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Molecular Systematics and Biogeography of the Southeast Asian Genus *Caryota* (Palmae)

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ABSTRACT. Restriction site variation of chloroplast DNA was analyzed in nine of the eleven currently recognized species of *Caryota*. Phylogenetic relationships were estimated and used to examine biogeographic patterns in the genus. Analysis of 49 populations of *Caryota* and six species from the putative outgroup genera *Arenga* and *Wallichia* demonstrated low levels of inter- and intraspecific variation as seen in other groups of palms and long-lived perennials. A total of 796 restriction sites representing 4,752 bp (ca. 4.0%) of the chloroplast genome was detected with 75 sites (9.4% of the total) showing phylogenetically informative variation. Phylogenetic analysis identified three main clades, each with one widespread variable species and one or more geographically restricted species. Hybridization was suggested as a probable explanation for patterns of variation detected in several instances of species sympatry. Biogeographic patterns among the three principal clades are largely congruent with Wallace's 1910 Line or Huxley's Line. The *Maxima* clade consists of three species restricted to the west of Huxley's Line. The *Mitis* clade consists of two species found west Huxley's Line and on the island of Sulawesi. The *Rumphiana* clade includes four species distributed, with one exception, to the east of Huxley's Line and on the border islands of Borneo and Palawan. The economically important *C urens*, the errant member of the *Rumphiana* clade, has a distribution disjunct to India and Sri Lanka, possibly a reflection of early human introduction, cultivation, and subsequent natural dispersal into local forests.

Southeast Asia has long fascinated biologists with its rich biological diversity and complicated biogeographic patterns. The meeting of predominantly Asian taxa from the west with those of Australian affinity from the east is accomplished in a geologic and climatic context of unsurpassed complexity (Audley Charles 1981; Hall and Blundell 1996). In recognition of the different floristic and faunistic elements that converge in the region, several biogeographic lines have been proposed to separate them (see George 1981 for review). Among these lines, perhaps the most famous is Wallace's Line (Wallace 1863, 1876; Simpson 1977; Whitmore 1981, 1987), which runs between the islands of Bali and Lombok, north between Sulawesi and Borneo, and to the east of the Philippines.

Wallace (1910) later presented a modified version of his Line that differed from the 1863 version by passing to the east of the island of Sulawesi. Huxley (1868) presented yet another line that was similar to Wallace's 1863 Line but ran to the west of the Philippines. In short, these lines differ only in how Sulawesi and the Philippines are treated (Simpson 1977; George 1981). While each line has strong geological correlates, from a biological perspective the relevance of any particular line is largely dependent upon the specific plant or animal taxa under study.

The geologic basis to the proposed lines is due to their tendency to follow features such as the edges of the continental Sunda and Sahul shelves, the relatively deep trenches of the Makassar and Lombok Straits, and the Isthmus of Kra in peninsular Thailand. While many biogeographic studies have discussed correlations between organismal distributions and geologic features, more recent interpretations of Southeast Asian biogeography have emphasized the geologic history of the region as well (e.g., Whitmore 1981; Michaux 1991; Holloway and Hall 1998).

Climatic factors have also been implicated as a causal basis for some of the biogeographic lines as several of the lines straddle transitions from one climatic zone to another (Morley 1998). For example, the Isthmus of Kra demarcates a transition from the seasonal monsoon forests of Indochina to everwet forests in south Thailand and peninsular Malaysia. Similarly, the everwet forests of Borneo give way to more seasonal forests to the east in the Moluccas, a transition demarcated by Wallace's and Huxley's Lines.

Plant biogeographic studies for the region have largely focused on the limits of distribution for various families, genera, and species (e.g., Steenis and

Steenis 1969; Steenis 1979; Baker et al. 1998). Subsequent analyses of these data include phenetic distance measures applied to describe overall species- or generic-level similarity of the different parts of the region (e.g., Balgooy 1987). An important element in each of these is consideration of the relative role of long distance dispersal in determining current plant distributions.

Cladistic biogeographic analysis of Southeast Asian plants has been conducted for several taxa (e.g., Turner 1985; Welzen 1989; Axelius 1990; Adema 1991; Schot 1991, 1998; Klackenberg 1995). Ridder-Numan (1998) has partially synthesized the results from these works in the form of a consensus area cladogram that demonstrated a clear biogeographic split centered on the Isthmus of Kra, a distinctive position for Borneo with considerable structure within that island, and an unresolved position for Sulawesi. Implicit in this approach is an underlying process of vicariance to explain current plant distributions. Nonetheless, several of these authors used dispersal hypotheses to explain incongruence among taxa or between putative areas of endemism.

Most of these plant studies focus on genera or parts of genera that do not span Wallace's or Huxley's lines and therefore do not directly address these lines as factors of speciation and differentiation within a monophyletic group (Crisp et al. 1995). Analysis of a clade that spans one or more of the major lines allows for examination of the validity of the lines themselves as well as the underlying biogeographic processes that gave rise to modern Southeast Asian plant distributions.

Palms are well represented in the flora of Southeast Asia and several genera of palms span Wallace's Line (Dransfield 1981, 1987; Baker et al. 1998). The genus *Caryota* is one of these genera with the ranges of many of its species bounded by one or more of the major biogeographic lines (Hahn 1993). In contrast, some of the more wide-ranging species in the genus cross one or more of the proposed biogeographic lines thereby challenging the biological relevance of the lines. Although these widespread species are known to vary intraspecifically in morphological characters (Hahn 1993), correlation of this variation with biogeographic information has been made only in the form of descriptions of endemic subspecific taxa (e.g., Beccari 1877). Given these distribution patterns, reconstruction of phylogenetic relationships within *Caryota* could offer insight into the geographic pattern of speciation in the genus as well as more general patterns of biogeography for Southeast Asia (Crisp et al. 1995).

Caryota is one of the most distinctive genera of palms with its concolorous bipinnate leaves, ruminate endosperm, bisexual inflorescences with triads of flowers throughout, and calcium oxalate raphides distributed throughout most tissues (especially the mesocarp). These features are considered strong evidence of monophyly for the genus (Uhl and Dransfield 1987; although see Dowe and Cabalion 1996). Within the genus, however, the large dimensions of many species (stems to 35m tall, leaves to 12m long, and inflorescences to 5m long) as well as the complex morphologies found in the bipinnate leaves and inflorescences have led to difficulties in sampling and species delimitation. Dransfield (1974, p. 87) considered *Caryota* to be one of the most difficult of palm genera to represent on an herbarium sheet and stated that a monograph of the genus would involve "tedious work matching up fragments, possibly with the aid of anatomical characters of the leaflets." As such, a taxonomic revision of the genus presents a particular challenge that demands integration of different data sets.

In general, phylogenetic analyses of palms using data from gross morphology have been limited due to high levels of homoplasy, difficulty in obtaining complete character information, and problems with polarity assessment (e.g., Dransfield et al. 1990; Zona 1990; Barfod 1991; Sanders 1991; Evans 1995; Uhl et al. 1995). Many of these problems are also seen in *Caryota* (Hahn 1993, 1999) where, as indicated above, the situation is often extreme.

In a monographic study of the genus (Hahn 1993), problems in species delimitation were partially overcome through the use of morphological, anatomical, and palynological character sets coupled with multivariate analyses. In that study (performed before the description of *C. ophiopellus* J. Dowe), 10 species were recognized based on phenetic analysis of 43 morphological, anatomical, and palynological characters. Unfortunately, attempts at reconstructing phylogenetic relationships among the recognized species with the same data were mostly unsuccessful. As seen in other phylogenetic studies of palms, many of the morphological characters varied in a continuous fashion or exhibited intraspecific polymorphisms that were difficult to code. More amenable data sets are needed for independent examination of the phenetic species delimitations and phylogenetic reconstruction of species relationships.

Molecular methods have provided alternatives to

morphological data in phylogeny reconstruction (e.g., Hillis et al. 1996). Large numbers of individuals can be sampled (as leaf tissue) in a relatively short period of time with each of these samples in turn examined for potentially thousands of discrete characters at the restriction site or nucleotide level. Because these characters are largely uncoupled from environmental or developmental influences, many of the problems encountered with analysis of morphological characters can be minimized. Ultimately, phylogenetic reconstructions based on molecular data sets provide opportunities for the study of evolutionary phenomena such as biogeography, speciation, and character evolution in an objective, non-circular fashion (e.g., Sytsma et al. 1991; Chase and Palmer 1992; Givnish and Sytsma 1997).

In the present study, phylogenetic reconstructions for the genus *Caryota* were generated using chloroplast DNA (cpDNA) restriction site data analyzed under the maximum parsimony and minimum evolution criteria. Phylogenetic reconstructions were then used to examine biogeographic patterns exhibited by members of the genus.

Materials and Methods

Sampling. Nine of the eleven currently recognized species of *Caryota* were surveyed for cpDNA restriction site variation. *Caryota sympetala* Gagnepain and the recently described *C. ophiopellus* were unavailable for the present study. Additionally, five species of *Arenga* and one of *Wallichia* were sampled as they together with *Caryota* form the clearly delimited tribe Caryoteae (Uhl and Dransfield 1987). Single accessions of the putatively related genera *Iriartea, Socratea,* and *Dictyocaryum* of tribe Iriarteae, subfamily Arecoideae (Uhl and Dransfield 1987) were also included in this study.

Previous cpDNA restriction site surveys of the Palmae resolved *Wallichia* of subtribe Caryoteae in a clade containing only members of the subfamily Coryphoideae (Uhl et al. 1995). While this position has been suggested in some previous taxonomies (Moore 1973), current morphological evidence (Uhl et al. 1995) argues against a direct connection between Caryoteae and Coryphoideae and no material of the latter subfamily was included in the present study.

Field collections were made from throughout the core of the range of *Caryota* and were augmented by material from more wide-ranging locales received from various collaborators (Table 1). Special attention was paid to intraspecific variation as limits of most species are poorly defined. Multiple populations (= localities) were sampled for seven of the species studied (all but *C. monostachya* and *C. obtusa*) to determine levels of intraspecific variation. As a test for variation within populations, a total of 15 populations for five species were sampled intensively (3–21 individuals per population) and screened for variation with the enzymes and probes that identified more variable restriction sites.

Material collected included fresh leaf tissue and/or seeds that were germinated under greenhouse conditions. General protocols for the collection and transport of such material are summarized in Sytsma et al. (1993). Voucher specimens are deposited at WIS, and additional information may be obtained by request to WJH.

Molecular Techniques. Leaf tissue was used fresh for DNA extractions, stored for up to a month at 4°C, or placed in long term storage at −80°C for later extraction. Nucleic acid extraction was performed using the CTAB method (see Smith et al. 1992 for a review) with the following modifications: 1) leaf tissue was soaked in liquid nitrogen for 5–30 min, then ground to a fine powder, 2) ground tissue was added to 10 ml of a warm (65°C) solution of 50 mM Tris-HCl, 10 mM EDTA, 2 M NaCl, 6% w/v CTAB (hexadecyltrimethylammonium bromide), and 200 µl 2-mercaptoethanol, and 3) all precipitations were performed with 10–15 ml cold EtOH (Bult et al. 1992).

Restriction digests followed the manufacturer's recommendations (New England Biolabs, Beverly, MA) and were initially conducted with 24 restriction endonucleases: *Ase*I, *Ava*I, *Bam*HI, *Ban*II, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*BI, *Bst*NI, *Cfo*I, *Cla*I, *Dra*I, *Eco*RI, *Eco*0109, *Hin*dIII, *Nci*I, *Nsi*I, *Pst*I, *Pvu*II, *Sac*I, *Sal*I, *Sca*I, *Sma*I, and *Xmn*I. Five enzymes (*Pst*I, *Sac*I, *Sal*I, *Sca*I, and *Sma*I) revealed very few restriction sites with no variation among initial samples of taxa and were omitted from all subsequent surveys and calculations. The digested DNAs were electrophoresed in 0.8–1% agarose gels for 12–16 hr then blotted to nylon membranes (MagnaGraph, Micron Separations, Inc., Westborough, MA) following the manufacturer's instructions. Specific restriction fragments were visualized via autoradiography of hybridized probes from the *Oncidium excavatum* Lindley cpDNA clone library (Chase and Palmer 1989) following the procedures outlined by Palmer (1986). Only 0.12% of the data matrix cells was scored as missing.

Phylogenetic Analysis. Phylogenetic analyses

of scored cpDNA restriction sites were conducted using maximum parsimony and distance matrix methods as implemented in PAUP* ver. 4.0 beta (Swofford 1998). Parsimony algorithms employed include equally weighted Wagner parsimony, Dollo parsimony, and several character-state weighting schemes as discussed by Holsinger and Jansen (1993). For the weighted parsimony approach, a step matrix was used to weight restriction site gains over site losses with values ranging from 1.05:1.0 to 1.5:1.0, as estimated by Albert et al. (1992) to be appropriate at the interspecific to intergeneric level.

Given the number of accessions analyzed and the special attention paid to intraspecific variation, all parsimony searches were conducted using heuristic methods to estimate minimum-length trees. Specific options used include tree bisection/reconnection (TBR) branch swapping and STEEPEST DESCENT. A simple taxon addition sequence was utilized in all initial analyses. These were later re-examined with 100 replications using a random order of taxon addition to see if other possible islands of shortest tree could be reached (via branch swapping) from different initial topologies (Maddison 1991).

Polarities were determined by the outgroup comparison method (Watrous and Wheeler 1981; Maddison et al. 1984) using *Arenga* and *Wallichia* as outgroups. Restriction site variation from the putatively related genera *Iriartea*, *Socratea*, and *Dictyocaryum* (tribe Iriarteae, subfamily Arecoideae) proved difficult to align with Caryoteae in side-by-side comparisons and was omitted from further analysis in this study.

Character support for monophyly of specific groups was evaluated using decay analysis, bootstrap analysis, and cladistic permutation tail (PTP) tests. For the decay analysis, strict consensus of all trees one to several steps longer were kept to calculate the group support or decay index (Bremer 1988), to search for regions of stability, and to provide a conservative estimate of relationships (Barrett et al. 1991). Bootstrap analysis was conducted using 1000 replications and a random order of taxon entry while saving all shortest trees during branch swapping. The PTP test of Faith and Cranston (1991) was used to determine the presence or absence of phylogenetic signal in the data set. One hundred random data sets were generated to explore the possibility of randomly generated trees shorter than those estimated by the true data set.

Phylogenetic relationships were also estimated using the minimum evolution method of Rhetzky and Nei (1992, 1993). Distance matrices were calculated based on the algorithms of Nei and Li (1979) and Upholt (1977) as implemented in PAUP* ver. 4.0 beta (Swofford 1998).

Results

Nature of Chloroplast DNA Restriction Site Variation. Results of filter hybridization studies showed colinearity of the *Caryota* chloroplast genome with that of *Oncidium* and the consensus angiosperm chloroplast genome (e.g., Chase and Palmer 1989) as previously reported for the palm genus *Nypa* (Uhl et al. 1995). For the 19 enzymes ultimately used in the study, a total of 796 restriction sites was detected, representing 4,752 bp or approximately 4.0% of the chloroplast genome. Of the 796 restriction sites observed, only 75 (9.4%) showed informative variation (Appendix 1). The relatively large heterologous probes used in this study allow only an approximation of the actual physical distribution of site mutations relative to gene location, a summary of which is presented in Table 2.

The observed restriction site variation is similar to that reported in other studies of palms (Wilson et al. 1990; Uhl et al. 1995). Estimated interspecific divergence values (as 100p; Nei and Li 1979) ranged from 0.023 between *C. bacsonensis* (Nan) and *C. maxima* (Chiang Mai) to 0.387 between *C. mitis* (Meijo) and *C. maxima* (Chiang Mai). No variation within individual populations was detected in any of the 13 localities intensively sampled (Table 1), an observation consistent with patterns seen in most plant taxa (Soltis et al. 1992). Significant variation among populations was detected in the five well-sampled and wide-ranging species. The ranges of estimated intraspecific divergences (as 100p; Nei and Li 1979) were: *C. bacsonensis* (0.0–0.011), *C. cumingii* (0.0–0.057), *C. maxima* (0.0–0.204), *C. mitis* (0.0–0.159), and *C. rumphiana* (0.0–0.102). Much of this variation was correlated with geographic distribution. Intraspecific variants in two populations (*C. bacsonensis* from Cuc Phuong, Vietnam and *C. mitis* from Palawan, Philippines) are excluded from these comparisons as they apparently are involved in hybridization and chloroplast capture (Rieseberg and Brunsfeld 1992; see discussion below).

Two deletions were detected in fragment patterns generated with *Ava*I, *Bam*HI, *Ban*II, *Bcl*I, *Bst*NI, *Cla*I, *Dra*I, *Nci*I, *Nsi*I, and *Xmn*I in probe region IV. The first deletion of approximately 400 bp is seen in all accessions of *C. bacsonensis*, *C. maxima*, and *C. obtusa*, with the single exception of *C. bac-*

TABLE 1. Collection information for accessions of *Caryota* and outgroups used in this study. Vouchers for all collections are deposited at WIS with some duplicates at BH, BKF, BO, K, LB, PNH, and SING. More complete locality information is available from WJH. Numbers in parentheses indicate number of individuals sampled in selected populations (see Materials and Methods).

Collection Name	Voucher Number (all Hahn)	Locality
Caryota bacsonensis Magalon		
1 *C. bacsonensis* VoNhai	6506	Vo Nhai, Vietnam
2 *C. bacsonensis* CucPhuong	6566	Cuc Phuong, Vietnam
3 *C. bacsonensis* Nan	6738	Sup Khun, Nan, Thailand
Caryota cumingii Loddiges ex Martius		
4 *C. cumingii* Pampanga	2846	Pampanga, Luzon, Philippines
5 *C. cumingii* Pangasinan	2986	Pangasinan, Luzon, Philippines
6 *C. cumingii* Cebu	3039	Manganilla, Cebu, Philippines
7 *C. cumingii* Palawan	6849	Palawan, Philippines
Caryota maxima Blume ex Martius		
8 *C. maxima* ChiangMai1 (4)	5901	Mae Sa, Chiang Mai, Thailand
9 *C. maxima* ChiangMai2	5916	N of Chiang Mai, Thailand
10 *C. maxima* Nan	5978	W of Phu Kha, Nan, Thailand
11 *C. maxima* Genting	6155	Genting, Malaysia
12 *C. maxima* Fraser (11)	6171	Bukit Fraser, Malaysia
13 *C. maxima* Java	6205	Ceramai, Java, Indonesia
14 *C. maxima* Sumatra	6209	Bukit Tingii, Sumatra, Indonesia
15 *C. maxima* ChiangMai3	6426	MaeSa Long, Chiang Mai, Thailand
Caryota mitis Loureiro		
16 *C. mitis* Palawan1 (12)	3170	Quezon, Palawan, Philippines
17 *C. mitis* Chiang Mai	5895	Mae Sa, Chiang Mai, Thailand
18 *C. mitis* KhaoYai (4)	6134	Khao Yai, Thailand
19 *C. mitis* Sumatra	6147	Surai, Sumatra, Indonesia
20 *C. mitis* Kachanburi	6154	Katchanburi, Thailand
21 *C. mitis* Kepong (17)	6157	Kepong, Malaysia
22 *C. mitis* Lampang	6404	Lampang, Thailand
23 *C. mitis* Sabah	6411	Sabah, Malaysia
24 *C. mitis* ChiangRai	6418	Chiang Rai, Thailand
25 *C. mitis* S Thail	6420	Southern Thailand
26 *C. mitis* Palawan2	6421	Palawan, Philippines
27 *C. mitis* Meijo	6425	Meijo, Thailand
28 *C. mitis* Pornchai	6428	Pornchai, Thailand
29 *C. mitis* Trang (12)	6627	Trang, Thailand
Caryota monostachya Beccari		
30 *C. monostachya* CucPhuong (8)	6537	Cuc Phuong, Vietnam
Caryota no Beccari		
31 *C. no* Sabah	2842	Sandakan, Sabah, Malaysia
32 *C. no* Kalimantan	6424	Kalimantan, Indonesia
Caryota obtusa Griffith		
33 *C. obtusa* Nan1 (10)	5920	Doi Phu Kha, Nan, Thailand
34 *C. obtusa* Nan2	6734	Doi Phu Kha, Nan, Thailand
Caryota rumphiana Martius		
35 *C. rumphiana* Sol Is	2847	Santa Ana, Solomon Islands
36 *C. rumphiana* PNG	2853	Milne Bay, Papua New Guinea
37 *C. rumphiana* Rizal (3)	2862	Rizal, Luzon, Philippines
38 *C. rumphiana* Laguna1 (6)	2864	Laguna, Luzon, Philippines

TABLE 1. Continued.

Collection Name	Voucher Number (all Hahn)	Locality
39 *C. rumphiana* Quezon1 (8)	2874	Quezon, Luzon, Philippines
40 *C. rumphiana* Quezon2 (21)	2897	Quezon, Luzon, Philippines
41 *C. rumphiana* Quezon3 (7)	2959	Quezon, Luzon, Philippines
42 *C. rumphiana* Quezon4 (2)	2971	Quezon, Luzon, Philippines
43 *C. rumphiana* Laguna2 (6)	3000	Laguna, Luzon, Philippines
44 *C. rumphiana* Seram Indo	6203	Seram, Indonesia
45 *C. rumphiana* Sumbar Indo	6208	Sumbar, Indonesia
46 *C. rumphiana* Australia1	6410	Cape York, Queensland, Australia
47 *C. rumphiana* Australia2	6412	Cape York, Queensland, Australia
Caryota urens Linneaus		
48 *C. urens* Kerala	6335	Kerala, India
49 *C. urens* W. Ghats	6417	Western Ghats, India
Outgroups		
Wallichia		
50 *W. caryotoides* Roxburgh	6150	Mae Sa, Chiang Mai, Thailand
Arenga		
51 *A. caudata* (Mart.) H.E. Moore	6204	Fairchild Tropical Garden
52 *A. listeri* Beccari	6146	Fairchild Tropical Garden
53 *A. microrocarpa* Beccari	6210	Fairchild Tropical Garden
54 *A. porphyrocarpa* (Mart.) H.E. Moore	6206	Fairchild Tropical Garden
55 *A. tremula* (Blanco) Beccari	6142	Fairchild Tropical Garden

sonensis from Cuc Phuong, Vietnam (see comments on hybridization below). An additional deletion of approximately 400 bp was detected in the Chiang Mai accessions of *C. maxima* as well as the Nan and Vo Nhai accessions of *C. bacsonensis.*

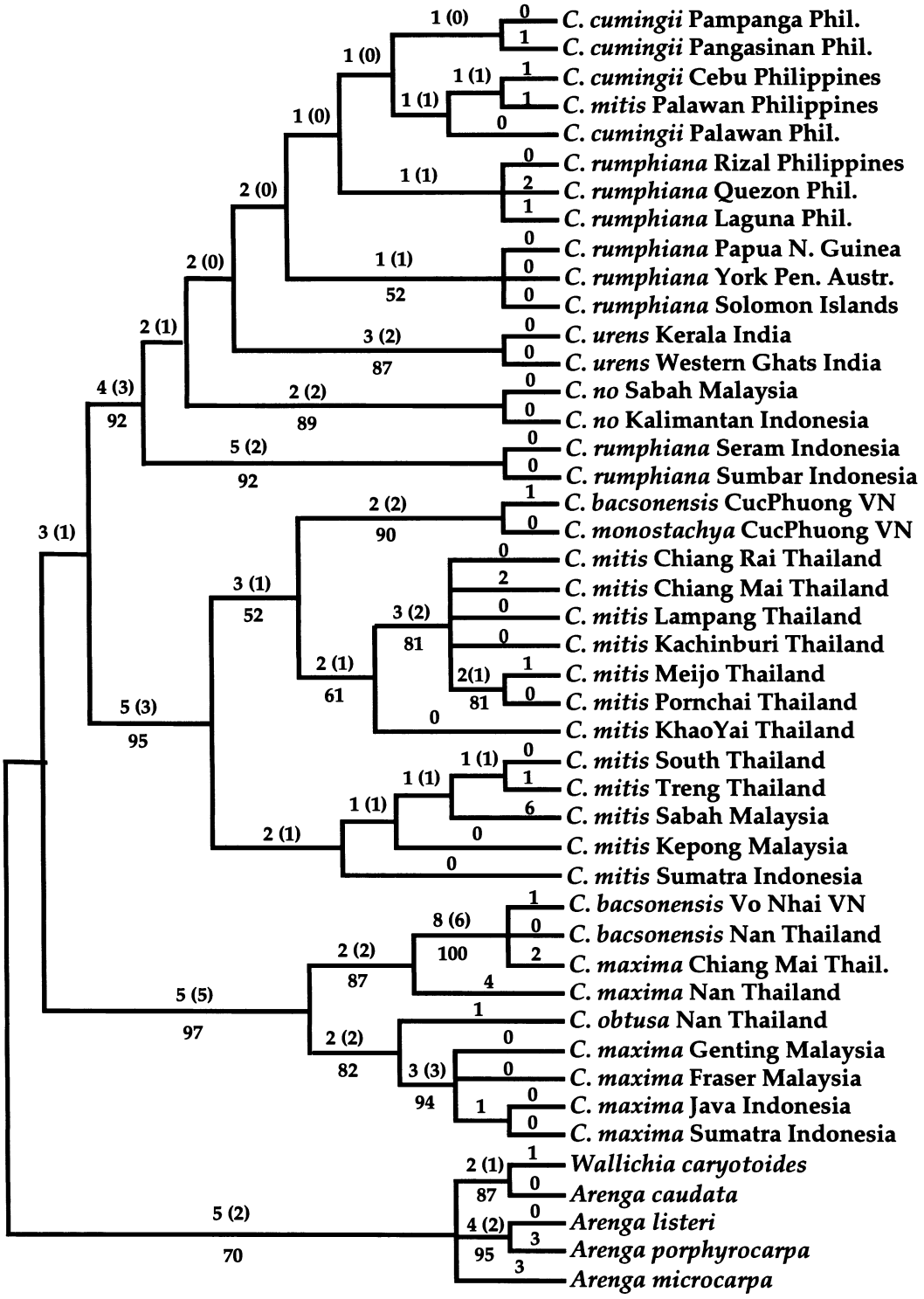
Results of Phylogenetic Analysis. The PTP tests showed significance at the 98% confidence interval indicating the presence of structure in the data set. The shortest random trees produced by this test required 4409 steps, considerably longer than the shortest trees obtained from the non-permutated data set. These results demonstrate the presence of significant structure in the data set and presumably indicate strong phylogenetic signal.

TABLE 2. Probe combinations used in this study and distribution of restriction site variation on the chloroplast genome (*Oncidium* fragment numbers from Chase and Palmer 1989).

Probe combination	*Oncidium* fragments (Chase and Palmer 1989)	Probe size	Number of mutations	#mutations/kb
I	19a,20,21,22	14.5	16	1.103
II	19b	5.7	7.5	1.316
III	18a, 18b	8.9	9.5	1.067
IV	16, 17	11.4	11	0.965
V	12c,13,14,15	10.3	3.5	0.340
VI	11, 12a, 12b	12.1	2.5	0.207
VII	10a,10b	10.7	4.5	0.421
VIII	9d	4.9	2	0.409
IX	1,2a,2b	13.3	2.5	0.188
X	3,4,6a	10.3	3.5	0.340
XI	6b	6.7	7	1.045
XII	7,8a	6.7	4.5	0.672

In all of the phylogenetic analyses, three principal clades were identified which are herein referred to as the *Maxima, Mitis,* and *Rumphiana* clades. Wagner parsimony analysis of the complete data set generated four most parsimonious trees of 116 steps with a consistency index (CI) of 0.606 (excluding uninformative characters), a retention index (RI) of 0.925, and a rescaled consistency index (RC) of 0.598. An analysis with removal of all but one accession from the same locality resulted in four shortest trees with the topology differing from the full data set analysis only in the nature of resolution within the *Rumphiana* clade (Fig. 1). These trees were also of 116 steps, a CI of 0.606, (with uninformative characters removed), a RI of 0.907, and a RC of 0.586.

The weighted parsimony analyses produced phylogenetic estimates that were topologically similar



to those produced in the equally-weighted Wagner analysis but with slightly stronger support for the branch uniting the *Mitis* and *Rumphiana* clades. Four shortest trees were detected at a weight of 1.3:1 with a consensus of these trees resulting in a loss of resolution only in small regions of the *Mitis* clade. Twenty-three shortest trees were recovered at a weight of 1.5:1 with results similar to that seen in the 1.3:1 trees. Dollo parsimony analysis produced eight shortest trees with a CI of 0.482 (excluding uninformative characters), a RI of 0.869, and a RC of 0.447. Differences between the Wagner and Dollo parsimony reconstructions are most noticeable in the pairing of the *Mitis* clade with the *Maxima* clade rather than with the *Rumphiana* clade. A strict consensus of the Dollo trees resulted in a loss of resolution in the *Rumphiana* clade and in parts of the *Mitis* and *Maxima* clades.

Minimum evolution reconstructions yielded a single tree with a topology similar to that of the Wagner parsimony analysis. The branch uniting the *Mitis* and *Rumphiana* clades was particularly short as were many of the branches within the *Mitis* and *Rumphiana* clades.

Discussion

Molecular Variation. For *Caryota,* the distribution of restriction site variation within the plastid is similar to that seen in other angiosperm cpDNAs (e.g., Kim et al. 1992). The highest levels of variation per kilobase (kb) were seen in probe regions I, II, III, and IV of the large single-copy region and XI of the small single-copy region (Table 2). The least amount of variation was detected in the inverted repeat as with most other plant groups studied to date.

Probe region I (1.103 mutations per kb), which contains a section of several ribosomal protein genes, is often involved in cpDNA rearrangements (e.g., Knox et al. 1993) and generally shows moderate to high restriction site variability. Probe region II (1.316 mutations per kb) includes several split genes (e.g., *clp*P, 5′ exon of *rps*12) and is also an inversion hotspot (Knox et al. 1993). Region III (1.067 mutations per kb) is an area that includes a large section of open reading frames and is well known for its variability (Golenberg et al. 1993; Kim et al. 1992). Probe region IV (0.965 mutations per kb) encompasses a large non-coding area between *atp*B and *psb*A, a region that showed the highest amounts of variability among species of Oncidiinae (Chase and Palmer 1989). This region also contained the two deletions detected in the present study as has been observed in other cpDNA surveys (e.g., Knox et al. 1993; Freudenstein and Doyle 1994; French et al. 1995). The small single-copy region XI (1.045 mutations per kb) contains several open reading frames and is known to vary in other monocots (e.g., Kanno et al. 1993).

Rates of Molecular Evolution. Previous studies of palms demonstrated relatively slow rates of molecular evolution and little divergence among taxa (Wilson et al. 1990; Uhl et al. 1995). In fact, palms have been shown to possess rates of molecular divergence considerably lower than all other groups of monocots studied (Gaut et al. 1992). Comparable studies on long-lived perennials such as Juglandaceae (Gunter et al. 1994; Smith and Doyle 1995) and Betulaceae (Bousquet et al. 1992) demonstrated similarly low levels of molecular divergence and implicate the effect of generation time in the calibration of any inferred molecular clock. The results of the present study include some of the lowest interspecific divergences recorded among angiosperms, and only by sampling with a larger array of restriction endonucleases was resolution of phylogenetic relationships possible.

Despite the overall low rates of molecular divergence, there was some difference in degree of internal differentiation between the three clades. Several biological features of *Caryota* may contribute to these different rates of molecular divergence including the unusual hapaxanthic (semelparous) reproductive biology, differences in growth form and generation time, the animal-dispersed fruits, geographic and ecological separation of species, and

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FIG. 1. One of 4 shortest Wagner parsimony reconstructions with only one sample per locality included. Numbers above the lines indicate the number of restriction site mutations supporting each branch. Numbers in parentheses indicate the decay index for each branch. Numbers below each line indicate the percentage of occurrence of each monophyletic group in the results of 1000 bootstrap resamplings when support was above 50%. (Tree length = 116 steps, consistency index = 0.647, consistency index excluding uninformative characters = 0.606, retention index = 0.907, rescaled consistency index = 0.586). Character transformations were optimized using the accelerated character transformation (ACCTRANS) option.

the potential for overlap of generations in some species. It should be noted, however, that potential hybridization among members of the genus (see below) could have the effect of exaggerating the degree of interpopulation and interspecific differentiation.

For the continental taxa with a caespitose growth habit (*C. mitis*, *C. monostachya*, and presumably the unexamined *C. sympetala*), extensive overlap among generations, relatively high local population densities, and apparent ease in dispersal probably account for the low levels of interspecific divergence. Among the three solitary-stemmed, continental taxa (*C. maxima*, *C. obtusa*, and *C. bacsonensis*) low population densities, very long generation times, apparently low dispersal abilities, and effective isolation by elevation or substrate barriers seem to have combined to produce high levels of interpopulation and interspecific divergence. Although the species from oceanic islands (*C. cumingii*, *C. rumphiana*, and *C. urens*) undoubtedly disperse with relative ease, geographic separation of populations by water barriers, medium-length generation times, and low to moderate population densities might account for the intermediate levels of interpopulation divergence.

Higher-level Phylogenetic Resolution. The distinctiveness of the genus *Caryota* relative to the outgroup genera *Arenga* and *Wallichia* is supported by 5 restriction site differences, a decay index of 2, and a bootstrap of 70% (Fig. 1). Due to a lack of suitable outgroups for the tribe, relationships among the three genera of Caryoteae were not resolved with restriction site data. Uhl and Dransfield (1987) have considered *Caryota* intermediate to the two other genera with a combination of both "primitive" character states (e.g., bisexual inflorescences with multiple bracts, triads throughout the inflorescence, sepals free and imbricate) and "derived" character states (e.g., bipinnate leaves, ruminate endosperm). Other features such as concolorous leaves and globose fruits serve to further distinguish *Caryota* from the other two genera. On developmental grounds, the bipinnate leaves (Fisher 1976) and ruminate endosperm could be considered apomorphies for *Caryota*.

The recent discovery of *Caryota ophiopellus* (Dowe and Cabalion 1995), which was not included in the current study, complicates this interpretation. The newly discovered taxon possesses bipinnate, concolorous leaves as found in all other species of *Caryota*. In contrast, the fruits of *C. ophiopellus* are ellipsoid, the seeds have homogeneous endosperm, and the inflorescences are unisexual; character states found in species of *Arenga* and *Wallichia* but not in any species of *Caryota*. This combination challenges the significance of at least some of the proposed synapomorphies for *Caryota* as well as the division of the tribe into three genera. A more complete analysis of all members of the tribe coupled with a better understanding of the position of the Caryoteae within the Palmae will be required to resolve this dilemma.

Within the sampled members of the genus, three principal clades were identified in all cpDNA analyses (Fig. 2). The first clade, the *Maxima* clade, is sister to the remainder of the genus in the Wagner parsimony and distance analyses. This clade consists of *C. maxima*, *C. obtusa*, and two accessions of *C. bacsonensis*; taxa that share the solitary growth habit, yellow petals, red fruits, and spinose pollen processes (Fig. 2). Members of this clade are restricted to the Asian continent and the Sunda Shelf islands of Sumatra and Java (Fig. 3). Indices of support for this clade are relatively high with a decay index of 5 and bootstrap value of 97%. Furthermore, internal relationships within this clade are resolved and well supported with bootstrap values ranging from 82–100% (Fig. 1).

A second group, the *Mitis* clade, is weakly supported as sister to the third group, the *Rumphiana* clade, in all analyses except Dollo parsimony. The *Mitis* clade consists of most accessions of *Caryota mitis*, the single accession of *C. monostachya*, and one accession of *C. bacsonensis* (Fig. 4). These latter two accessions are both from Cuc Phuong, Vietnam and the apparent misplacement of *C. bacsonensis* may be due to hybridization with *C. monostachya* (see below). *Caryota mitis* and *C. monostachya* share the clustering habit, purple petals, and maroon or purple fruits (Fig. 4).

Like all members of the *Rumphiana* clade, *Caryota mitis* has clavate pollen processes whereas *C. monostachya* has a smooth exine devoid of ornamentation (Hahn 1993; M. Harley pers. comm.). Integrity of the *Mitis* clade as a whole is well supported with a decay index of 3 and a bootstrap of 95% (Fig. 1). Resolution within this clade is largely correlated with geography with a southern *Mitis* clade sister to the remainder of the group and the northern and central populations of *C. mitis* sister to *C. monostachya* (Fig. 2).

The final group, the *Rumphiana* clade, consists of *C. cumingii*, *C. no*, *C. rumphiana*, *C. urens*, and one accession of *C. mitis*. The single, seemingly misplaced sample of *C. mitis* is possibly of hybrid ori-

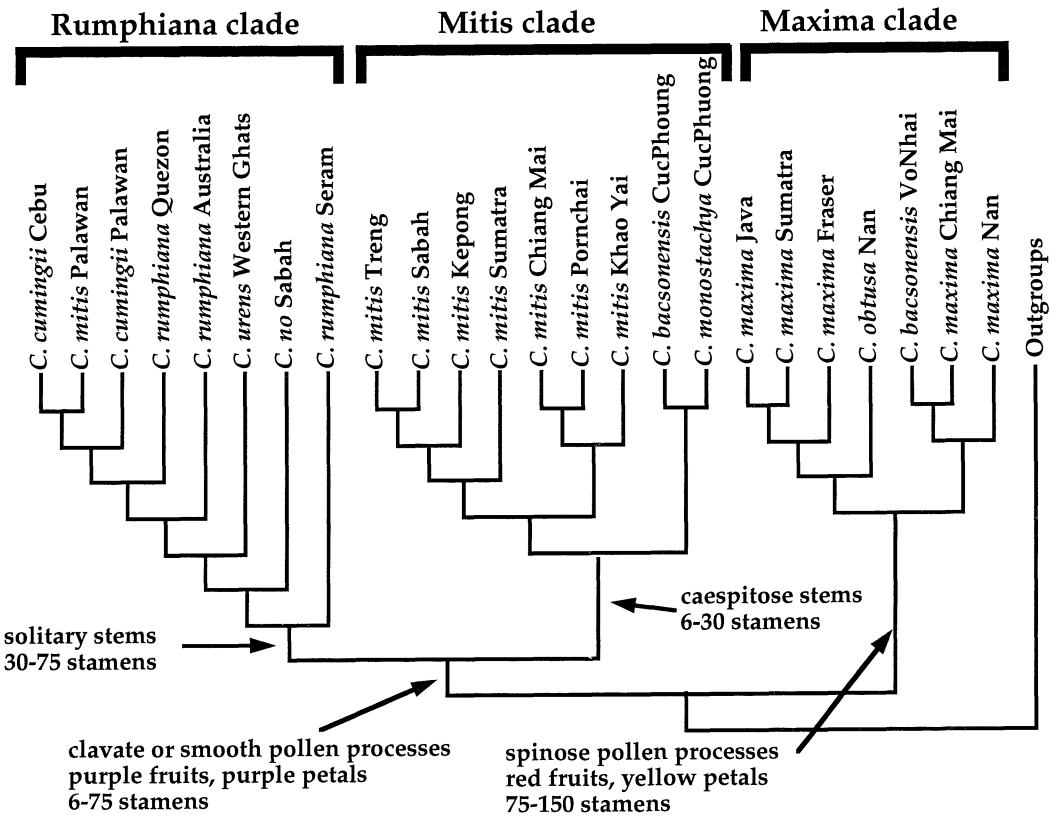

FIG. 2. Summary phylogeny for the species of *Caryota* with geographic distributions indicated. The tree is rooted with the outgroup genera *Arenga* and *Wallichia* (Uhl and Dransfield 1987). The three major clades are identified based on molecular characters but are consistent with morphological features (see text).

gin (see below). Otherwise, all of these species share the solitary stem growth habit, purple petals and fruits, and clavate pollen processes. With the exceptions of the Palawan population of *C. cumingii*, *C. no* on Borneo, and *C. urens* in India and Sri Lanka, this clade is restricted to the east of Huxley's Line (Fig. 5). Character support for the *Rumphiana* clade is strong with decay at 3 additional steps and a bootstrap value of 92% (Fig. 1). The narrowly distributed *Caryota no* and *C. urens* were each strongly supported as monophyletic (a decay of 2 steps for each and bootstraps of 89% and 87% respectively) although some interpretations of their restriction site patterns were difficult. Three lineages of the widely distributed and apparently paraphyletic *C. rumphiana* were detected, representing the eastern (Australia, New Guinea, and the Solomon Islands), central (Seram and Sumbar), and western (Philippines) parts of its range.

Measures of character support for the Wagner parsimony reconstructions vary considerably within the trees. Relaxing the parsimony criterion to allow trees of 117 steps or shorter produced 1258 trees with both strict and majority-rule consensus of these showing a collapse of support for the *Mitis*/*Rumphiana* bifurcation and many of the branches within the *Mitis* and *Rumphiana* clades. Several of the branches internal to these two clades are supported by only one or two character-state changes and show low decay and bootstrap indices. However, relatively good support and resolution are maintained within the *Maxima* clade.

The pairing of the *Mitis* and *Rumphiana* clades is seen in the shortest equally and differentially-weighted Wagner parsimony analyses as well as the minimum evolution analyses. Although support for this pairing is not strong and is contradicted in the Dollo parsimony analyses, it is more consistent with several morphological characters (pollen morphology, petal and fruit pigmentation, stamen num-

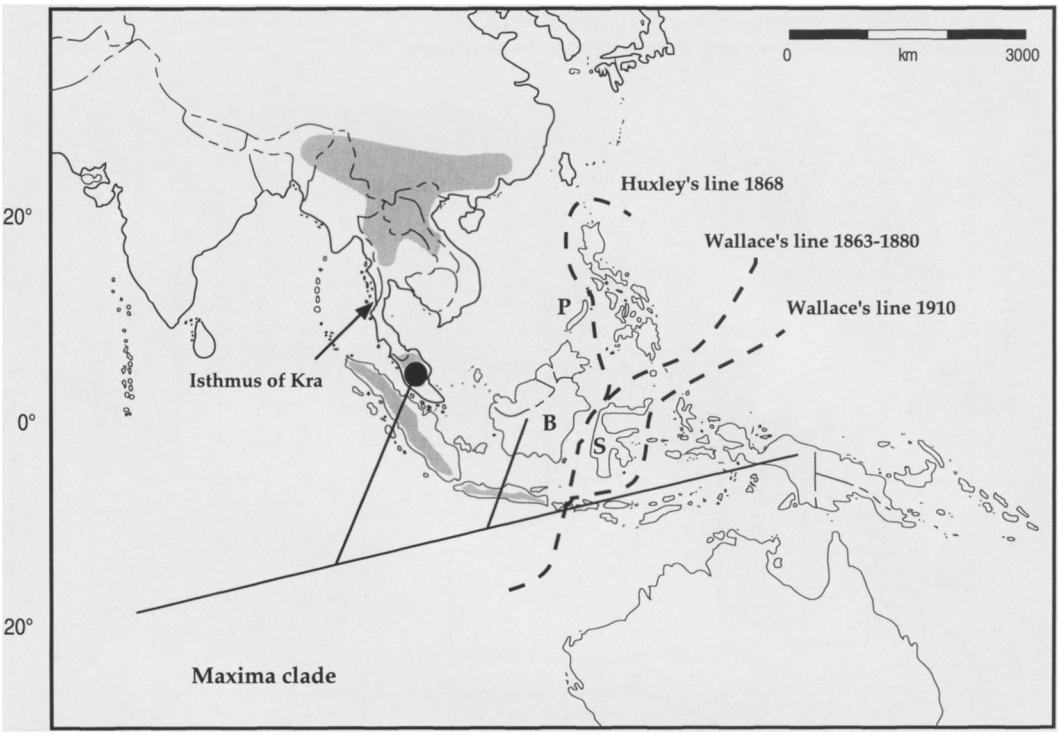

FIG. 3. Distribution of the *Maxima* clade in relation to major biogeographic boundaries. B = Borneo, P = Palawan, S = Sulawesi.

ber—see Fig. 2) and is herein taken as the best supported topology of intrageneric relationships for *Caryota*.

Species Delimitation. In a phenetic analysis of morphological and anatomical data (Hahn 1993), ten species of *Caryota* were recognized based on clustering of specimens via principal components and canonical variates analyses. Material of the recently described *C. ophiopellus* was not available for that study but the species is so distinct that it would probably not be confused with any other member of the genus. In fact, as discussed above, this species may best be assigned to *Arenga* given some of the character states that it possesses. The phenetic groupings identified served as species hypotheses to be examined for monophyly under a phylogenetic species concept (e.g., Cracraft 1983). Unfortunately, the morphological data available did not allow for phylogenetic resolution at the species level, so it was the goal of the present study to test these phenetic species delimitations for monophyly.

Chloroplast DNA evidence supports monophyly for all of the narrowly distributed species (*C. bacsonensis*, *C. cumingii*, *C. no*, and *C. urens*) with the exception of a few cases of species sympatry and possible hybridization (see below). For the two taxa sampled only once, (*C. monostachya* and *C. obtusa*), the phylogenetic placement was consistent with the phenetic analyses (Hahn 1993) but monophyly of the taxa could not be directly tested. *Caryota monostachya* is a clearly circumscribed taxon with a comparatively narrow range and would probably not be confused with any other species. *Caryota obtusa*, on the other hand, is a more widespread taxon with an uncertain delimitation. Additional material from southern China and northeastern India is needed to verify the limits of this species.

All three of the widespread species (*C. mitis*, *C. rumphiana*, and *C. maxima*) were resolved as paraphyletic with one or more of the narrowly distributed species nested within (Fig 1). Several different explanations for this paraphyly are possible including previous episodes of hybridization and introgression (Arnold 1992, 1997), lineage sorting of ancestral polymorphisms (Niegel and Avise 1986), or failure to recognize cryptic species within a species complex.

Evidence of paraphyly of widespread plant spe-

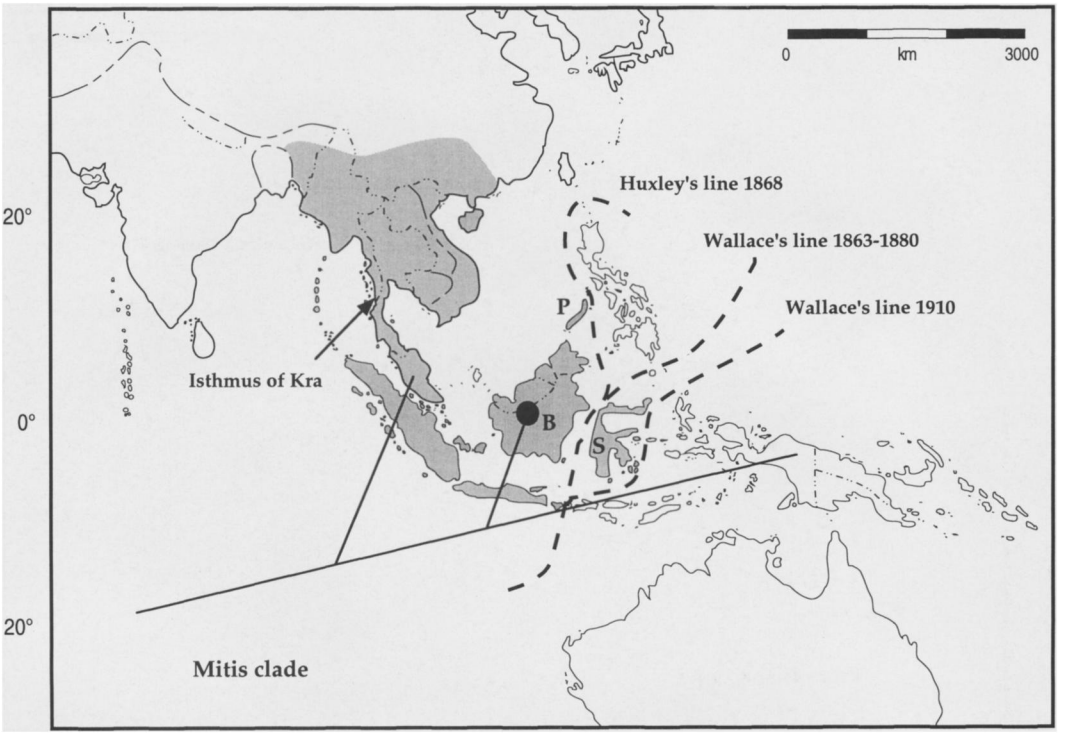

FIG. 4. Distribution of the *Mitis* clade in relation to major biogeographic boundaries. B = Borneo, P = Palawan, S = Sulawesi.

cies is not uncommon but the actual acceptance of non-monophyletic species ultimately depends upon the species concept employed as well as the degree and nature of phylogenetic resolution available. Some have argued that at least some paraphyletic taxa are inevitable (e.g., Rieseberg and Broulliet 1994), and the interim solution in cases of poor resolution is simply to accept paraphyletic species. Because of the incomplete understanding of species limits in *Caryota,* it is this approach that is taken in the current study.

As an example, the loosely delimited *C. maxima* interdigitates with the other members of the *Maxima* clade (*C. bacsonensis* and *C. obtusa)* and is resolved as paraphyletic in the cpDNA analyses. Several clearly related species have been described including *C. aequatorialis* Ridley, *C. gigas* Hahn ex Hodel, *C. kiriwongensis* Hodel, and *C. ochlandra* Hance but, for lack of clearly defined morphological differences among them, all of these taxa were lumped in *C. maxima* for the current study. Resurrection of one or more of these described species might possibly alleviate the problems of paraphyly in this clade but the lack of convincing morphological evidence argues against such an approach. Furthermore, the potential for misleading phylogenetic resolution based on organellar data alone strongly suggests that that further tests using nuclear data, additional populations, and more complete morphological studies are required for proper delimitation of species in this clade.

Hybridization. Most of the species of *Caryota* identified by phenetic analyses (Hahn 1993) show very little sympatry with each other and are limited biogeographically or ecologically by elevation or substrate preference. Nonetheless, in some of the cases where species co-occur there are indications of hybridization and introgression.

Hybridization has been implicated between species of *Caryota* in the Philippines (Beccari 1919) and morphological evidence for hybridization was detected at several locations in the present study. Some collections from the island of Cebu in the Philippines show morphological intermediacy between the Philippine endemic *C. cumingii* and the widespread *C. rumphiana,* two taxa otherwise well characterized on morphological grounds. However, subsequent molecular analyses of plants from the

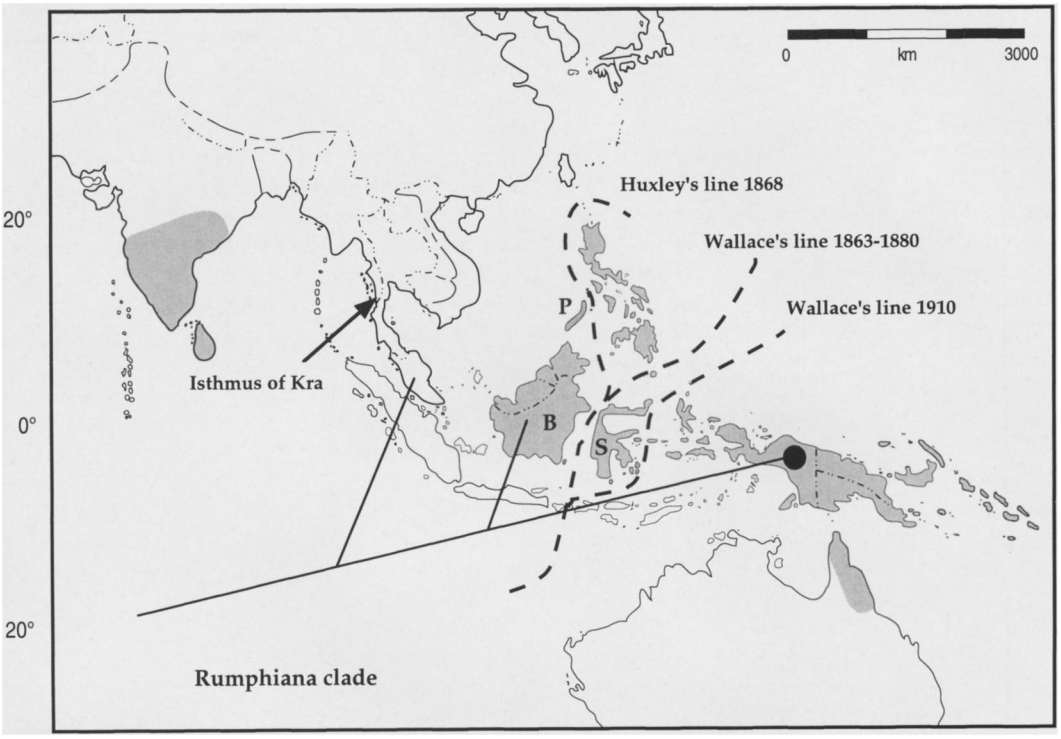

FIG. 5. Distribution of the *Rumphiana* clade in relation to major biogeographic boundaries. B = Borneo, P = Palawan, S = Sulawesi.

same locality failed to demonstrate any evidence of hybridization, at least in terms of cpDNA variation. Similarly, in the central highlands of Vietnam, the endemic *C. sympetala* shows a number of features intermediate to the native and sympatric *C. mitis* and *C. monostachya*. Among these are small clustering stems and a sparsely branched, semi-erect panicle with flowers and fruits of an intermediate size and shape. Unfortunately, material of *C. sympetala* was not available for the molecular aspects of this study.

In two other localities, however, there was good molecular evidence of interspecific hybridization and resultant chloroplast introgression (Fig. 1). Interestingly, in neither case was there morphological evidence of hybridization. On the island of Palawan, both accessions of *C. mitis* included in this study showed plastid genotypes most similar to that of the sympatric *C. cumingii*. In contrast, all accessions of *C. mitis* from other localities were resolved as a monophyletic group distinct from the *C. cumingii* subclade. Although these two species are morphologically similar, leaf material used in this study was derived from complete collections (Hahn 1993) and the plants sampled conform well to the respective species delimitations.

At the single collection site (Cuc Phuong, Vietnam) for *C. monostachya*, some sympatric individuals of *C. bacsonensis* possess a plastid genome similar to that observed in *C. monostachya*. These two species are morphologically very distinct with *C. bacsonensis* characterized by medium to large solitary stems, spreading leaves, pendulous panicles with ca. 100–150 rachillae, yellow petals, and red fruits. *Caryota monostachya* is a small, caespitose, understory species with thin stems, spicate inflorescences, and purple petals and fruits. No morphologically intermediate individuals were seen at this locality, suggesting that repeated backcrosses have erased any morphological evidence of hybridization.

The contrast between morphological and molecular data seen in these taxa is not unprecedented. Many molecular studies have confirmed morphological evidence of hybridization but other molecular studies have identified hybrids even though there was no obvious morphological evidence (Rieseberg and Ellstrand 1993; Arnold 1992, 1997). In

particular, several studies reviewed by these authors suggest that the plastid may introgress much more readily than nuclear or morphological markers.

Intraspecific Variation. Initial studies of intraspecific cpDNA restriction site variation assumed relatively low levels of intraspecific divergence and tended to sample poorly within the boundaries of individual species (reviewed in Soltis et al. 1992). Subsequent studies have shown that intraspecific variation can occasionally be quite high and that such variation has possible effects on phylogenetic reconstruction, estimates of interspecific divergence, and inference of mode of speciation (e.g., Harris and Ingram 1991; Rieseberg and Soltis 1991). Additionally, episodes of hybridization, introgression, and chloroplast capture have been well documented in a number of plant taxa (Rieseberg and Brunsfeld 1992) which may further complicate patterns of intraspecific variation. While intraspecific variation studied in some taxa has been correlated with geographic and geologic factors (e.g., Soltis et al. 1992; Avise 1996), the need for additional studies and more intensive sampling is clear.

In the present study, special attention was paid to intraspecific variation and hybridization for several reasons. First, the limits of some of the recognized species are not well defined and additional taxonomic realignments may be necessary. Second, the broad geographic ranges of several of the species coupled with numerous barriers to gene flow among different populations present the opportunity for significant isolation and intraspecific differentiation. Third, although sympatry is not common in the genus, hybridization has been suggested for *Caryota* in a number of areas based on morphological evidence (Beccari 1919). Fourth, there are relatively few studies of trees and long-lived perennials, particularly for tropical tree groups (although see Sytsma and Schaal 1985; Bruneau and Doyle 1993; Byrne and Moran 1994; Givnish et al. 1995). The low population densities typical of many tropical trees further enhance the effects of population-level phenomena and have been implicated as factors involved in tropical speciation (Bawa and Beach 1981; Bawa 1992). Finally, *Caryota* exhibits both mammal and avian fruit dispersal on insular and continental systems (Zona and Henderson 1988) which may result in different patterns of differentiation.

The intraspecific variation seen in *Caryota* is noteworthy in both its magnitude and distribution. Six of the eight species sampled at multiple sites exhibited some degree of variation, the exceptions being *C. no* and *C. urens.* The former is confined to the island of Borneo and has one of the longest generation times in the genus (ca. 20 yrs.) *Caryota urens*, on the other hand, has a relatively wide distribution throughout the tropical and subtropical parts of India and Sri Lanka and is common in cultivation. As such, one possible explanation for a lack of variation in this taxon is a recent origin related to human-mediated dispersal and a history of cultivation.

Biogeography. In the current analysis, differences between the various parsimony and distance-based reconstructions give only slightly different interpretations of biogeography for *Caryota.* At higher levels of diversification, distributions within the genus correlate well with previously established biogeographic delineations and major geologic features for Southeast Asia. The three main clades are largely confined by Huxley's Line and three of the four deviations involve islands that border this line. Within each of the major clades, geological factors, habitat specialization, and mechanisms of dispersal are correlated with patterns of distribution and diversification.

With only one exception, all species found east of Huxley's Line are in the *Rumphiana* clade (Fig. 5). In contrast, most taxa found west of this line are in the *Mitis* or *Maxima* clades (Figs. 3, 4). The deviations from this biogeographic separation of the clades are: 1) *C. urens* of the *Rumphiana* clade in India and Sri Lanka, 2) *C. no* of the *Rumphiana* clade on Borneo, 3) *C. cumingii* of the *Rumphiana* clade on Palawan, and 4) *C. mitis* of the *Mitis* clade on Sulawesi. The first exception may involve human mediated dispersal while the three latter exceptions all involve islands that border Huxley's and Wallace's Lines.

This general adherence to the fundamental split of the region by Wallace's or Huxley's Line is consistent with broader trends seen in most other plant and animal taxa (e.g., Wallace 1876, Steenis 1979). In particular, where the lines break down, the differences usually involve taxa on the same border islands or island complexes—Borneo plus Palawan, the Philippines, and Sulawesi (e.g., Simpson 1977).

For *Caryota*, the choice of lines is problematic but Huxley's seems to have the greatest support. With Wallace's 1863 Line, two of the three clades are severed with conflicts on Borneo (*C. no*), Sulawesi (*C. rumphiana, C. mitis*), and the Philippines including Palawan (*C. rumphiana, C. cumingii*). For Wallace's 1910 Line, only the *Rumphiana* clade is disrupted but three species are involved: *C. rumphiana* on Su-

lawesi, C. *no* on Borneo, and C. *cumingii* and C. *rumphiana* in the Philippines including Palawan. For Huxley's Line, the same problems with Wallace's 1863 line exist (*C. cumingii* on Palawan, *C. mitis* on Sulawesi, and *C. no* on Borneo,) but the distribution of C. *rumphiana* is no longer split.

Wallace's 1863 Line is violated by almost half of the recognized species in *Caryota* and does not explain patterns of variation as well as the alternatives. Wallace's 1910 Line emphasizes the connection between Sulawesi and the Sunda Shelf but also claims a strong connection between the Philippines and the Sunda Shelf. While the former is supported in *Caryota* (with *C. mitis*), evidence for a connection between the Philippines and the Sunda Shelf is limited to a few collections of C. *cumingii* on the island of Palawan. In contrast, Huxley's Line emphasizes the Australian affinities of the Philippines over the Asiatic influences as seen in the wide ranging *C. rumphiana* as well as the general structure of the three clades. In sum, Huxley's Line seems to better describe biogeographic patterns in *Caryota.*

Within each of the three major clades, additional geographic correlations are evident. The well-characterized *Maxima* clade shows a strongly supported and highly defined phylogenetic structure that coincides with geographic and geologic features. Broad relationships within this clade are mostly resolved as northern and southern subclades divided by the Isthmus of Kra (Fig. 2). All accessions of *C. maxima* from south of the Isthmus of Kra are united in a single monophyletic group, as are all accessions from northern Thailand. The two accessions of *C. bacsonensis* are included in the northern subclade in agreement with their geographic origin. The only geographic aberration is the single population sampled of *C. obtusa* that is included in the southern subclade despite its northern origins.

Levels of molecular variation among the members of the northern subclade are the highest seen within the entire genus. In addition to restriction site differences, both of the two deletions detected in this study are confined to members of the northern subclade. In contrast to the variation found in the northern subclade, comparatively little cpDNA diversity was detected among the accessions of *C. maxima* from south of the Isthmus of Kra. This would be consistent with a relatively recent origin of the southern accessions of *C. maxima* relative to the northern accessions. Drier and cooler periods during the Pleistocene resulted in generally lower sea levels and a more or less continuous connection among the Sunda Shelf Islands of Sumatra, Java, Bali, Borneo, and Palawan (Morley and Flenley 1987; Morley 1998). These connections could have allowed direct diffusion of *C. maxima* across the continuous land mass as suitable climatic conditions for *C. maxima* would have been at lower elevations than seen today. Subsequent rising of sea levels separated the islands and increasing temperatures would have forced the species to the higher elevations it inhabits today.

Monophyly for the *Mitis* clade is well supported but that of *C. mitis* is not. The single accession of *C. monostachya* from Cuc Phuong, Vietnam, is weakly nested among accessions of *C. mitis* although its placement is consistent on biogeographic grounds. As seen in the *Maxima* clade, a fundamental split among the 13 accessions in the *Mitis* clade coincides with the biogeographic boundary of the Isthmus of Kra in peninsular Thailand (Fig. 2). Within the two subclades in the *Mitis* clade, little or no variation among populations was detected with the exception of *C. mitis* from Borneo (Sabah). As with *C. maxima,* the current distribution of *C. mitis* on the Greater Sunda Islands can be explained by simple diffusion across contiguous habitat during previous periods of lower sea levels.

The presence of *C. mitis* on the island of Sulawesi is more problematic unless a very early origin is postulated for *C. mitis* or a hypothesis of long distance dispersal is invoked. Sulawesi is a composite island composed of fragments of several different origins with at least one fragment derived from material once connected to Borneo (Hall 1996). Based on the date of separation for this fragment, any vicariance explanation for the current distribution of *C. mitis* would require an extremely early origin for the species—at least 10 Mya, more likely 25 Mya (Hall 1996; Morley 1998)—with little subsequent morphological divergence within the species.

Alternatively, long distance dispersal across the Makassar Strait or via some of the proposed stepping stone fragments could explain the current distribution of *C. mitis* on Sulawesi. This species is widespread in the lowlands throughout Sulawesi as well as the presumed source area of Borneo. Additionally, the species is widespread and common throughout the remainder of its natural range and has some weedy tendencies when introduced into other tropical areas. The colonial mode of growth, ability to self-pollinate, and apparently good dispersal characteristics for the fruits would support a dispersalist hypothesis. Nonetheless, the absence of *C. mitis* in the Philippines then becomes noteworthy although it may simply be replaced by *C. cumingii*

there. Unfortunately, material of *C. mitis* from Sulawesi was not available for this study.

Within the *Rumphiana* clade, rigorous biogeographic inference is not possible with the current level of phylogenetic resolution. However, as mentioned above, the geographic distribution of three of the four species in this clade challenge the integrity of Wallace's and Huxley's Lines. *Caryota no,* on the island of Borneo, is the only species of the *Rumphiana* clade to occur exclusively on the Sunda Shelf, west of Wallace's and Huxley's Lines (Fig. 6). Borneo itself is a composite island having been formed through both autochthonous volcanic activity as well as accretion of terranes formed elsewhere (Hall 1996). Although *C. no* is not well collected, it is known from some widely dispersed localities suggesting comparatively little habitat specificity. Possible biogeographic hypotheses for *C. no* are that: 1) it is a remnant of the early cladogenic event that gave rise to the *Rumphiana* clade, or 2) it represents a back-disperal event after the clade had occupied the lands east of Huxley's Line.

For the former hypothesis, an early point of origin would need to be invoked as with *C. mitis* for Sulawesi. Unlike *Caryota mitis,* which is found on both sides of Wallace's and Huxley's Lines, *C. no* is a distinct species easily distinguished from the other members of the *Rumphiana* clade. Nonetheless, source areas for dispersal are abundant with both Sulawesi and Mindanao in the Philippines, home to the closely related *C. rumphiana.* In particular, while collections of *C. no* differ morphologically from *C. rumphiana,* it is precisely the collections of *C. rumphiana* from Mindanao that are most similar to *C. no* (Hahn 1993).

Caryota cumingii, also of the *Rumphiana* clade, is widely distributed throughout the Philippines including the Sunda Shelf island of Palawan. For a species that is clearly capable of dispersal among the many islands of the Philippine archipelago, its presence across Huxley's Line on Palawan is not exceptional. Nonetheless, the question of its absence on Sulawesi and Borneo is notable. In this case, it is possible that the species is replaced by *C. mitis* on these latter islands. For Palawan, replacement is not complete as both *C. cumingii* and *C. mitis* are recorded and appear to hybridize on occasion.

The third problematic distribution within the *Rumphiana* clade concerns the toddy palm, *Caryota urens,* of India and Sri Lanka. This species is widely cultivated in India and Sri Lanka and used for a multitude of purposes (Balick and Beck 1990). Several lines of evidence suggest that this species may not be native to these countries. First, on both morphological and molecular grounds, the phylogenetic affinities of this species are clearly with the *Rumphiana* clade, which is otherwise found strictly to the east of Wallace and Huxley's Lines or on the border islands of Borneo and Palawan. In fact, apart from locality data, there are virtually no morphological characters that consistently separate collections of *C. urens* from those of *C. rumphiana* (Hahn 1993). Second, *C. rumphiana* is frequently cultivated in the Philippines for many of the same products (Balick and Beck 1990). Third, collections of the species are almost always of cultivated plants, from feral plants in formerly cultivated areas, or from secondary forest. Only rarely are collections made from relatively undisturbed forest. Several species of the genus *Caryota* are well known to escape from cultivation in other parts of the world (e.g., Florida, Costa Rica). Finally, the long history of cultivation argues for at least some human-mediated dispersal. Nonetheless, additional resolution of relationships within the *Rumphiana* clade is needed to test this hypothesis.

***Summary Model of Evolution in* Caryota.** In many phylogeny-based interpretations of Southeast Asian biogeography (e.g., Schuh and Stonedahl 1986; Axelius 1990), vicariance due to tectonic events or sea level changes are inferred as the principal mechanisms determining current distributions. In other studies (e.g., Adema 1991; Turner 1995), one or more dispersal events were invoked to explain distributions in taxa that crossed Wallace's or Huxley's Lines. For *Caryota,* a combination of the two mechanisms seems to be involved. Broad patterns of relationship among the three principal clades correlate well with vicariance coupled with the geologic history of SE Asia. However, at least some of the deviations from this correlation seem best explained by dispersal. In terms of directionality, Dransfield (1987) has suggested a pattern of west to east expansion for SE Asian palms in general. This scenario is supported by the current phylogenetic analysis of *Caryota* as well as available geologic evidence.

A general model of evolution for *Caryota* would posit an early origin on the mainland of SE Asia followed by two separate episodes of cladogenesis. Each of these would involve habitat preference shifts, morphological evolution, and subsequent range expansion due to both vicariance and dispersal. In this model, the common ancestor would most closely resemble a member of the *Maxima* clade. Apart from the basal resolution of this group,

the *Maxima* clade has some of the deepest nodes and longest branches in the molecular analyses, a pattern consistent with an early origin for the group. Furthermore, the extant members of the clade occur on relatively old and geologically stable terrains. Finally, this clade contains several morphological character states, such as spinose pollen processes, that could be considered plesiomorphic.

The first cladogenic event would have involved invasion of the lowlands and shifts in floral and fruiting biology as seen in the *Mitis* and *Rumphiana* clades. Morphologically this would include the evolution of dark-pigmented petals and fruits as well as pollen with gemmate processes. Presumably, these morphological changes would have an effect on the pollination and fruit dispersal of these species although little is known about these biological features. Even though members of both the *Mitis* and *Rumphiana* clades are found on either side of Huxley's Line, only the *Mitis* clade is found on the mainland of SE Asia and might reasonably represent the product of this first cladogenic event. Morphological evolution in the form of a caespitose growth form and long petioles may well be associated with the colonization of the understory.

Although resolved within the *Mitis* clade by cpDNA data, the comparatively local endemic *C. monostachya* shows transitional stages between the *Maxima* and *Mitis* clades in several morphological features. Similarities with the *Maxima* clade include broad ovate petals, 75 or more stamens, and comparatively large fruits with a leathery pericarp. Similarities with the *Mitis* clade include the caespitose habit, the long petioles, thin leaflets, purple petals and fruits, and the lack of spinose pollen processes. Much further study is needed for this species and the closely related *C. sympetala* to properly ascertain their phylogenetic affinities.

The widespread species, *C. mitis*, is found throughout the mainland from Burma and China through Peninsular Malaysia and throughout all of the Sunda Shelf islands as well as to the east of Huxley's Line on Sulawesi. Through a process of diffusion, this species could have colonized all of the Sunda Shelf islands during periods of lower sea levels in the Pleistocene or Pliocene (Morely and Flenley 1987). While Cenozoic reconstructions of the geology of SE Asia indicate previous geologic connections between Borneo and Sulawesi (Hall 1996; Morley 1998), a vicariance explanation for the presence of *C. mitis* on Sulawesi would require a very slow rate of morphological change. Herbarium material from that island shows very few morphological differences relative to material from the remainder of its range. A hypothesis of relatively recent dispersal across the Makassar Strait would more easily account for the presence of *C. mitis* on that island. Morley (1998) has provided a wealth of evidence for this scenario in that fossil pollen records indicate biotic exchange across Wallace's Line at 17, 14, 9.5, 3.5, and about 1 Ma.

The second cladogenic event would involve differentiation of a solitary stemmed group, the *Rumphiana* clade, from its common ancestor with the caespitose *Mitis* clade. The current distribution of the *Rumphiana* clade is predominantly to the east of Huxley's Line although three exceptions are noted. In two cases (*C. cumingii* on Palawan and *C. urens* in India and Sri Lanka), long distance dispersal seems to be involved. The very widespread distribution of *C. rumphiana* (Philippines to the Solomon Islands) suggests that dispersal has played a very large role in the evolution of this group. In the case of *C. no*, however, its status as a distinct species relative to the remainder of the *Rumphiana* clade would more strongly support speciation and cladogenesis due to vicariance of Borneo and Sulawesi rather than dispersal between these two islands.

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APPENDIX 1. Chloroplast DNA restriction site polymorphisms detected in this study. Probes were prepared from the *Oncidium* cpDNA library (Chase and Palmer 1989) provided courtesy of M. Chase, Royal Botanic Gardens, Kew. The probe combinations are described in TABLE 2. Plesiomorphic and apomorphic character states determined using *Arenga* and *Wallichia* as outgroups are listed as fragment sizes, in kilobases.

Character number	Enzyme	Probe combination	Mutation
1	*Ase*I	I	0.95 + 0.6 = 1.55
2		II	2.0 = 1.9 + 0.1
3		II/III	4.0 = 2.3 + 1.7
4		VII	3.5 = 2.8 + 0.7
5		VIII	4.5 = 4.3 + 0.2
6	*Ava*I	I	3.1 = 2.85 + 0.25
7		I	3.1 + 0.6 = 3.7
8		III	7.6 = 6.7 + 0.9
9	*Bam*HI	I/VIII	6.0 = 2.8 + 3.2
10		IV	5.0 = 4.3 + 0.7
11		V	4.0 + 2.4 = 6.4
12		VI	6.8 = 5.45 + 1.35
13		VII	2.4 + 1.1 = 3.5
14		IX	2.5 + 1.5 = 4.0
15		X	3.0 + 0.7 = 3.7
16	*Ban*II	II/III	7.7 = 3.9 + 3.8
17		III	1.3 = 0.,9 + 0.4
18		VI	4.2 = 2.1 + 2.1
19		VI	4.2 = 3.3 + 0.9
20		VI	1.8 + 0.7 = 2.5
21		X	1.0 + 0.6 = 1.6
22		XI/XII	23.0 = 21.4 + 1.6
23	*Bcl*I	I	2.15 = 1.90 + 0.25
24		I	5.2 = 3.8 + 1.6
25		II	2.7 + 0.6 = 3.3
26		II	4.0 = 3.2 + 0.8
27		II/III	6.5 + 3.2 = 9.7
28		IV	4.6 + 3.6 = 8.2
29		IV	4.3 + 3.6 = 7.9
30		XI/XII	6.3 = 6.0 + 0.3
31		XI/XII	3.0 + 1.0 = 4.0
32	*Bgl*I	V/VI	17.1 = 10.3 + 6.8
33	*Bgl*II	III/IV	4.1 + 1.0 = 5.1
34		III/IV	2.7 = 2.3 + 0.4
35		IV	1.7 + 1.8 = 2.5
36		X/XI	10.2 = 6.1 + 4.1
37	*Bst*BI	III	3.1 + 2.0 = 5.1
38		IV	3.3 + 1.8 = 5.1
39	*Bst*NI	I	4.4 + 0.9 = 5.3
40		I	5.3 = 4.7 + 0.6
41		III	3.0 = 2.4 + 0.6
42		III	3.0 = 1.9 + 1.1
43	*Cfo*I	X	2.0 = 1.6 + 0.4
44		XI/XII	6.5 = 4.7 + 1.8
45	*Cla*I	I	5.9 = 5.7 + 0.2
46		III	5.2 + 1.3 = 6.5
47	*Dra*I	V	2.5 = 1.5 + 1.0
48		IV	2.3 + 1.8 = 4.1
49		IX	15.0 = 9.4 + 4.6
50		XI	2.2 = 1.1 + 1.1
51	*Eco*O109	VII/VIII	15.1 = 9.5 + 5.6
52	*Eco*RI	I	2.1 + 0.45 = 2.55
53		I/IX	2.6 + 0.2 = 2.8
54		III	4.9 = 2.6 + 2.3
55	*Hin*dIII	I/II	10.5 = 5.6 + 4.9
56		VII	2.5 + 0.3 = 2.8
57		XI/XII	7.1 = 2.3 + 5.8
58	*Nci*I	I	2.2 = 1.9 + 0.5
59		I	1.5 = 1.0 + 0.5
60		IV	7.6 = 5.0 + 2.6
61		IV	7.6 = 4.8 + 2.8
62		V	1.9 = 1.7 + 0.2
63		VII	4.3 + 2.7 = 7.0
64		XI/XII	13.0 = 9.4 + 3.6
65		XI/XII	13.0 = 6.5 + 6.5
66		XI/XII	7.0 = 4.0 + 3.0
67	*Nsi*I	I	9.0 = 4.7 + 4.3
68		XI/XII	1.1 + 1.2 = 2.3
69	*Pvu*II	I	16.0 + 2.5 = 18.5
70		II	3.7 + 18.3 = 22.0
71		IV	21.2 = 14.3 + 6.9
72		IV	20.7 = 10.7 + 10.5
73	*Xmn*I	I/II	2.5 = 1.3 + 1.2
74		II	1.5 = 0.7 + 0.8
75		XI	3.4 = 2.5 + 0.9

APPENDIX 1. Continued.

APPENDIX 2. Chloroplast DNA Restriction site data matrix for *Caryota*.

	1111111111222222222233333333334444444445555555555666666666677777777
	1234567890123456789012345678901234567890123456789012345678901234567890123456789012345
C. *bacsonensis* VoNhai VN	10100100001000001000111000000001000000010010001?000000101100100000000100100101
C. *bacsonensis* VoNhai VN	10100100001000001000111000000001000000010010001?000000101100100000000100100101
C. *bacsonensis* CucPhuong VN	10100000100000000100111000000011000000001010000000010000000000000000000000000
C. *bacsonensis* Nan Thail	10100100001000001000111000000001000000010010001?000000101100100000000100100101
C. *cumingii* Pampanga Phil	00101000000000000010010011000000001000000010101000001000000000000000000100000
C. *cumingii* Pangasinan Phil	00101000000000000010010011000000001000000010101000001000000000000000000100000
C. *cumingii* Cebu Phil	00101000000000000010010011001000001000000010101001001000000000000000000100000
C. *cumingii* Palawan Phil	00101000000000000010010011001000001000000010101001001000000000000000000100000
C. *maxima* ChiangMai1 Thail	10110100001000001000111000000001000000011010010000000101100100000000100100101
C. *maxima* ChiangMai2 Thail	10110100001000001000111000000001000000011010010000000101100100000000100100101
C. *maxima* ChiangMai3 Thail	10110100001000001000111000000001000000011010010000000101100100000000100100101
C. *maxima* Nan Thail	10100000001000000000111000000001000000010000000010110000000100000000100000001
C. *maxima* Genting Mal	11100001001000000000110000000000000000010000000010001000000000000010100000001
C. *maxima* Fraser Mal	11100001001000000000110000000000000000010000000010001000000000000010100000001
C. *maxima* Java Indo	11100001001000000000110000000000000000010000000010001000000010000010100000001
C. *maxima* Sumatra Indo	11100001001000000000110000000000000000010000000010001000000010000010100000001
C. *mitis* ChiangRai Thail	10100000100100000100001000000000000000011000010010110000000010001010100000001000
C. *mitis* Chiang Mai Thail	10100000100100001?000100000000000000011000010010110000000011000101010100000001000
C. *mitis* Lampang Thail	10100000100100001000010000000000000000011000010010110000000010001010100000001000
C. *mitis* Kachinburi Thail	10100000100100001000010000000000000000011000010010110000000010001010100000001000
C. *mitis* KhaoYai Thail	10100000100100001000010000000000000000011000010010110000000010000010100000001000
C. *mitis* Meijo Thail	10100000100100001000010001000000000000011000010010110000010001010100000001000
C. *mitis* Pornchai Thail	10100000100100001000010001000000000000011000010010110000000010001010100000001000
C. *mitis* S Thail	10100000100000000100001000010000000000011000010010110000000010001010000000010000
C. *mitis* Treng Thail	10100000100000000100001000?00000000000011000010010110000000010001010000000010000
C. *mitis* Kepong Mal	10100000000000000000010000100000000000011000010010110000000010001010000000010000
C. *mitis* Sumatra Indo	10100000000000000000010000100000000000011000?00101000000010001010000000010000
C. *mitis* Sabah Mal	10100010000000000000010000100000111001000001001010000001000101000000010000
C. *mitis* Palawan1 Phil	00101000000000000010010011001000010000000100000010001000010000000000100000
C. *mitis* Palawan2 Phil	00101000000000000010010011001000010000000100000010001000010000000000100000
C. *monostachya* CucPhuong VN	10100000100000000100010000011000000011000000010100000000010000000000000000000

APPENDIX 2. Continued.

	1111111111222222222233333333334444444445555555556666666667777777 12345678901234567890123456789012345678901234567890123456789012345
C. *no* Sabah Mal	00101001000000000000010010100000100000000000010000001000000010000000100000
C. *no* Kalimantan Indo	00101001000000000000010010100000100000000000010000001000000010000000100000
C. *obtusa* Nan1 Thail	10100000001000000000110000000000000000000100000000000100010000000000001010000011
C. *obtusa* Nan2 Thail	10100000001000000000110000000000000000000100000000000100010000000000001010000011
C. *rumphiana* Rizal Phil	00101000000010000010010010100000100000010000001010101001000000000000001000000
C. *rumphiana* Quezon1 Phil	00101000000010000010010010100000100100001001000010101001000000000000001000000
C. *rumphiana* Quezon2 Phil	00101000000010000010010010100000100100001001000010101001000000000000001000000
C. *rumphiana* Quezon3 Phil	00101000000010000010010010100000100100001001000010101001000000000000001000000
C. *rumphiana* Quezon4 Phil	00101000000010000010010010100000100100001001000010101001000000000000001000000
C. *rumphiana* Laguna1 Phil	00101000000010000010010011100000100000001000000010101001000000000000001000000
C. *rumphiana* Laguna2 Phil	00101000000010000010010011100000100000001000000010101001000000000000001000000
C. *rumphiana* Seram Indo	00101000100000000010010010100000100000001000000010100001000000000000001000000
C. *rumphiana* Sumbar Indo	00101000100000000010010010100000100000001000000010100001000000000000001000000
C. *rumphiana* PNG	00101000000000000010010010100000100000001000000010100001000000000000001000000
C. *rumphiana* Australia1	00101000000000000010010010100000100000001000000010001001000000000000001000000
C. *rumphiana* Australia2	00101000000000000010010010100000100000001000000010001001000000000000001000000
C. *rumphiana* Solomon Is	00101000000000000010010010100000100000001000000010001001000000000000001000000
C. *urens* Kerala India	00101001000000000010010010100000000000001000000010001001000000000000001000000
C. *urens* W. Ghats India	00101001000000000010010010100000000000001000000010001001000000000000001000000
Wallichia caryotoides	00000000010000000000000000000000000000000000000000000000000000000000000000000
Arenga caudata	00000000010000000000000000000000000000000000000000000000000000000000000000000
Arenga listeri	00001000000000010000000000000000000000000000000000000000000000000000000000000
Arenga microcarpa	00000000000000010000000000000000000000000000000000000000000000000000000000000
Arenga porphyrocarpa	00001000000000010000000110000000000000000000000000000000000000000000000000000
Arenga tremula	00000000000000000000000000000000000000000000000000000000000000000000000000000