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PHYLOGENY OF THE TROPICAL TREE FAMILY DIPTEROCARPACEAE BASED ON NUCLEOTIDE SEQUENCES OF THE CHLOROPLAST *rbcl* GENE¹

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The Dipterocarpaceae, well-known trees of the Asian rain forests, have been variously assigned to Malvales and Theales. The family, if the Monotoideae of Africa (30 species) and South America and the Pakaraimoideae of South America (one species) are included, comprises over 500 species. Despite the high diversity and ecological dominance of the Dipterocarpaceae, phylogenetic relationships within the family as well as between dipterocarps and other angiosperm families remain poorly defined. We conducted parsimony analyses on *rbcl* sequences from 35 species to reconstruct the phylogeny of the Dipterocarpaceae. The consensus tree resulting from these analyses shows that the members of Dipterocarpaceae, including *Monotes* and *Pakaraimaea*, form a monophyletic group closely related to the family Sarcocaulaceae and are allied to Malvales. The present generic and higher taxon circumscriptions of Dipterocarpaceae are mostly in agreement with this molecular phylogeny with the exception of the genus *Hopea*, which forms a clade with *Shorea* sections *Anthoshorea* and *Doona*. Phylogenetic placement of *Dipterocarpus* and *Dryobalanops* remains unresolved. Further studies involving representative taxa from Cistaceae, Elaeocarpaceae, *Hopea*, *Shorea*, *Dipterocarpus*, and *Dryobalanops* will be necessary for a comprehensive understanding of the phylogeny and generic limits of the Dipterocarpaceae.

Key words: Dipterocarpaceae; Gondwana; *Hopea*; Malvales; Phylogeny; *Shorea*.

Three entities are often considered to comprise the tropical tree family Dipterocarpaceae. The original family circumscription, based on the genus *Dipterocarpus* (Blume, 1825), is confined to Asia and the Seychelles. The Asian dipterocarps include 470 species and dominate the canopy of lowland equatorial forests. Subfamily Monotoideae (Gilg, 1925) comprises ~30 species in three genera, one of which, *Monotes*, was earlier associated with Tiliaceae (Heim, 1892). The monotypic subfamily Pakaraimoideae (Maguire and Ashton, 1977) based on *Pakaraimaea dipterocarpacea* has been described from the Guyana Highlands in South America. The recent discovery of *Pseudomonotes tropinbosii* from Colombia (Londono et al., 1995; Morton, 1995) extends the range of

Monotoideae to the Neotropics. If all three entities are accepted as one family, the Dipterocarpaceae include over 500 species (Table 1). The Asiatic dipterocarps (subfamily Dipterocarpoideae) are further divided into two tribes, Dipterocarpeae and Shoreae (Brandis, 1895), with haploid chromosome numbers of 11 and 7, respectively. The tribe Shoreae is richer in species than Dipterocarpeae due to species richness in the genera *Shorea* and *Hopea*. *Shorea* comprises almost 200 species in 11 sections, while *Hopea* has over 100 species (Ashton, 1982).

The phylogenetic placement of the Dipterocarpaceae within the angiosperms has long been problematic, with arguments favoring its placement either in the order Malvales or the order Theales (Bessey, 1915; Hutchinson, 1926; Wettstein, 1935; Ashton, 1982). In more recent classifications, Cronquist (1988) placed Dipterocarpaceae in the Theales, while Dahlgren (1983) and Thorne (1992) placed it in the Malvales, again emphasizing the uncertainty of its phylogenetic position. Some of the morphological characters that support the placement of Dipterocarpaceae in the Theales are the possession of persistent imbricate sepals and frequently contorted corolla. Other characters, such as stellate, tufted, or glandular hairs, connective appendages, mucilage cells, wood rays, floral vascular supply, seed coat, and geniculate petiole, support its placement in the Malvales (Ashton, 1982). Previous analyses of *rbcl* sequences robustly place Dipterocarpaceae allied to Malvales in a clade with Sarcocaulaceae (Chase et al., 1993; Dayanandan, 1996). A recent comprehensive study of the phylogeny of the Malvales based on *rbcl* sequence data has provided a more conclusive evidence for the Malvacean affinity of the Dipterocarpaceae (Alverson et al., 1998).

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TABLE 1. Number of species in different genera of Dipterocarpaceae and their geographical distribution. Data are from Ashton (1980, 1982).

Subfamily	Genus	No. of species	Distribution
Pakarimoideae	<i>Pakaraimaea</i>	1	Guyana highland, South America
Monotoideae	<i>Monotes</i>	30	Africa and Madagascar
	<i>Marquesia</i>	3	Africa
	<i>Pseudomonotes</i>	1	Colombia, South America
Dipterocarpoideae			
Tribe: Dipterocarpeae	<i>Dipterocarpus</i>	69	Sri Lanka, India, Burma, Thailand, Indo-China, Yunnan, Malaya, Sumatra, Java, Bali, Borneo, Philippines
	<i>Anisoptera</i>	11	Chittagong and Indo-China to New Guinea
	<i>Upuna</i>	1	Malesia, Borneo
	<i>Cotylelobium</i>	6	Sri Lanka, Peninsular Thailand, Malesia, Sumatra, Lingga, Anabas Islands, Borneo
	<i>Vatica</i>	65	Sri Lanka, South and East India, Burma, Thailand, Indo-China, South China, and Malesia
	<i>Vateria</i>	2	Sri Lanka and India
	<i>Vateriopsis</i>	1	Seychelles
	<i>Stemonoporus</i>	15	Sri Lanka
Tribe: Shoreae	<i>Dryobalanops</i>	7	Malaya, Central Sumatra, Borneo and intervening islands
	<i>Parashorea</i>	14	South Burma, Thailand, Indo-China, South China, Malesia, Sumatra, Borneo, Philippines, and intervening islands
	<i>Neobalanocarpus</i>	1	Malesia
	<i>Hopea</i>	102	Sri Lanka, Andamans, South and east India, Burma, Thailand, Indo-China, South-China, Hainan, Malesia
	<i>Shorea</i>	194	Sri Lanka, India, Burma, Thailand, Indo-China, Malesia, Malaya, Sumatra, Borneo, intervening islands, Java, Philippines, Moluccas

Maury (1978), after analyzing the morphology of pollen, fruits, embryos, and seedlings of many species in the family Dipterocarpaceae, suggested that the Monotoideae represent a distinct entity and could be treated as a separate family with an alliance to Asiatic dipterocarps. Kostermans (1985) argued that presence of extrafloral glands on the leaf, uniseriate wood rays, androgynophore with numerous stamens, and tricolporate pollen grains in both *Monotes* and members of Tiliaceae, and the absence of these characters in Asiatic dipterocarps, indicate affinities of the Monotoideae with Tiliaceae. However, he failed to recognize that extrafloral nectaries are widespread among Asiatic dipterocarps. Furthermore, glandular hairs are present in some Asiatic dipterocarps, but absent in *Monotes* and Tiliaceae. According to Kostermans (1985), the only character shared between Asiatic dipterocarps and *Monotes*, but not in Tiliaceae, is the imbricate flower sepals, which he did not consider important enough to link *Monotes* to the dipterocarps. Consequently, Kostermans (1989) created a new family Monotaceae to include both Pakarimoideae and Monotoideae, recognizing the close affinity between the two subfamilies. He placed Monotaceae close to Tiliaceae and distant from Dipterocarpaceae.

One of the reasons for these disagreements may be due to reversals or parallel evolution of the limited number of morphological characters. It is often difficult to weigh the relative importance of different morphological characters. On the other hand, none of the morphological analyses (Maury, 1978; Ashton, 1982) were done cladistically. An alternative approach is clearly necessary to resolve the relationships of the Dipterocarpoideae, Monotoideae, and Pakarimoideae.

Present understanding of phylogenetic relationships of Dipterocarpaceae is based on a few comparative studies

(Maury, 1978, 1979; Ashton, 1979). Suggested affinities among dipterocarps have been based on wood anatomy (Gotwald and Parameswaran, 1966; Brazier, 1979; Parameswaran and Gotwald, 1979), secondary metabolites (Ourisson, 1979), and morphological characters (Ashton, 1979, 1982). Within Dipterocarpaceae, Maury (1978, 1979), on the basis of seed, embryo, and seedling characters, recognized two main groups, one with imbricate fruit sepals and the other with valvate fruit sepals. The imbricate group included two monophyletic genera, *Hopea* and *Shorea*, while the valvate clade included *Dipterocarpus* and *Vatica*. The placement of most genera within these two clades remained unresolved. Ashton (1979) also pointed out the difficulties involved with classification of *Hopea*, *Shorea*, and *Neobalanocarpus* due to their morphological similarities. The taxonomic position of *Hopea brevipetiolaris* has long been a point of controversy. This species was originally placed in the genus *Balanocarpus*. Ashton (1963, 1972, 1980) transferred *B. brevipetiolaris* into *Hopea*. Kostermans (1992), however, favored its placement in the genus *Balanocarpus*. The presence of a terminal band of apotracheal parenchyma, silica (SiO₂) in ray cells, and sparsely distributed resin canals in *H. brevipetiolaris* supports its placement in a separate genus from *Hopea*, as those characters are absent in other *Hopea* species (Parameswaran and Gotwald, 1979). However, the type species of *Balanocarpus*, *B. utilis*, lacks these characters.

Two alternative hypotheses exist to explain the origin and phylogeography of dipterocarps (Merrill, 1923; Croizat, 1952, 1964; Ashton, 1982). The first suggests that dipterocarps originated on the Eurasian plate, possibly in the Malesian region, and migrated westward towards south Asia and Africa. This is based on the assumption that the high species diversity of dipterocarps found in

TABLE 2. Taxa selected for *rbcL* sequencing and their source (GenBank or voucher number). Classification is based on Ashton (1982) and Cronquist (1988).

Family	Genus	Section	Species selected	GenBank or voucher no. ^a
Bixaceae	<i>Bixa</i>		<i>B. orellana</i>	GBAN-AF022128
	<i>Cochlospermum</i>		<i>C. vitifolium</i>	GBAN-AF022129
Sphaerocepalaceae	<i>Rhopalocarpus</i>		<i>R. lucidus</i>	GBAN-AF022130
Thymeliaceae	<i>Phaleria</i>		<i>P. chermsideana</i>	GBAN-PCU26332
	<i>Daphne</i>		<i>D. mezereum</i>	GBAN-AF022132
	<i>Dirca</i>		<i>Dirca</i> sp.	GBAN-DSU26322
Sarcolaenaceae	<i>Sarcolaena</i>		<i>S. oblongifolia</i>	GBAN-SOU26337
Dipterocarpaceae				
Subfamily: Pakaraimoideae	<i>Pakaraimaea</i>		<i>P. dipterocarpacea</i>	Ashton, 001
Subfamily: Monotoideae	<i>Monotes</i>		<i>Monotes</i> sp.	DKH3134
Subfamily: Dipterocarpoideae				
Tribe: Dipterocarpeae	<i>Dipterocarpus</i>		<i>D. zeylanicus</i>	91D003
			<i>D. insignis</i>	91D004
	<i>Anisoptera</i>		<i>Anisoptera</i> sp.	92D011
	<i>Upuna</i>		<i>Upuna borneensis</i>	92D010
	<i>Cotylelobium</i>		<i>C. scabriusculum</i>	91D005
	<i>Vatica</i>	<i>Vatica</i>	<i>V. affinis</i>	91D006
		<i>Sunaptea</i>	<i>V. cinerea</i>	91D007
	<i>Stemonoporus</i>		<i>S. canaliculatus</i>	91D008
			<i>Stemonoporus</i> sp.	91D009
			<i>S. gilimalensis</i>	91D010
	<i>Vateria</i>		<i>V. copalifera</i>	91D011
Tribe: Shoreae	<i>Dryobalanops</i>		<i>D. aromatica</i>	91D012
	<i>Neobalanocarpus</i>		<i>N. heimii</i>	91D013
	<i>Hopea</i>	<i>Hopea</i>	<i>H. jucunda</i>	91D014
			<i>H. brevipetiolaris</i>	91D015
		<i>Dryobalanoides</i>	<i>H. dryobalanoides</i>	91D016
	<i>Shorea</i>	<i>Shorea</i>	<i>S. dyeri</i>	91D017
			<i>S. lissophylla</i>	91D018
			<i>S. robusta</i>	91D019
		<i>Anthoshorea</i>	<i>S. stipularis</i>	91D020
			<i>S. assamica</i>	91D021
		<i>Mutica</i>	<i>S. curtisii</i>	91D022
		<i>Ovales</i>	<i>S. ovalis</i>	91D023
		<i>Doona</i>	<i>S. affinis</i>	91D024
			<i>S. ovalifolia</i>	91D025
			<i>S. zeylanica</i>	91D001

^a The prefix GBAN- has been added for linking the online version of *American Journal of Botany* to GenBank but is not part of the actual GenBank accession number.

the Far East is associated with the origin of the family (Merrill, 1923; Prakash, 1972; Meher-Homji, 1979). The second hypothesis suggests that dipterocarps originated in Gondwana (Croizat, 1952, 1964; Ashton, 1982). One clade, comprising the subfamilies Monotoideae and Pakaraimoideae, extended its range westward from Africa to South America; the other clade, the subfamily Dipterocarpoideae, migrated eastward through the Deccan plate (Ashton and Gunatilleke, 1987), entered the Eurasian plate, and diversified in the Far Eastern region (Ashton, 1980). Ashton (1982) pointed out that dipterocarp dispersal must be overland due to limited seed dispersal ability, obligate species-specific mycorrhizal symbiosis, lack of seed dormancy, and salt intolerance of seeds. This hypothesis is consistent with fossil evidence (Ramanujan, 1968; Lakanpal, 1970), tectonic events (Sclater and Harrison, 1971; Audley-Charles, Hurley, and Smith, 1981; Brock, 1981; Klootwijk and Radhakrishnamoorthy, 1981), the existence of the wingless-fruited dipterocarpoid *Vateriopsis* on the Gondwana fragment of Mahe, Seychelles, and comparative anatomy and morphology

(Ashton, 1982). Reconstructing the phylogeny of dipterocarps using an additional set of characters such as molecular data may shed light on the understanding of the historical biogeography of the family Dipterocarpaceae.

In the present study, we reconstructed the phylogeny of Dipterocarpaceae based on *rbcL* sequence data of selected species. We provide evidence favoring the placement of the Asian dipterocarps in the order Malvales allied to Sarcolaenaceae and show that *Monotes* and *Pakaraimaea* are closely related to Asiatic dipterocarps.

MATERIALS AND METHODS

Taxon sampling—The *rbcL* gene sequences of 35 species representing five families and 20 genera were used in the present analysis (Table 2). Leaves were either frozen in liquid nitrogen or dried in silica gel in the field and transported to the laboratory at Boston University for DNA extractions. Published *rbcL* sequences representing all families in a monophyletic clade with Dipterocarpaceae (Alverson et al., 1998) were included in the present analysis. The *rbcL* sequences of Cistaceae and *Muntingia* of Elaeocarpaceae were not available from GenBank.

DNA extraction, *rbcL* amplification, and sequencing—Total genomic DNA was extracted from leaf samples using the methods of Doyle and Doyle (1987) and Dayanandan, Bawa, and Kesseli (1997). The *rbcL* gene was amplified by the polymerase chain reaction (PCR) using oligonucleotide primers G1F and G1460R, modified from primers designed by Gerard Zurawski (Zurawski et al., 1981; Zurawski, Clegg, and Brown, 1984; Zurawski and Clegg, 1987), to be complementary to the *Gossypium rbcL* gene. Amplification reactions contained 200 $\mu\text{mol/L}$ each of dATP, dCTP, dGTP, dTTP, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 9.0), 0.1% Triton X-100, 10 pmol/L of each primer, 2 units of Taq DNA Polymerase (Promega), and 1.5 mmol/L MgCl₂ in a total volume of 100 μl . Thermal cycling was performed in a MJ Research PTC100 thermal cycler at 94°C for 90 sec, 40°C for 60 sec and 72°C for 120 sec for 40 cycles. Amplified DNA was purified using Promega Magic PCR purification columns and directly sequenced by the Sanger dideoxy chain termination method with thermal cycling using the fmol Sequencing System (Promega Corporation City, Madison, Wisconsin). Thermal cycling was performed for 30 sec at 50°C, 60 sec at 70°C, and 90 sec at 94°C for 30 cycles. Internal primers originally designed by G. Zurawski (DNAX Institute, Palo Alto, California) were modified to complement dipterocarp *rbcL* sequences and used for sequencing both strands of *rbcL*. Sequences were aligned using the IBI/PUSTELL sequence analysis program using an IBM personal computer. Accuracy of sequences was rechecked by translating to peptide sequences and examined for premature stop codons and frame shifts.

Cladistic analysis—Aligned sequences and resulting trees were analyzed using PAUP 3.1.1 (Swofford, 1993) and MacClade 3.04 (Madison and Maddison, 1993) on a PowerMacintosh computer. A heuristic search was performed with equal weights, 100 replicates of random sequence addition, and TBR (tree bisection reconnection) branch swapping with MULPARS (multiple parsimonious trees) on (Swofford and Begle, 1993). Bootstrap analysis was performed for 100 replicates with simple sequence addition, MULPARS on and steepest descent off. The *rbcL* sequences of *Bixa*, *Cochlospermum*, and *Rhopalocarpus* were used as outgroups.

RESULTS

The heuristic search resulted in 12 equally parsimonious trees, each with a length of 359 steps with consistency index (CI) of 0.50 and rescaled consistency index of (RC) of 0.37 (after excluding uninformative characters). The strict consensus tree of 12 equally parsimonious trees is given in Fig. 1. Ten key morphological characters of Dipterocarpaceae were compiled (Gotwald and Parameswaran, 1966; Maury, 1978; Ashton, 1979, 1982; Jong and Kaur, 1979) and mapped on the phylogenetic tree derived from *rbcL* sequence data. All branches were resolved in the strict consensus tree, except for the branches from *Dryobalanops* to the remaining Asiatic dipterocarps, and the *Anisoptera*, *Cotylelobium*, and *Vatica* to the *Stemonoporus*, *Upuna*, and *Vateria* clade.

Dipterocarpaceae and Sarcolaenaceae formed a strongly supported monophyletic clade sister to Thymelaeaceae. Within the dipterocarp clade, *Monotes* occupied a basal position and *Pakaraimaea* occupied the next position basal to Asiatic dipterocarps. However, the branches among *Sarcolaena*, *Monotes*, and *Pakaraimaea* were weakly supported with bootstrap values of <50%.

The monophyly of the subfamily Dipterocarpoideae is well supported. Members of Dipterocarpoideae formed four clades (*Dryobalanops*, *Dipterocarpus*, and remaining species of the tribes Shoreae and Dipterocarpeae) and relationships among these clades remain unresolved (Fig.

1). Among the remaining Dipterocarpeae taxa, *Vateria*, *Stemonoporus*, *Upuna*, *Anisoptera*, *Vatica*, and *Cotylelobium* formed a monophyletic group with a close affinity among *Vateria*, *Stemonoporus*, and *Upuna*.

The monophyly of the tribe Shoreae is supported except for the position of *Dryobalanops* (Fig. 1). Species of *Shorea* section *Shorea* (*S. dyeri*, *S. lissophylla*, *S. robusta*) formed a clade sister to the clade of *S. curtisii* (section *Mutica*) and *S. ovalis* (section *Ovales*). *Shorea* species of section *Anthoshorea* and the species of the genus *Hopea* formed a monophyletic clade sister to *Shorea* section *Doona*. *Neobalanocarpus* was sister to the *Anthoshorea*, *Hopea*, and *Doona* clade. Within the Dipterocarpoideae clade, bootstrap results are in agreement with the strict consensus tree obtained from the heuristic search, with few exceptions. All branches showed a bootstrap consensus value of >50%, except for branches supporting the monophyly of *Shorea*, *Hopea*, and *Neobalanocarpus*; *Neobalanocarpus*, *Doona*, *Hopea*, and *Anthoshorea*; *Doona*, *Hopea*, and *Anthoshorea*; and *Upuna*, *Vateria*, and *Stemonoporus*.

Mapping of morphological characters on the phylogenetic tree reconstructed from *rbcL* sequence data (Fig. 1; Tables 3 and 4) showed five character state changes (wood parenchyma, resin canals, flower petals, pollen aperture, and pericarp dehiscence) on the branch supporting the monophyly of the Dipterocarpoideae. Within Dipterocarpoideae, monophyly of the tribe Shoreae is supported by five characters (grouped xylem vessels, resin canal grouped into series, chromosome number of seven, imbricate fruit sepal aestivation, and thickened fruit sepal base). The monophyly of the tribe Dipterocarpeae is supported by three characters (solitary resin canals, chromosome number of 11, and free flower petals). Two morphological character state changes (solitary resin canals and chromosome number of 11) were observed on the branch with the genus *Dipterocarpus*, and three character state changes (resin canals grouped into series, chromosome number of seven, and thickened fruit sepal base) were observed on the branch with *Dryobalanops*.

DISCUSSION

Analysis of *rbcL* sequence data suggests that the family Dipterocarpaceae, including *Monotes* and *Pakaraimaea*, is monophyletic and is sister to Sarcolaenaceae. Although the Sarcolaenaceae have been considered as a member of Theales (Cronquist, 1988), recent studies showed a malvalean affinity (Dahlgren, 1983, 1989; Thorne, 1992; Conti, Litt, and Sytsma, 1996; Alverson et al., 1998). Close affinity of Dipterocarpaceae to Sarcolaenaceae had earlier been suggested based on wood anatomical and other features (Ashton, 1982). A common origin of Dipterocarpaceae and Sarcolaenaceae from an ancient tiliaceous stock has also been suggested (Maguire et al., 1977). The *rbcL* data support earlier authors who argued a malvalean affinity for Dipterocarpaceae (Lindley, 1846; Heim, 1892; Hallier, 1912; Corner, 1946; Ashton, 1982; Thorne, 1992) but disputes the thealean affinity (Endlicher, 1840; Lindley, 1846; De Candolle, 1868; Cronquist, 1981). According to Corner (1976), palisade cells in the seed coat of *Dipterocarpus* and *Vatica* show similarity to *Durio* and *Cullenia* of Bombacaceae (Mal-

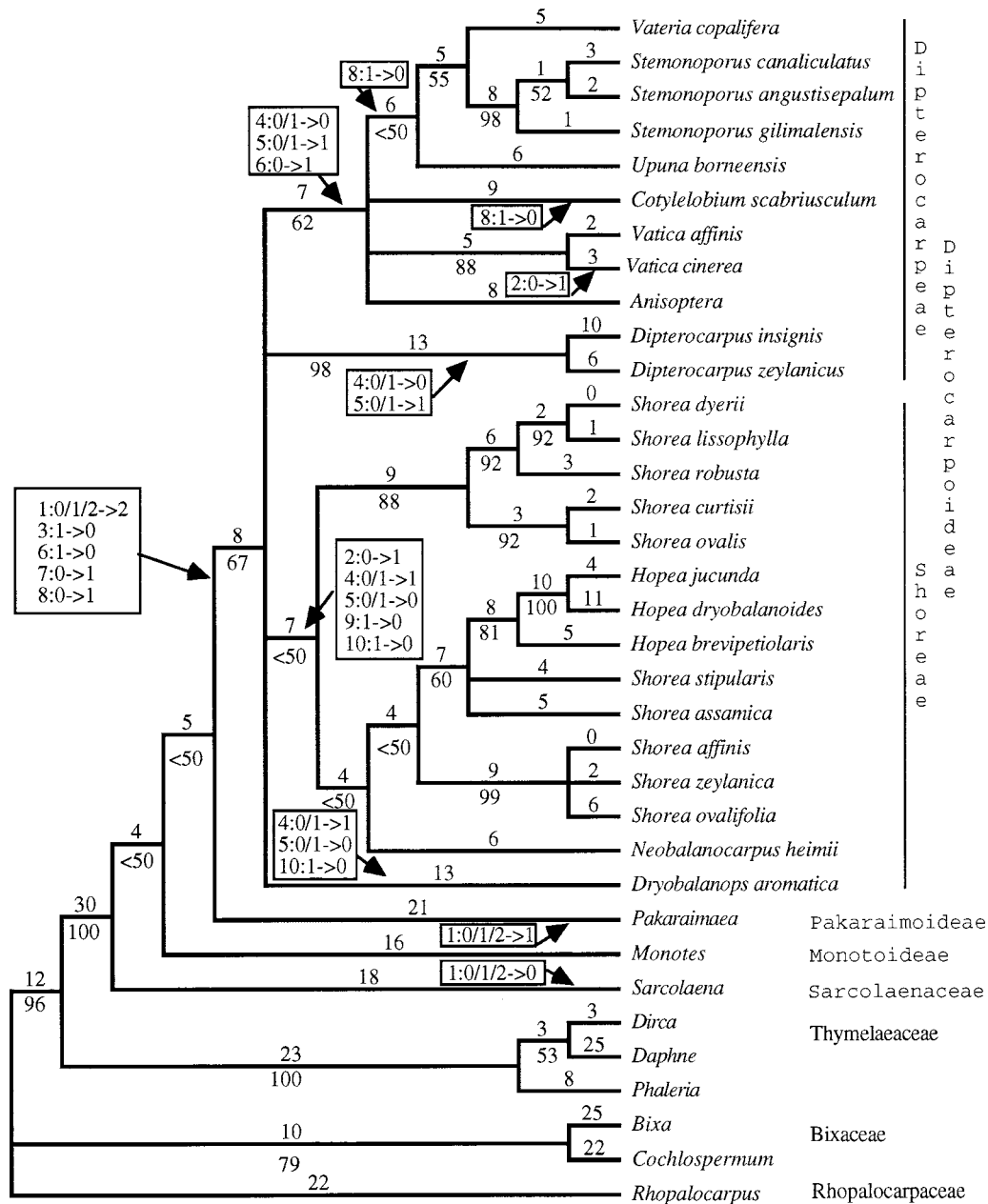


Fig. 1. The strict consensus tree of 12 equally parsimonious trees (Length = 359 steps, CI = 0.50, and RC = 0.37, after excluding uninformative characters) identified by equally weighted parsimony analysis using *rbcL* sequence data from selected taxa of Dipterocarpaceae. A heuristic search was performed with random sequence addition (100 replicates) with TBR branch swapping, steepest descent off, and MULPARS on, using PAUP 3.11. Branch length (above the line) and bootstrap values (below the line) are given at each branch. Changes in morphological character states are given inside boxes at branches. Characters and character states are numbered as in Tables 3 and 4. *Bixa*, *Cochlospermum*, and *Rhopalocarpus rbcL* sequences were used as outgroups.

vales). Analysis of *rbcL* sequences also supports a close affinity of that Dipterocarpaceae with Cistaceae and Malvales s. str. (Bombacaceae, Malvaceae, Sterculiaceae, and Tiliaceae; Chase et al., 1993; Alverson et al., 1998).

The evidence presented here suggests that *Monotes* and *Pakaraimaea* are more closely related to Asiatic dipterocarps than to Tiliaceae. This is in agreement with other recent interpretations (Maguire et al., 1977; Maury, 1978). Wood anatomical characters also support the close alliance between *Monotes* and Asiatic dipterocarps (Ban-

croft, 1935; Gotwald and Parameswaran, 1966). However, this is contrary to Kostermans (1989) who considered that *Monotes* is more closely related to Tiliaceae than to Dipterocarpoideae. Kostermans (1989) also raised Monotoideae to a family status, including both *Monotes* and *Pakaraimaea*. In phylogenetic trees based on *rbcL* sequence data, *Monotes* and *Pakaraimaea* form two distinct, though weakly supported clades, paraphyletic to Asian dipterocarps, and therefore formation of a new family, Monotaceae, cannot be justified. However, all

TABLE 3. Morphological characters and character states of Dipterocarpaceae used for mapping on the phylogenetic tree derived from *rbcL* sequence data. All characters were used as unordered.

1. Wood parenchyma: 0 = uniseriate; 1 = biseriate; 2 = multiseriate.
2. Xylem vessels: 0 = solitary; 1 = grouped.
3. Resin canals: 0 = present; 1 = absent.
4. Resin canal groups: 0 = solitary; 1 = series.
5. Chromosome number: 0 = seven; 1 = eleven.
6. Petals: 0 = connate; 1 = free.
7. Pollen aperture: 0 = tricolporate; 1 = tricolpate.
8. Pericarp dehiscence: 0 = loculicidal; 1 = non-loculicidal.
9. Fruit sepal aestivation: 0 = imbricate; 1 = valvate.
10. Fruit sepal base: 0 = thick; 1 = non-thick.

three groups, Monotoideae, Pakaraimoideae, and Dipterocarpoideae, together form a monophyletic group and therefore treating the whole clade as one family is justified. However, a detailed study involving members of Cistaceae and Elaeocarpaceae, particularly *Muntingia*, would be necessary for a comprehensive understanding of the relationships between Sarcolaenaceae and subfamilies of the Dipterocarpaceae.

Phylogenetic relationships within Dipterocarpaceae based on *rbcL* data are mostly in agreement with present taxonomic treatments (Ashton, 1980, 1982) as well as phylogenetic speculations (Maury, 1978, 1979; Ashton, 1982). The inferred phylogenetic tree topology is in agreement with the division of Dipterocarpaceae into two tribes, mainly based on chromosome numbers (Jong and Kaur, 1979). The tribes Dipterocarpeae and Shoreae form monophyletic groups with the exception of *Dipterocarpus* and *Dryobalanops*, for which phylogenetic positions remain unresolved. However, presence of solitary resin canals and the base chromosome number of 11 support the placement of *Dipterocarpus* within the clade of the tribe Dipterocarpeae. Similarly, grouping of resin canals into groups, chromosome number of seven, and the thick-

ened fruit sepal base support the placement of *Dryobalanops* within tribe Shoreae.

Meijer (1979) postulated that *Dipterocarpus* may represent the basal clade of Dipterocarpoideae. Maury (1978) recognized *Dipterocarpus* as a primitive group among the members of the tribe Dipterocarpeae, and she placed *Dipterocarpus* along with other taxa of Dipterocarpeae as sister to the group with species of the tribe Shoreae. *Dipterocarpus* has a number of unique characters, including the winged free calyx tube and large flowers associated with pollination by macrolepidoptera. *Dipterocarpus* may be a clade with early divergence, but most of the primitive morphological characters appear to have been lost in *Dipterocarpus* over the course of evolution.

The monophyly of the remaining taxa of the tribe Dipterocarpeae (*Vateria*, *Stemonoporus*, *Vatica*, *Cotylelobium*, *Anisoptera*, and *Upuna*) is in agreement with previous taxonomic and phylogenetic treatments of the dipterocarps (Maury, 1978, 1979; Ashton, 1982). The separate status of *Cotylelobium* and *Vatica* and the unity of the latter genus, from which we sampled species from each of its two sections, are upheld. This is contrary to Kostermans' (1987) decision to unite *Cotylelobium* with *Vatica* section *Sunaptea* and separate both from the type section of *Vatica*. Morphological data suggest that *Vateriopsis* also belongs to this group, and further molecular studies will be necessary to infer the phylogenetic position of *Vateriopsis*.

The phylogenetic placement of the genus *Dryobalanops* remains unresolved. Ashton (1979) considered *Dryobalanops* as a member of the tribe Shoreae, while Maury (1978) placed *Dryobalanops* in the tribe Dipterocarpeae. The presence of solitary vessels suggests the affinity of *Dryobalanops* to the tribe Dipterocarpeae (Gotwald and Parameswaran, 1966), while chromosome number indicates an affinity to the tribe Shoreae (Jong and Kaur, 1979). Among other morphological characters,

TABLE 4. Morphological character states of selected taxa of Dipterocarpaceae used to map on the phylogenetic tree derived from *rbcL* sequence data. Characters and states are numbered as given in Table 3. Data were compiled from Ashton (1979, 1982), Gotwald and Parameswaran (1966), Jong and Kaur (1979), and Maury (1978).

Genus—Section	Character									
	1	2	3	4	5	6	7	8	9	10
<i>Monotes</i>	0	0	1	?	?	1	0	0	0/1	1
<i>Pakaraimaea</i>	1	0	1	?	?	1	0	0	1	1
<i>Dipterocarpus</i>	2	0	0	0	1	0	1	1	1	1
<i>Anisoptera</i>	2	0	0	0	1	1	1	1	1	1
<i>Vatica—Vatica</i>	2	0	0	0	1	1	1	0/1	1	1
<i>Vatica—Sunaptea</i>	2	1	0	0	1	1	?	1	1	1
<i>Cotylelobium</i>	2	0	0	0	1	1	1	0	1	1
<i>Vateria</i>	2	0/1	0	0	1	1	1	0	1	1
<i>Upuna</i>	2	0	0	0	1	1	1	0	1	1
<i>Stemonoporus</i>	2	0	0	0	1	1	1	0	1	1
<i>Dryobalanops</i>	2	0	0	1	0	0	1	1	1	0
<i>Neobalanocarpus</i>	2	1	0	1	0	0	1	1	0	0
<i>Shorea—Doona</i>	2	1	0	1	0	0	1	1	0	0
<i>Shorea—Anthoshorea</i>	2	1	0	1	0	0	1	1	0	0
<i>Shorea—Mutica</i>	2	1	0	1	0	0	1	1	0	0
<i>Shorea—Ovales</i>	2	1	0	1	0	0	1	1	0	0
<i>Shorea—Shorea</i>	2	1	0	1	0	0	1	1	0	0
<i>Hopea—Hopea</i>	2	1	0	1	0	0	1	1	0	0
<i>Hopea—Dryobalanoides</i>	2	1	0	1	0	0	1	1	0	0

valvate fruit sepals (Maury, 1978) support the placement of *Dryobalanops* in Dipterocarpaceae, while connate petals (Ashton, 1982) and grouped resin canals support the placement of *Dryobalanops* in the tribe Shoreae. A detailed analysis using several species of *Dryobalanops* would be valuable in further resolving the phylogenetic position of this genus.

The present study shows that *Neobalanocarpus*, *Hopea*, and *Shorea* sections *Anthoshorea* and *Doona* form a clade sister to the remaining species of *Shorea*. The close affinity between *Neobalanocarpus*, *Hopea*, and section *Doona*, is supported by wood anatomical characters (Parameswaran and Gotwald, 1979). Although the close alliance between sections *Doona* and *Anthoshorea* has been recognized by Maury (1978), she considered *Hopea* as a separate clade sister to all species of *Shorea*.

Neobalanocarpus occupies a weakly supported basal position in the clade comprising section *Doona*, section *Anthoshorea*, and *Hopea*. Floral biology, especially the diurnal anthesis and the stamen structure of *Neobalanocarpus*, shows similarity to section *Doona* (Dayanandan et al., 1990). Presence of medium-sized vessels and storied rays and absence of silica in *Neobalanocarpus* led Parameswaran and Gotwald (1979) to place it in a special position allied to *Hopea*. Anthocyanin development (Bate-Smith and Whitmore, 1959) and bark morphology (Whitmore, 1962) in *Neobalanocarpus* are similar to *Hopea*. Jong and Kaur (1979) observed abnormalities in meiosis in *Neobalanocarpus*: failure in pairing of chromosomes at metaphase I, irregular disjunction of chromosomes, and tendency to associate in pairs at metaphase II and anaphase II. Therefore, they speculated that *Neobalanocarpus* may be a result of hybridization between *Hopea* and *Shorea*.

Although Maury (1978) placed the genus *Hopea* and section *Anthoshorea* in two different but closely related groups, *rbcL* data support the monophyly of section *Anthoshorea* and the genus *Hopea*. The floral morphologies of the genus *Hopea* and section *Anthoshorea* are similar, both having an urceolate corolla and stamens with an acicular connectival appendage. The positioning of *Hopea* and *Neobalanocarpus* in a clade with *Shorea* disrupts the monophyly of the genus *Shorea*. Since the objective of a natural system of classification is to have monophyletic groups as taxonomic units, this relationship needs further evaluation using representative taxa from all sections of both *Hopea* and *Shorea* for subsequent consideration in the classification of the genera *Shorea* and *Hopea*. According to *rbcL* data, *Hopea brevipetiolaris* forms a clade with other *Hopea* species, and therefore it is reasonable to include it in *Hopea* rather than in a separate genus.

The monophyly of the three species of *Shorea* of section *Shorea* studied is in agreement with taxonomical grouping (Ashton, 1982). Despite the disjunct distribution pattern of *Shorea robusta* in India and *S. dyeri* and *S. lissophylla* in Sri Lanka, these three species form a clade sister to species of *Shorea* of section *Mutica*, which are distributed in Malesia. Section *Ovales* is closely related to *Mutica* and is also distributed in Malesian forests. Although *S. ovalis* is a polyploid (Ashton, 1982), the wood anatomy of *S. ovalis* is similar to that of section *Mutica*. Therefore, *S. ovalis* (section *Ovales*) and section

Mutica may be a result of recent divergence from a common ancestor.

The distribution of the taxa of the basal clades, Sarcolaenaceae and *Monotes*, in Madagascar and Africa where Sarcolaenaceae are now extinct and the absence of such taxa in the Asian region are in agreement with the Gondwanan origin hypothesis of dipterocarps. Presence of fossils of *Dipterocarpoxyton* in Africa (Chiarugi, 1933) and fossil records of *Dryobalanoxylon* in India (Ramanujan, 1968) together with the basal position of *Dipterocarpus* and *Dryobalanops* in the present cladogram are also consistent with the hypothesis that dipterocarps originated in Gondwana and migrated eastwards through India to the Malesian region. However, the disjunct distribution of closely related taxa both in Sri Lanka and Malesia suggests that dipterocarps must have already diverged to generic or even infrageneric sections before they entered the Laurasian plate from the Deccan plate. For instance, *Hopea jucunda* and *Shorea stipularis*, both of section *Anthoshorea*, are present in Sri Lanka (Ashton, 1980), while their respective sister taxa, *Hopea dryobalanoides* and *Shorea assamica*, are present in Malesia (Ashton, 1982).

In summary, this study shows that taxa of Dipterocarpaceae, including *Monotes* and *Pakaraimaea*, form a monophyletic group closely related to the family Sarcolaenaceae. The present taxonomic grouping of Dipterocarpaceae is mostly in agreement with the phylogeny with the exception of the genus *Hopea*, which forms a clade with *Shorea* section *Anthoshorea*. Phylogenetic placement of *Dipterocarpus* and *Dryobalanops* remains unresolved. Further studies involving representative taxa from both *Hopea* and *Shorea* as well as *Dipterocarpus* and *Dryobalanops* are needed for a comprehensive understanding of the phylogeny within the Dipterocarpaceae.

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