

## Secondary Structures of Chloroplast *trnL* Intron in Dipterocarpaceae and its Implication for the Phylogenetic Reconstruction

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Unambiguous insertion-deletion events were previously identified in *trnL* intron of 110 species of subfamily Dipterocarpoideae (Dipterocarpaceae). These indels are associated with the formation of four stem loop structures and featuring characteristic for generic/intra-generic level depended upon which taxonomic classifications are followed. Phylogenetic analyses were performed by including and excluding these structures to examine the robustness of resulted topologies. Results indicated that inclusion of such structures yielded more resolved topologies, and that none of the stemloop structures were homoplasious. Results of this present study was also in agreement with the previous molecular phylogenetic studies that using several genes of cp genomes in that tribe Dipterocarpaceae was polyphyletic by the placement of all members of the genus *Dipterocarpus* within tribe Shoreae, and that tribe Shoreae was a potential monophyletic group. The phylogenetic relationships between variable genera of *Hopea* and *Shorea* was also in accordance to earlier studies that suggested a potential monophyly of the two with inclusion of *Parashorea* and *Neobalanocapus heimii*. Genera that were received strong branch support (*Dipterocarpus*, *Dryobalanops*, *Vatica*, and *Stemonoporus*) possessed certain indels exclusive to each and this may contributed to the monophyletic nature of these genera.

Key words: secondary structures, dipterocarpaceae, *trnL*, intron, phylogeny

### INTRODUCTION

The *trnL*-F of chloroplast genome of land plants consists of the transfer RNA genes *trnL*<sub>uaa</sub> and *trnF*<sub>gaa</sub> arranged in tandem and separated by noncoding spacer regions. The region is positioned in large single copy region, approximately 8 kb downstream of *rbcL*. The conserved nature of *trnL*-F region made the design of plant universal primers possible (Tarbelet *et al.* 1991), thus this region has become one of the most widely used chloroplast markers for phylogenetic analyses in plants (Borsch *et al.* 2003; Hamilton *et al.* 2003; Pirie *et al.* 2007; Shaw *et al.* 2007; Koch *et al.* 2007). The *trnL* gene is part of *trnL*-F region of chloroplast genome that split by group I intron, the intergenic spacer and *trnF* exons (Figure 1) and is co-transcribed (Bakker *et al.* 2000). The intron is positioned between the U and the A of the UAA anticodon loop. Secondary structures within the *trnL* intron is important because the function of the transfer RNA for which the *trnL* gene codes is related to it and that of the intron within it (Pirie *et al.* 2007). Hence, deduction of positional homology -which is the most important part for the phylogenetic reconstruction- of the structure is important during the process of DNA alignment.

Sequences from *trnL*-F regions in combination with other cp and nuclear genomes have been used in phylogenetic reconstruction of Dipterocarpaceae (Tsumura *et al.* 1996; Kajita *et al.* 1998; Dayanandan *et al.* 1999; Kamiya *et al.* 2005; Yulita *et al.* 2005; Gamage *et al.* 2006), population genetic study (Aoki *et al.* 2003) and even DNA *barcoding* (Tarbelet *et al.* 2007). However, none of the studies have examined the evidence of secondary structure of *trnL* intron into detail. Four unambiguous indels were previously described in Dipterocarpaceae (Yulita 2007). These indels made stem loop structures located at position 70-105 bp (Stem Loop/SL 1), 153-171 (SL 2), 257-328 (SL 3), and 360-386 (SL 4) (Figure 2). Large indels have mostly been excluded from the data set (Koch *et al.* 2007) since it may provide 'noise' within the phylogenetic analysis, although structural mutation built from indels can be reliable markers for phylogenetic reconstruction in

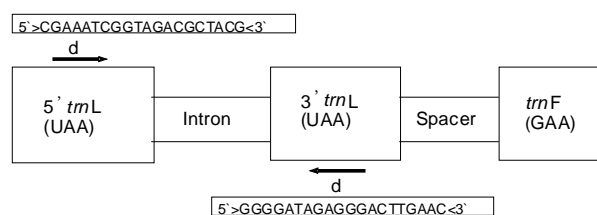


Figure 1. Diagram of *trnL*-F gene with primer sequences of intron *trnL* (c and d) (after Yulita 2007).

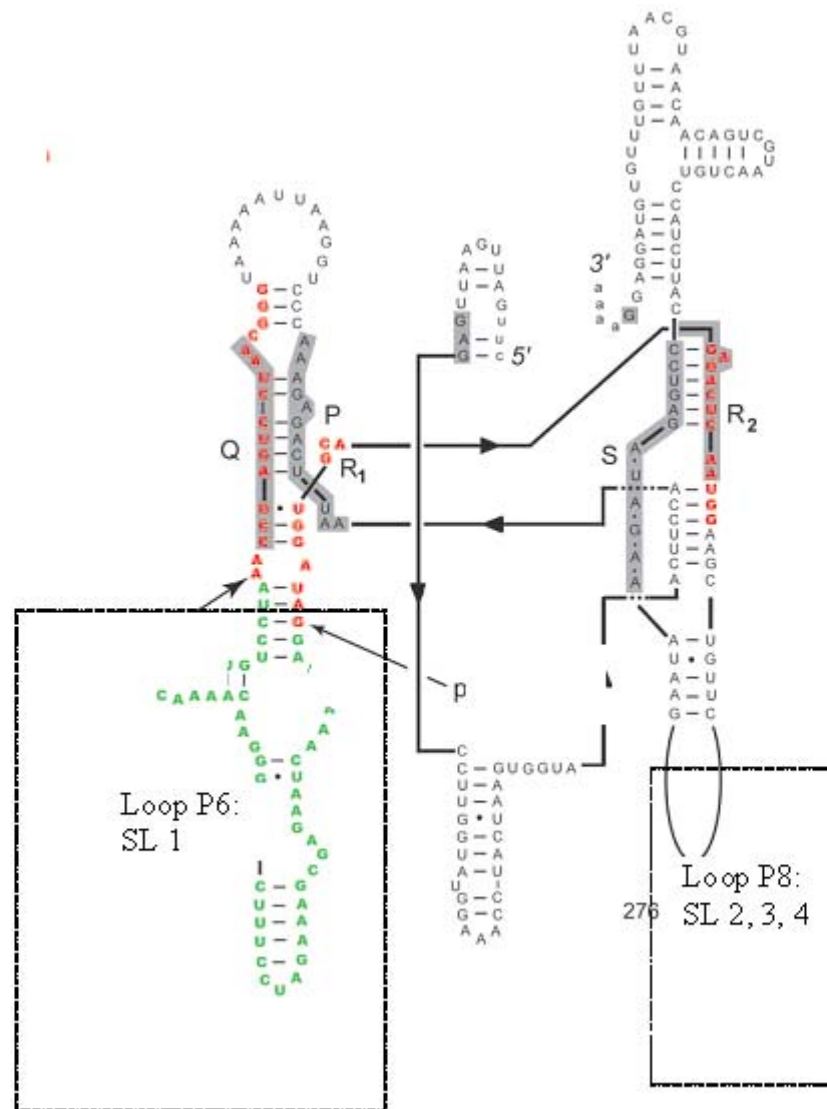


Figure 2. Secondary structure of *trnL* intron of Dipterocarpaceae that was modified from *Nymphaea odorata* (Tarbelet *et al.* 2007). Location of *stem loop* 1 (SL1) was in loop P6, locations of stem loop 2, 3, dan 4 (SL 2, 3, 4) were in loop P8 (after Yulita 2007).

some plant groups (Soltis *et al.* 1992). Examination for these structures, however, suggested that these have implications on taxonomic diagnostic characters as certain indels were possessed by certain taxa in Dipterocarpaceae. This present study was aimed to test the utility of the indels in assessing phylogenetic relationships among species of Dipterocarpaceae.

## MATERIALS AND METHODS

The *trnL* intron sequences of 110 species of 14 genera of Dipterocarpaceae were obtained from the genbank database (<http://www.ncbi.nlm.nih.gov/>). The list of genbank accession number in those samples is detailed in Table 1. The raw sequences were aligned using Clustal X (Thompson *et al.* 1997) and eyed refined to determine the positional homology. The existence of inverted repeat was examined by GENETYX and eyed refined. These structures were

particularly built in regions that have long repeat, insertions and deletions, and hotspot for base substitution.

Two cladistic analyses were performed using PAUP (Swofford 1998) by including and excluding secondary structures. The optimal tree was estimated using a heuristic search strategy with maximum parsimony criterion. A hundred replicate searches were conducted using random addition to search across multiple islands of trees. This strategy was used for all final tree searches. Initial MAXTREES was set to 230,000 (auto-increased by 100). Tree Bisection Reconnection (TBR) branch-swapping was used, with the steepest descent option off and using ACCTRAN (Accelerated Transformation) optimisation. The MULPARS (multiple parsimonious trees) option was on and minimum branches of zero were collapsed. Ten equally parsimonious trees were held following each replicate.

Table 1. Species samples and Genbank accession numbers

Species	Abreviation	Genbank accession number
<i>Anisoptera laevis</i>	ALAEV	AB006387
<i>Anisoptera oblonga</i>	AOBLO	AB006388
<i>Cotylelobium malayanum</i>	CMALA	AB006389
<i>Cotylelobium scabriusculum</i>	CSCRO	AB246545
<i>Dipterocarpus alatus</i>	DALAT	AB246603
<i>Dipterocarpus confertus</i>	DCONF	AY026528
<i>Dipterocarpus cornutus</i>	DCORN	AB246602
<i>Dipterocarpus glandulosus</i>	DGLAN	AB246607
<i>Dipterocarpus hispidus</i>	DHISP	AB246606
<i>Dipterocarpus insignis</i>	DINSI	AB246605
<i>Dipterocarpus kerrii</i>	DKERI	AB006392
<i>Dipterocarpus retusus</i>	DRETU	AY026529
<i>Dipterocarpus zeylanicus</i>	DZEYL	AB246604
<i>Dryobalanops aromatica</i>	DRARO	AY026530
<i>Dryobalanops lanceolata</i>	DRLAN	AY026531
<i>Dryobalanops oblongifolia</i>	DOBLO	AB006395
<i>Hopea apiculata</i>	HAPIC	AY026532
<i>Hopea brevipetiolaris</i>	HBREV	AY026533
<i>Hopea celebica</i>	HCELE	AY026534
<i>Hopea celtidifolia</i>	HCELT	AY026535
<i>Hopea cernua</i>	HCERN	AY026536
<i>Hopea cordifolia</i>	HCORD	AY026537
<i>Hopea discolor</i>	HDISC	AB246588
<i>Hopea dryobalanoides</i>	HDRYO	AY026538
<i>Hopea ferruginea</i>	HFERR	AY026594
<i>Hopea helferi</i>	HHELF	AB246587
<i>Hopea jucunda</i>	HJUCU	AY026540
<i>Hopea latifolia</i>	HLATI	AB246586
<i>Hopea mengerawan</i>	HMENG	AY026541
<i>Hopea nervosa</i>	HNERV	AB006401
<i>Hopea nigra</i>	HNIGR	AY026542
<i>Hopea pierrei</i>	HPIER	AY026543
<i>Hopea pubescens</i>	HPUBE	AY026544
<i>Hopea subalata</i>	HSUBA	AB246585
<i>Hopea wightiana</i>	HWIGH	AY026545
<i>Monotes madagascariensis</i>	MMADA	AB246608
<i>Neobalanocarpus heimii</i>	NHEMI	AB006400
<i>Parashorea lucida</i>	PLUCI	AB006399
<i>Shorea acuminata</i>	SACUM	AB006399
<i>Shorea affinis</i>	SAFFI	AB246601
<i>Shorea assamica</i>	SASSA	AB246583
<i>Shorea balangeran</i>	SBALA	AY026546
<i>Shorea beccariana</i>	SBECC	AY026547
<i>Shorea bracteolata</i>	SBRAC	AB006398
<i>Shorea bullata</i>	SBULLA	AB246565
<i>Shorea congestiflora</i>	SCONG	AB246593
<i>Shorea cordifolia</i>	SCORD	AB246592
<i>Shorea curtisii</i>	SCURT	AB246563
<i>Shorea disticha</i>	SDIST	AB246595
<i>Shorea dyeri</i>	SDYER	AB246576
<i>Shorea elliptica</i>	SELLI	AB246574
<i>Shorea exelliptica</i>	SEXEL	AY026548
<i>Shorea fauetiana</i>	SFAGU	AY026549
<i>Shorea fallax</i>	SFALL	AB246564
<i>Shorea foxworthyi</i>	SFOXW	AY026550

Table 1. Continue

Species	Abreviation	Genbank accession number
<i>Shorea gardneri</i>	SGARD	AB246598
<i>Shorea guiso</i>	SGUIS	AY026551
<i>Shorea hopeifolia</i>	SHOPE	AY026552
<i>Shorea isoptera</i>	SISOP	AY026553
<i>Shorea johorensis</i>	SJOHO	AY026555
<i>Shorea kunstleri</i>	SKUNS	AY026556
<i>Shorea laevis</i>	SLAEV	AY026557
<i>Shorea leprosula</i>	SLEPR	AY026558
<i>Shorea lissophylla</i>	SLYSS	AB246577
<i>Shorea longisperma</i>	SLONG	AY026559
<i>Shorea macrophylla</i>	SMACR	AY026560
<i>Shorea macroptera</i>	SMACT	AB006396
<i>Shorea materialis</i>	SMATE	AY026561
<i>Shorea maxima</i>	SMAXI	AY026562
<i>Shorea maxwelliana</i>	SMAWX	AY026563
<i>Shorea megistophylla</i>	SMEGI	AB246594
<i>Shorea multiflora</i>	SMULT	AY026565
<i>Shorea ovalis</i>	SOVAL	AY026566
<i>Shorea palembanica</i>	SPALE	AY026567
<i>Shorea pallescens</i>	SPALL	AB246578
<i>Shorea parvifolia</i>	SFOLI	AY026568
<i>Shorea parvistipulata</i>	SPARV	AY026569
<i>Shorea pilosa</i>	SPILO	AY026570
<i>Shorea pinanga</i>	SPING	AY026571
<i>Shorea quadrinervis</i>	SQUAD	AB246566
<i>Shorea richetia</i>	SRICH	AY026572
<i>Shorea roxburghii</i>	SROXB	AY026573
<i>Shorea scaberrima</i>	SSCAB	AY026574
<i>Shorea selanica</i>	SSELA	AY026575
<i>Shorea seminis</i>	SSEMI	AY026576
<i>Shorea singkawang</i>	SSING	AY026577
<i>Shorea smithiana</i>	SSMIT	AY026578
<i>Shorea splendens</i>	SSPLN	AB246573
<i>Shorea splendida</i>	SSPLE	AY026579
<i>Shorea stenoptera</i>	SSTEN	AY026580
<i>Shorea stipularis</i>	SSTIP	AB246584
<i>Shorea trapezifolia</i>	STRAP	AB246596
<i>Shorea virescens</i>	SVIRE	AY026581
<i>Shorea worthingtonii</i>	SWORT	AB246599
<i>Stemonoporus acuminatus</i>	STACU	AB246552
<i>Stemonoporus bullatus</i>	STBUL	AB246556
<i>Stemonoporus canaliculatus</i>	STCAN	AB246555
<i>Stemonoporus gilimalensis</i>	STGIL	AB246553
<i>Stemonoporus kanneliyensis</i>	STKAN	AB246559
<i>Stemonoporus lancifolius</i>	STLAN	AB246560
<i>Stemonoporus reticulatus</i>	STRET	AB246557
<i>Stemonoporus scalarinervis</i>	STSCA	AB246554
<i>Stemonoporus wightii</i>	STWIG	AB246558
<i>Upuna borneensis</i>	UBORN	AB006391
<i>Vateria copallifera</i>	VCOPA	AB246561
<i>Vateriopsis seychellarum</i>	VSEYC	AB246562
<i>Vatica affinis</i>	VAFFI	AB246551
<i>Vatica bella</i>	VBELL	AB246546
<i>Vatica chinensis</i>	VCHIN	AB246550
<i>Vatica coriacea</i>	VCORI	AB246548

The character states were treated as unordered only (Fitch 1971). Statistical measures of the Consistency Index (CI), Homoplasy Index (HI) (Kluge & Farris 1994), Rescaled Consistency Index (RC), and Retention Index (RI) (Farris 1989) were

also calculated. Clade support was estimated by performing 100 bootstrap replicates (Felsenstein 1985) by using 50% majority-rule of MPT input as trees but with MULPARS off. Definition of bootstrap supports were following Richardson *et al.* (2004):

50-74% represents weak support, 75-84% moderate support, 85-100% strong support.

## RESULTS

**Inclusion of Secondary Structures.** The aligned sequences used for this study was 524 bp. The high content of adenine and thymine within *trnL* intron was therefore suggesting that this region was relatively A+T rich. The four stem loop structures present in intron *trnL* were consisted of seven indels: indel 1 was deletion of 5 bp within the loop of SL 1 (Figure 3), indel 2, 3, 4, and 5 were present in SL3 (Figure 4), and indels 6 and 7 were observed in SL 4 (Figure 5). SL 2, however, did not contain any indels. These seven indels were coded as additional characters, thus made up the total of 531 characters. Of these, only 59 were parsimony-informative characters.

A total of 107 of mostly parsimonius trees of 215 steps were obtained. The CI (0.83), RC (0.77), and RI (0.92) values suggest that the changes are mostly apomorphic, despite homoplasy occurring in 17% of the characters. Most of the clades were defined by apomorphic changes rather than synapomorphic changes. Apomorphic changes are mostly provided by base substitutions.

The cladogram (Figure 6) shows two paraphyletic groups with *Monotes madagascariensis* fall excluded from two groups. The first group is moderately supported (BSV of 81%) consisted of most member of tribe Dipterocarpeae except for *Dipterocarpus*. Of these members of tribe

Dipterocarpeae, only *Stemonoporus* and *Vatica* was supported 90 and 84% respectively.

The second main clade did not receive support from bootstrap. *Dipterocarpus* that was at the basal clade as the sister of Tribe Shoreae, containing *Dryobalanops*, *Parashorea*, *Neobalanocarpus heimii* and *Hopea-Shorea* clades. *Hopea* and *Neobalanocarpus heimii* formed a group probably monophyletic, while *Shorea* and *Parashorea* were scattered over the lineages. The only potential monophyletic group of *Shorea* was Section *Richetioides* (Yellow Meranti) and Section *Doona* (Sri-Lankan endemic).

**Exclusion of Secondary Structures.** Excluding the 4 SL characters resulted in 370 characters to which 265 characters are constant, 67 characters were parsimony-uninformative, and only 38 are parsimony informative characters. There were 1196 most parsimonius trees of 136 steps were obtained. The CI (0.6935), RC (0.7979), and RI (0.9275) values suggest that the changes are mostly apomorphic, despite homoplasy occurring in 14% of the characters. Most of the clades were defined by apomorphic changes rather than synapomorphic changes. Apomorphic changes were mostly provided by base substitutions.

The cladogram still showed similar grouping as of inclusion of indels. *Monotes madagascariensis* still form a single lineage. Two main paraphyletic groups were recognized whose divisions were almost in accordance to tribal divisions except for inclusion of *Dipterocarpus* spp. within Tribe Shoreae. Tribe Dipterocarpeae (B) was strongly supported (BSV

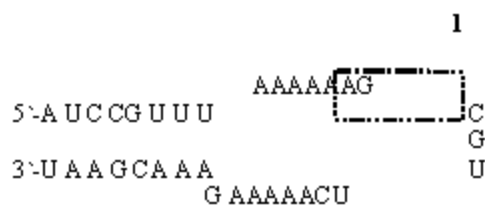


Figure 3. Structure of stem loop 1 (70-105 bp). This model was derived from RNA sequence of *Neobalanocarpus heimii* (after Yulita 2007). Nucleotides in dotted box indicates location of indel 1.

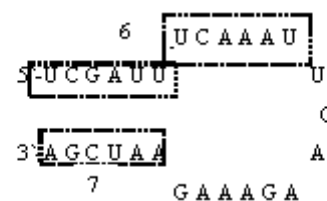


Figure 5. Structure of stem loop 4 (360-386). This model was derived from RNA sequence of *Neobalanocarpus heimii* (after Yulita 2007). Nucleotides in dotted boxes indicate locations of indel 6 and 7.

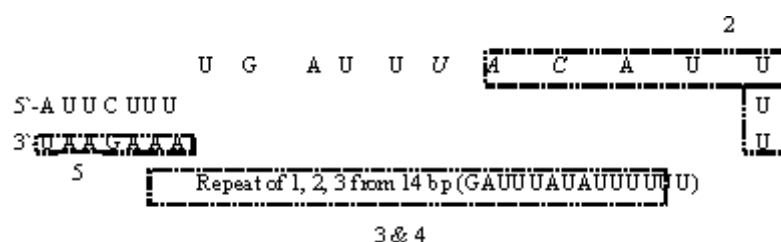


Figure 4. Structure of stem loop 3 (257-328). This model was derived from RNA sequence of *Dipterocarpus kerrii* (after Yulita 2007). Nucleotides in dotted boxes indicate locations of indel 2,3, and 4.

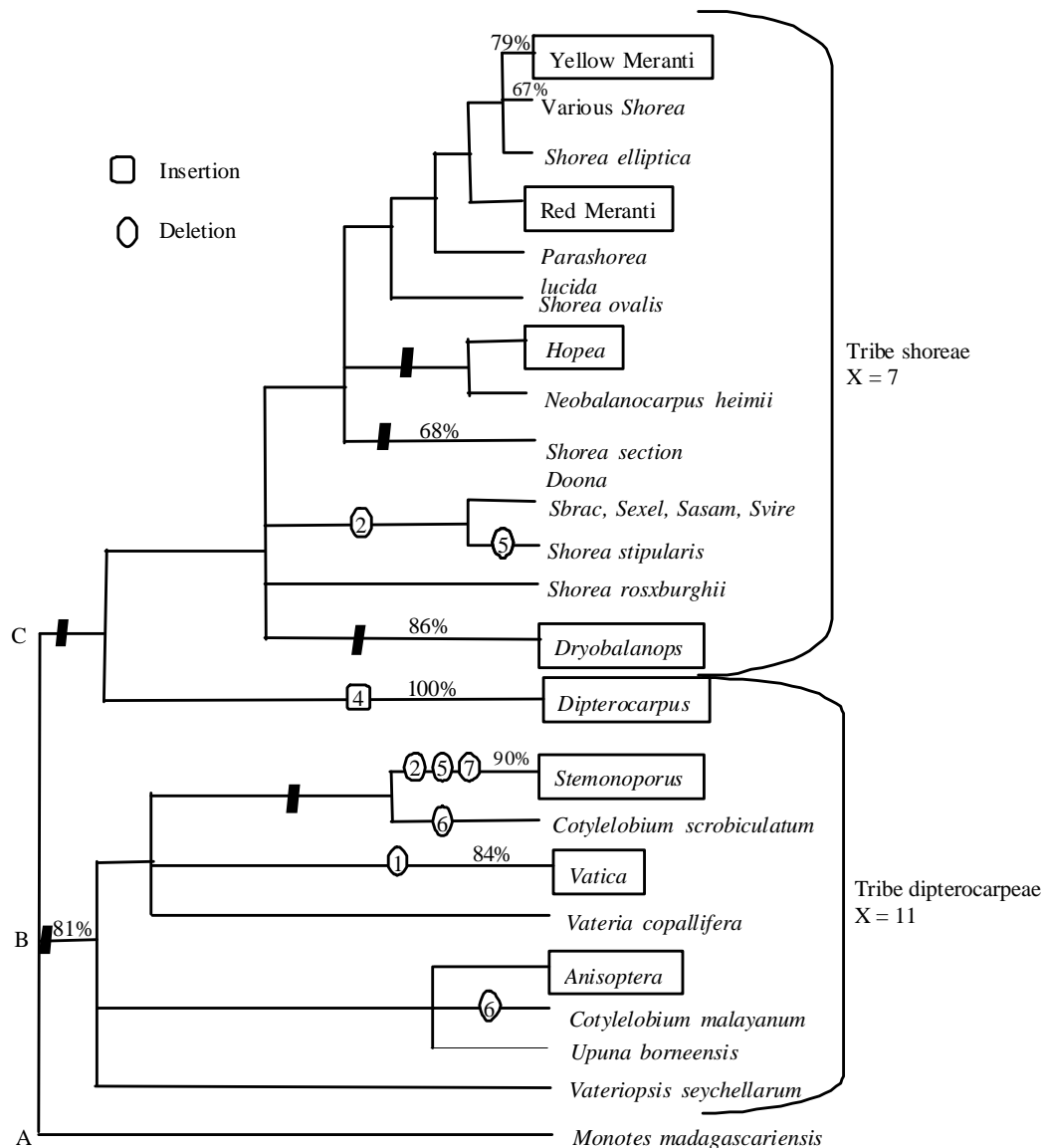


Figure 6. Phylogenetic tree of 110 species of Dipterocarpaceae based on *trnL* intron sequences by including structural mutations. Thick lines are branches appear in strict consensus trees. Taxa in boxes contain all of their species members included in the analysis. Bootstrap supports > 50% are above branches.

89%), while Tribe Shoreae (C) did not received any support from bootstrap (Figure 7). Within tribe Dipterocarpeae, only species of *Stemonoporus* that was weakly supported, other genera/species were not supported. Meanwhile, within Tribe Shoreae only *Shorea* section *Doona* and *Dryobalanops* were weakly supported (61 and 76% respectively).

## DISCUSSION

The common practice for phylogenetic reconstruction using molecular evidences is to set foundation of the study on the basis of sequence homology by performing alignment of DNA sequences. Variations within the data set might due to base substitution and/or indel event. The consequence of assigning indels within alignment is

length polymorphism (length mutation) within the data set to which secondary structures can be built upon. Secondary structures of *trnL* intron was often built to infer positional homology, for example in Annonaceae (Pirie *et al.* 2007). This was important because inclusion of homoplasious indels into the data set it can be misleading, thus producing incorrect phylogenetic tree. Examination through diagnostic characters (Table 2, Homoplasly Index/HI) revealed that none of the characters within the stem loop structures were homoplasious. Thus these characters were properly suit to be included within a phylogenetic analysis.

On the other hand, the existence of such structures is also useful when such structure is consistently found within certain taxonomic level so that they can be used as molecular marker to detect

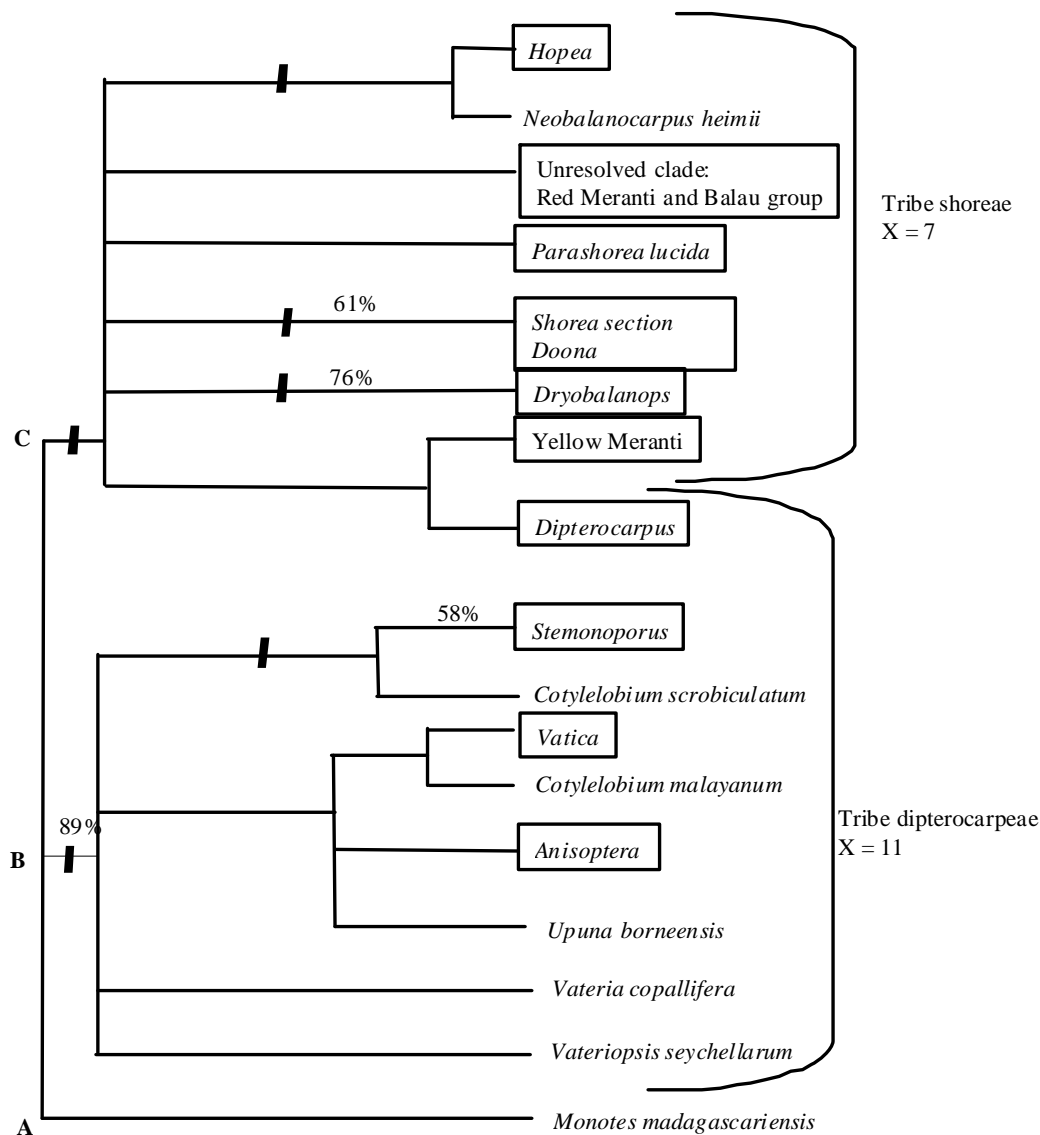


Figure 7. Phylogenetic tree of 110 species of Dipterocarpaceae based on *trnL* intron sequences by excluding structural mutations. Thick lines are branches appear in strict consensus trees. Taxa in boxes contain all of their species members included in the analysis. Bootstrap supports >50% are above branches.

variation at certain taxonomic levels. In this study, inclusion of structural mutations within the data set provided more robust topology for clade C (Figure 6 & 7). The resolved branch includes *Parashorea lucida*, *Shorea* section *Doona*, Red Meranti, Balau, and *Dryobalanops*.

Several classification systems of Dipterocarpaceae were recognized, *i.e.* on the basis of timber grouping (Symington 1943), anatomy (Maury-Lechon & Curtet 1998) and natural group Ashton (1982). The accepted classification system (Ashton 1982) divided this family into 3 sub-families, Dipterocarpoideae (in Asia), Monotoideae (in Africa) and Pakaramoideae (Guayana and Africa). The Asian Dipterocarpoideae contributed the largest number of species within the family. The subfamily Dipterocarpoideae is further divided into two tribes based on the basic

chromosome number: 1) tribe Dipterocarpeae (x = 11) consisted of genus *Dipterocarpus*, *Anisoptera*, *Upuna*, *Cotylelobium*, *Vatica*, *Stemonoporus*, *Vateria*, and *Vateriaopsis*; 2) tribe Shoreae (x = 7) comprises *Dryobalanops*, *Parashorea*, *Neobalanocarpus*, *Shorea*, and *Hopea*. Recent molecular phylogenetic studies of the family using multi cp regions have two different findings in regard to tribal division of subfamily Dipterocarpoideae. The first was the polyphyly of tribe Dipterocarpeae and the monophyly of tribe Shoreae (Tsumura *et al.* 1996; Kajita *et al.* 1998; Gamage *et al.* 2006) and the vice versa: tribe Dipterocarpeae is monophyletic and tribe Shoreae is polyphyletic (Indrioko *et al.* 2006). Indrioko *et al.* 2006 used PCR-RFLP of 17 cp regions, while others employed direct DNA sequencing of some cp genes. These may contributed

Table 2. Character diagnostics for parsimony informative indels. Constant characters are not shown. Location of SL1: 70-105 bp, SL2: 153-171, SL3: 257-328, SL4: 360-386

Char. No.	Tree steps	RI	RC	HI	G-fit
70	1	0/0	0/0	0.000	1.000
79	3	0.000	0.000	0.333	0.750
80	1	0/0	0/0	0.000	1.000
81	1	0/0	0/0	0.000	1.000
82	1	0/0	0/0	0.000	1.000
85	1	0/0	0/0	0.000	1.000
86	2	0/0	0/0	0.000	1.000
87	1	1.000	1.000	0.000	1.000
88	1	0/0	0/0	0.000	1.000
Char. No.	Tree steps	RI	RC	HI	G-fit
89	1	0/0	0/0	0.000	1.000
95	2	0.800	0.400	0.500	0.750
97	1	0/0	0/0	0.000	1.000
99	1	0/0	0/0	0.000	1.000
153	1	1.000	1.000	0.000	1.000
160	3	0.000	0.000	0.333	0.750
162	1	0/0	0/0	0.000	1.000
165	1	0/0	0/0	0.000	1.000
169	1	0/0	0/0	0.000	1.000
Char. No.	Tree steps	RI	RC	HI	G-fit
170	2	0.000	0.000	0.500	0.750
258	1	0/0	0/0	0.000	1.000
261	1	0/0	0/0	0.000	1.000
264	2	0.500	0.250	0.500	0.750
265	1	0/0	0/0	0.000	1.000
269	2	0/0	0/0	0.000	1.000
270	5	0.912	0.365	0.600	0.500
271	2	0.976	0.488	0.500	0.750
277	1	0/0	0/0	0.000	1.000
279	1	0/0	0/0	0.000	1.000
308	1	0/0	0/0	0.000	1.000
309	1	1.000	1.000	0.000	1.000
317	1	0/0	0/0	0.000	1.000
318	1	0/0	0/0	0.000	1.000
320	1	0/0	0/0	0.000	1.000
321	1	0/0	0/0	0.000	1.000
324	1	0/0	0/0	0.000	1.000
Char. No.	Tree steps	RI	RC	HI	G-fit
325	1	0/0	0/0	0.000	1.000
360	1	0/0	0/0	0.000	1.000
363	1	0/0	0/0	0.000	1.000
366	1	0/0	0/0	0.000	1.000
369	1	0/0	0/0	0.000	1.000
372	1	1.000	1.000	0.000	1.000
373	1	0/0	0/0	0.000	1.000
375	1	1.000	1.000	0.000	1.000
380	2	1.000	1.000	0.000	1.000
383	1	1.000	1.000	0.000	1.000
385	2	1.000	1.000	0.000	1.000
525	1	1.000	1.000	0.000	1.000
526	5	0.750	0.150	0.800	0.429
527	1	0/0	0/0	0.000	1.000
528	1	1.000	1.000	0.000	1.000
529	2	0.889	0.444	0.500	0.750
530	2	0.000	0.000	0.500	0.750
531	1	1.000	1.000	0.000	1.000

to the major difference on their results. Second was the inclusion of *Parashorea* within *Shorea* and the monotypic genus *Neobalanopcarpus heimii* within *Hopea*. Not only of these molecular studies (Yulita *et al.* 2005, Indrioko *et al.* 2006; Gamage *et al.* 2006; Tsumura *et al.* 2007) suggested this findings, Symington (1943) has earlier suggested to include *Parashorea* within *Shorea* due to many similarities on morphological traits.

The phylogenetic inference resulting from this study only came from 59 parsimony informative characters but the results of this present study was in accordance to the first finding in that the major groupings tend to follow tribal division to which tribe Dipterocarpeae was polyphyletic and tribe Shoreae is monophyletic. The polyphyletic of tribe Dipterocarpeae was caused by the placement of genus *Dipterocarpus* within tribe Shoreae. Examination of SL structures found that there was a large insertion within *Dipterocarpus* located in SL 3. This large insertion is a repeat of 14 nucleotides (GAUUUAUAUUUUUU) exclusively present only in *Dipterocarpus* that may have evolved independently within *Dipterocarpus* (Yulita 2007). Similar findings also suggested by Vijverberg and Bachmann (1999) that structural mutation <1000 bp may have been repeated independent origin of closely related taxa in *Microseris* (Asteraceae). The unresolved polytomy feature in *Dryobalanops* found in previous studies (Dayanandan *et al.* 1999; Yulita *et al.* 2005, Indrioko *et al.* 2006) was well resolved in this study *Dryobalanops* was well supported by 86% BV and 76% BV respectively (Figure 6 & 7). *Dryobalanops* have morphological features (wood anatomy, pollen and floral aestovations) resembled tribe Shoreae and Dipterocarpeae (Maury-Lechon & Curtet 1998). *Dryobalanops* even received 100% support from bootstrap analysis (Gamage *et al.* 2006) when they included more cp genes (*trnL-F* and *matK*). In addition, the phyletic nature of long debated complex genera, *Shorea* and *Hopea*, was also in accordance to previous studies (Yulita *et al.* 2005; Kamiya *et al.* 2005; Indrioko *et al.* 2006) in which both genera was to form a potential monophyletic group. This could indicated that intron *trnL* consisted of DNA sequences that was evolutionary well preserved. Borsch *et al.* (2003) have demonstrated that the secondary structure of the *trnL* intron is highly conseved in basal Angiospermae, in that only 20% of the 95 positions corresponding to proposed stem structures were variable across their study group. Intron *trnL* was suggested to have been present in the cyanobacterial ancestor of the plastid lineages of Rhodophyta, Chlorophyta (Besendahl *et al.* 2000) to

different orders of flowering plants (Bakker *et al.* 2000).

The results from this study was therefore indicated that indels of *trnL* intron in Dipterocarpaceae was of no homoplasious. Similarity of results obtained from this present study to the previous studies that included more cp genes may indicated that DNA sequence of *trnL* intron contained phylogenetic signals that was sufficiently used to reconstruct phylogeny of the subfamily Dipterocarpoideae.

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