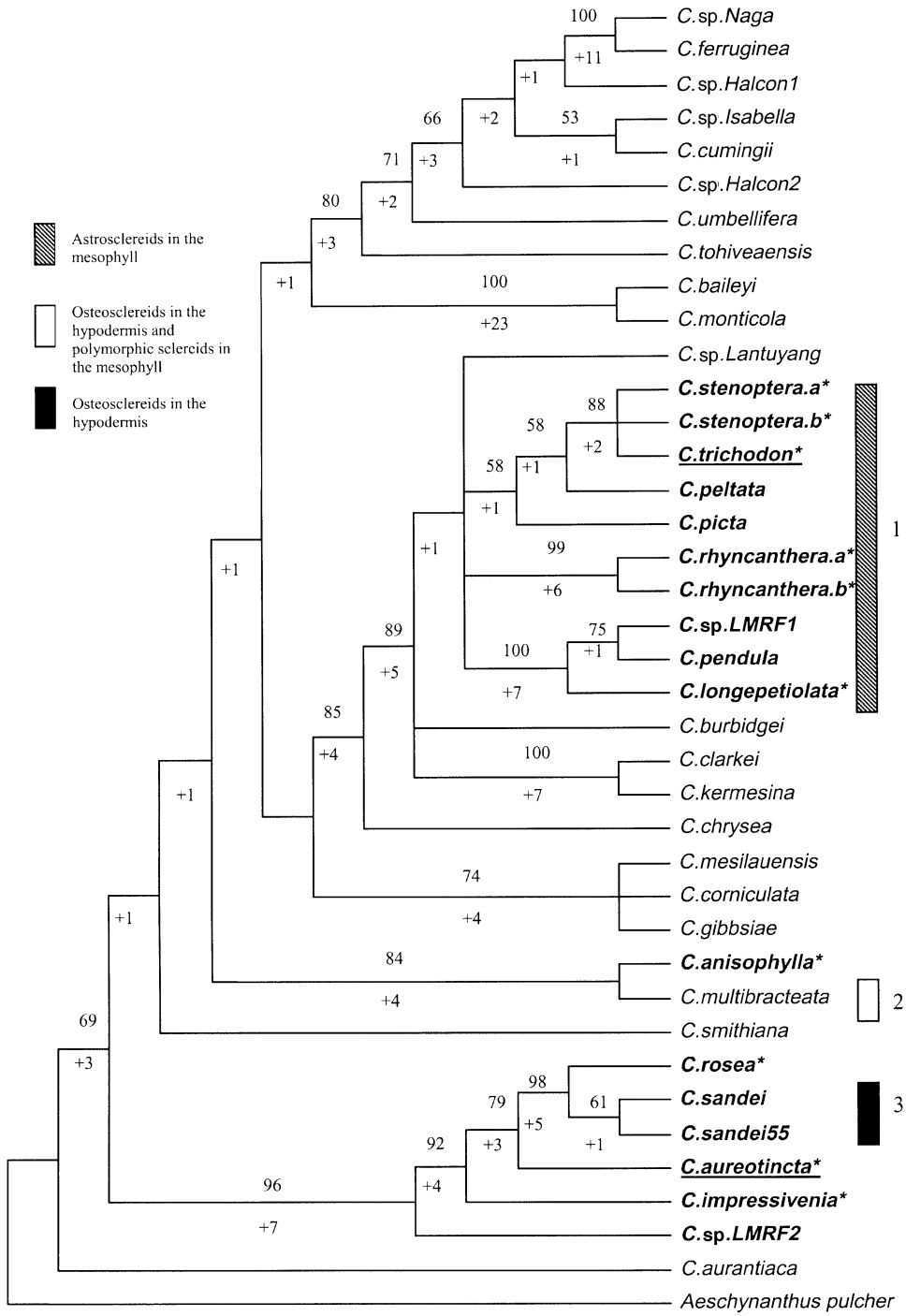

Figure 4. Strict consensus tree of five most parsimonious trees of 650 steps. Numbers above the branches are bootstrap values, numbers below are decay indices. Species from Sumatra are in bold type; asterisks mark species from Mount Kerinci; those thought to be endemic to Mount Kerinci are underlined. Bars denotes pattern of foliar sclereids.

according to the available rate estimates summarized in Richardson *et al*. (2001), which range from 1.72×10^{-9} to 7.83×10^{-9} substitutions per site per year. Using this rate, the minimum ages for the three crown groups of Sumatran *Cyrtandra* were calculated by summing the minimum number of substitutions separating any two terminal taxa, as measured by parsimony branch lengths, via the most basal node of the clade. The maximum ages were calculated using exactly the same method, but using the maximum

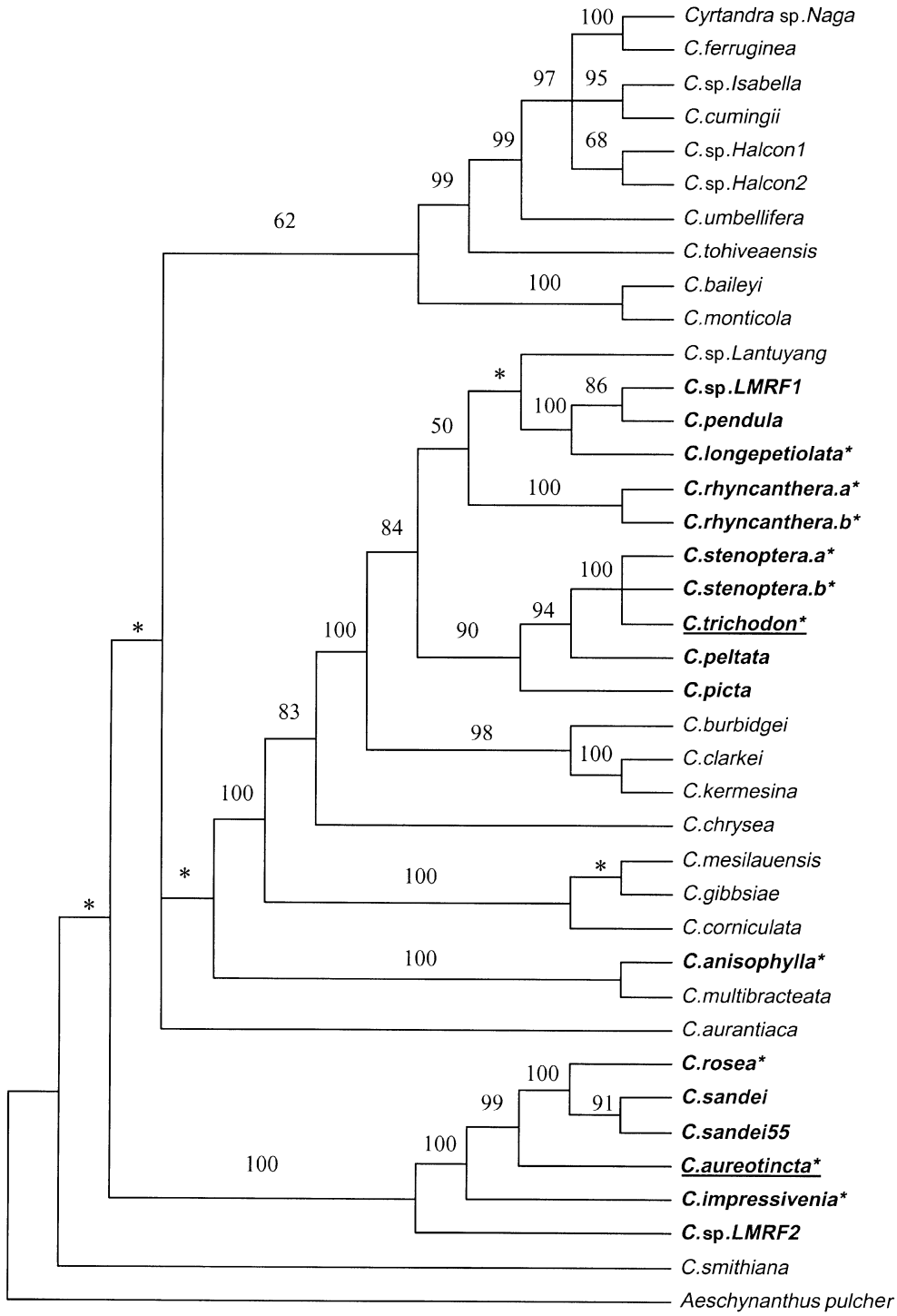

Figure 5. ML tree. Values on nodes are the Bayesian majority rule consensus percentages. Asterisks denote nodes that conflict with or are unsupported by the Bayesian analysis. Species from Sumatra are in bold type; asterisks mark species from Mount Kerinci; those thought to be endemic to Mount Kerinci are underlined.

number of substitutions between any two terminal taxa via the most basal node of the clade. At the chosen substitution rate, the implied ages of the three crown groups of Sumatran *Cyrtandra* are as follows: group one 1.9–3.4 million years old; group two 6.6 million years old; group three 7.0–8.7 million years old. These dates can only be considered as extremely rough estimates, and are not integral to the hypotheses

posed in this paper, but nevertheless do provide support for a pre-Pleistocene start of diversification for the three lineages present on Mount Kerinci.

Morphological characters and phylogeny

Each group tends to have a typical morphology (Fig. 6). Group one is supported by the presence of astrosclereids in the mesophyll of the leaves of all of its members. Apart from *C. rhyncanthera* C.B.Clarke, which has very thin leaves, group one species have thick mesophyll layers compared with the epidermis and hypodermis. The species also tend to be herbaceous in habit, with the exception of *C. stenoptera* and *C. trichodon*. Radiation within the group seems to have allowed the development of many morphological forms. For example, *C. pendula* Blume has flowers that are held on long trailing peduncles, *C. peltata* Jack has peltate leaves and *C. rhyncanthera* C.B.Clarke has creeping stems. All species bear fairly large (usually > 3 cm), predominantly white flowers.

Group two is supported by a number of morphological characters: marked anisophylly in the leaves; a zygomorphic calyx with the upper three lobes united into a tridentate tip, the lower two being divided to the base; small rather fleshy white corollas; and the species have very characteristic cells of the upper leaf epidermis, which have a conical outer wall (Burtt & Bokhari, 1973). The sclereid pattern seems to vary: *C. anisophylla* has no sclereids, but *C. multibracteata* has osteosclereids in the hypodermis and polymorphic sclereids in the mesophyll (M. H. Bokhari, unpublished note on specimen label E).

Group three is a group of woody Sumatran shrubs. No foliar sclereids appear to be present in the specimens examined except in *C. sandei* de Vriese, of which two accessions from West Sumatra are analysed here: this species has osteosclereids in the hypodermis. The species have large hairy leaves with prominent venation with no distinct anisophylly. Flowers are small (usually 1.5 cm or less), hairy and vary in colour from white to red or purple. They are usually enclosed in bracts.

DISCUSSION

Patterns of evolution in Sumatran *Cyrtandra*

The phylogeny gives clear evidence to support the existence of three groups of Sumatran *Cyrtandra*, all of which occur on Mount Kerinci. It seems likely that these groups are the result of three independent developments because there are, at least within this sample, distinct morphological characters associated with each group, therefore giving confidence in the single locus molecular results. Each group and its characteristic features are discussed individually.

Group 1 Group 2 Group 3

Figure 6. Representative species from each group of Sumatran *Cyrtandra*. Group one: *C. longepetiolata* de Vriese; group two: *C. anisophylla* C.B.Clarke; group three: *C. rosea* Ridl.

Group one: Sumatran species nested within a larger Bornean clade

Group one (eight Sumatran species) is suggested by the phylogeny to form part of a large clade of Bornean species. Also present in this clade is one Philippine species (*C.* sp. *Lantuyang*). This species also fell in a Bornean clade in the study of Atkins *et al*. (2001) .

All species appear to have astrosclereids present in the mesophyll of their leaves. Sclereids are thought to provide leaf rigidity and perhaps also act as defence against herbivores. The fact that the sclereid pattern found in this group is the same in all species might suggest that this is a species group with a particular ecological uniformity (Burtt & Bokhari, 1973). This is perhaps supported by the herbaceous habitat shared by the majority of the species; the exceptions, *C. stenoptera* and *C. trichodon*, are pole plants with stiff erect unbranched stems, enabling them to grow taller, presumably to increase gain of light.

It seems likely that group one, and the clade of Bornean species in which it is nested, would form part of a larger Bornean clade should the sampling be increased. This suggests that Sumatran and Bornean species of *Cyrtandra* are closely related. This may be the result of lowering of sea levels in intermittent periods from the Oligocene to the Pleistocene that allowed extensive land bridges to connect the now isolated areas of Sundaland (Morley & Flenley, 1987; Hall, 1998; Voris, 2000). Geographical separation resulting from sea-level rises may have since furthered the radiation of *Cyrtandra* on each individual land area.

Group two: the anisophylla–multibracteata pair

This pair belongs to a monophyletic group of *Cyrtandra* that can be defined by a number of morphological synapomorphies. It is a rare example (Burtt, 1990) of a group consistent with the last overall treatment of the genus (Clarke, 1883), corresponding to Clarke's section *Dissimiles* (11 species, G. L. C. Bramley, unpubl. data). Its members have the characteristic calyx, corolla and anisophyllous leaves described in the Results.

It is interesting that group two (section *Dissimiles*) is unusually widespread in Sundaland. Furthermore, a number of the species in this group seem to have quite wide distributions and exist at fairly low altitudes, e.g. *C. anisophylla* has been recorded between altitudes of 450 and 2000 m and is widespread in Sumatra. Most *Cyrtandra* species tend to occupy habitats above 1000 m, perhaps where the forest becomes slightly less dense and they are able to survive more successfully in the understorey layer. Two possible explanations could be advanced for this widespread distribution: (i) occurrence at lower altitudes may allow a wider range; (ii) dispersal of the fruit, which is unusually fleshy for western Malesian species, may be more effective.

Group three: the woody shrubs

This monophyletic group of Sumatran *Cyrtandra* appears to be a radiation of woody shrubs (sometimes pole plants). They seem to have evolved from a different lineage to the other groups of *Cyrtandra* found and from their morphology appear to be exploiting a different range of niches. Altitudinal range varies within the group, but tends to be 2000–2500 m.

It is expected, owing to morphological similarities, that other species of Kerinci *Cyrtandra* that could not be sequenced (*C. patentiserrata* Bramley & Cronk, *C. ampla* C.B.Clarke, *C. flabelligera* Ridl.) would also fall in this clade.

Assembly of *Cyrtandra* biodiversity on Mount Kerinci

All three groups of Sumatran *Cyrtandra* identified here are found on Mount Kerinci. The *Cyrtandra* community on Kerinci is composed of species from three different phylogenetic lineages: there has been phyletic assembly rather than local diversification. Hypothesis two proposed that the Kerinci *Cyrtandra* community would have a multiple origin, appearing in various clades in the phylogenetic tree. It also stated that there would have been an accumulation of diversity in many phylogenetic lineages over time as a result of these lineages co-occurring and ancient speciation. Although the results support the first statement, there is less evidence in support of the second statement. It seems that there have been differing degrees of speciation in each lineage, and diversity has not accumulated due to equal amounts of speciation within all the lineages. In group one, there appears to have been some recent speciation as there are morphologically very similar and therefore closely related species, e.g. *C. trichodon* and *C. stenoptera*. However, group two has shown no growth in terms of species numbers, with only one representative species on Mount Kerinci, and group three, for the most part, shows evidence of older speciation events, with species on much longer branches, i.e. with a greater amount of nucleotide substitutions between them, e.g. *C. impressivenia* and *C. aureotincta* Bramley & Cronk. The composition of the community therefore seems to be the result of a balance of not only ancient but also recent speciation, but the recent speciation appears to have occurred in one lineage. Hypothesis one, which suggested that the *Cyrtandra* community on Mount Kerinci would be composed of one lineage that had undergone rapid and recent speciation, is not supported. However, if speciation continues to occur in

group one only, the community may eventually become predominantly composed of one lineage.

Endemic species

The analysis included two species known only from Kerinci, *C. trichodon* and *C. aureotincta*. The results tend to suggest that one of these endemics is relatively recent and the other a member of an older lineage. In group one, the endemic *C. trichodon* is very closely related to *C. stenoptera*, which has a slightly wider range and has been collected on the neighbouring Mount Tudjuh. These species have only 1–6 nucleotide substitutions between them, and although it is possible to distinguish them morphologically, it is also obvious from their morphology that they are closely related. However, in group three, *C. aureotincta*, sister to *C. rosea*, is characterized by more nucleotide changes (12) and has a number of unique indel events. It also has a number of unique morphological characters such as a hairy disk, and may be a relict species. This single species lineage may be older than Mount Kerinci and is expected to occur (or to have occurred) elsewhere.

ORIGINS OF THE *CYRTANDRA* COMMUNITY ON MOUNT KERINCI

The rough estimates of the ages of these lineages indicated that the primary diversification of *Cyrtandra* was pre-Pleistocene. It therefore seems likely that the lineages are older than the volcano, which probably has a late Pliocene/Pleistocene origin. Many of the species present on Mount Kerinci are also present on other volcanoes such as Mt Singgalang (Bramley & Cronk, 2003), perhaps indicating that such areas were colonized by a similar *Cyrtandra* community.

Investigations involving palynological, geological, fossil and termite data (e.g. Flenley, 1979; Newsome & Flenley, 1988; Morley, 2000; Gathorne-Hardy *et al*., 2002) have suggested that during Quaternary glaciations, the Barisan Mountain range remained an area of forest, acting as a rain forest refugium. The stable environment within this refugium is likely to have allowed the low extinction rates and gradual accumulation of species suggested by the museum hypothesis, resulting in the present-day assembly of the Kerinci *Cyrtandra* community, which is composed of different ancient lineages. Further evidence provided by these studies has suggested that montane forest migrated downhill during colder times in the Pleistocene, then gradually receded to higher levels, leaving higher altitude species confined to peaks. All of this evidence could explain the similarity of *Cyrtandra* species on the Barisan mountains, and perhaps also why the more widespread species, such as *C. anisophylla*, are those that are successful at lower altitudes.

Conservation

The identification of the three lineages of *Cyrtandra* on Mount Kerinci shows that the species in this community are diverse genetically, most probably as a result of the Barisan mountains acting as a rain forest refugium during Quaternary climate changes. Information such as this demonstrates the importance of refugia sites in providing long-term stability, and highlights the need to conserve them as modern rain forest refugia (Gathorne-Hardy *et al*., 2002).

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