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Secondary Structures of Chloroplast *trn*L Intron in Dipterocarpaceae and its Implication for the Phylogenetic Reconstruction

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Unambiguous insertion-deletion events were previously identified in *trn*L 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/infra-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 resoved 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 Dipterocarpae 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 recived strong branch support (*Dipterocarpus, Dryobalanops, Vatica,* and *Stemonoporus*) possessed certains indels exclusive to each and this may contributed to the monophyletic nature of these genera.

Key words: secondary structures, dipterocarpaceae, *trn*L, intron, phylogeny

INTRODUCTION

The *trn*L-F of chloroplast genome of land plants consists of the transfer RNA genes $trnL_{uaa}$ and $trnF_{gaa}$ arranged in tandem and separated by noncoding spacer regions. The region is positioned in large single copy region, approximately 8 kb downstream of *rbc*L. The conserved nature of *trn*L-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 *trn*L gene is part of *trn*L-F region of chloroplast genome that split by group I intron, the intergenic spacer and *trn*F 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 *trn*L intron is important because the function of the transfer RNA for which the *trn*L 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.

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Sequences from *trn*L-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 *trn*L 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

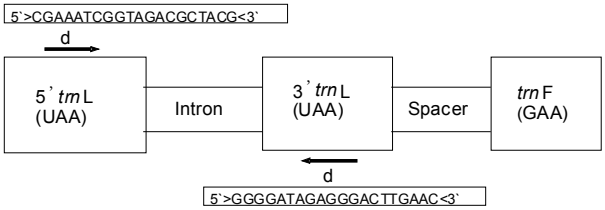

Figure 1. Diagram of *trn*L-F gene with primer sequences of intron *trn*L (c and d) (after Yulita 2007).

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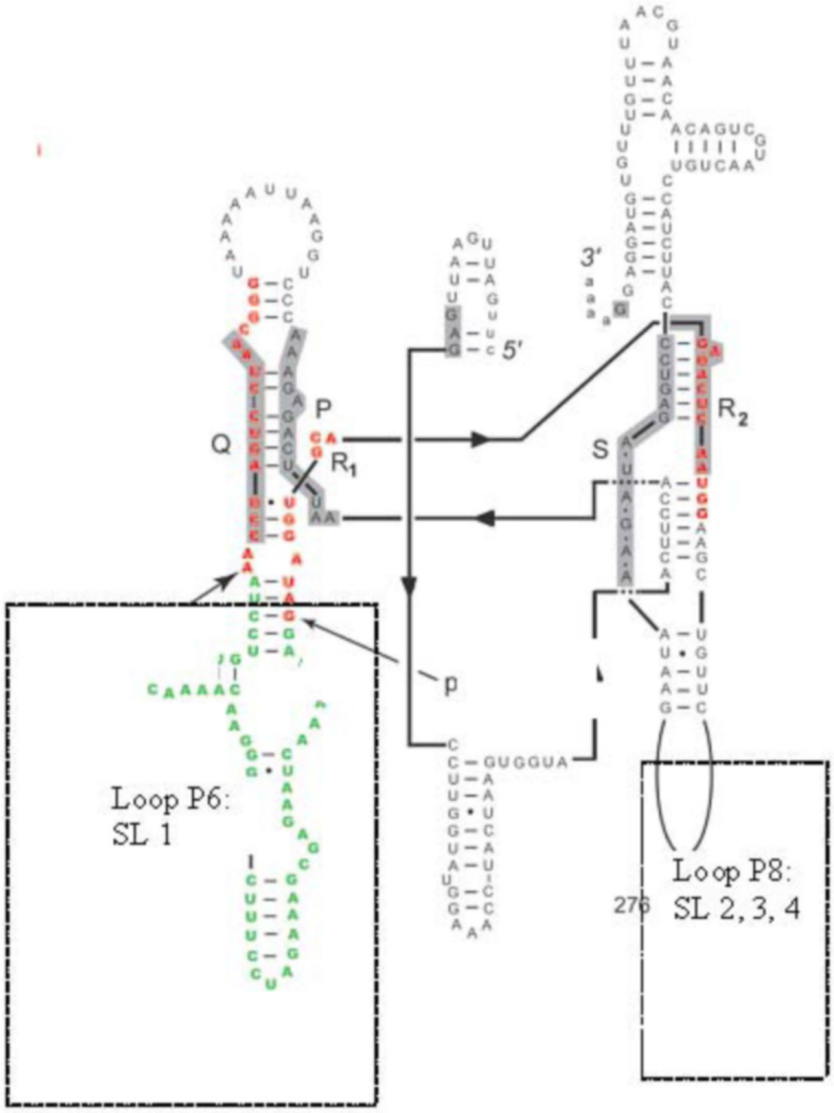

Figure 2. Secondary structure of *trn*L intron of Dipterocarpaceae that was modified from *Nymphaea odorata* (Tarbelet *et al*. 2007). Location of *stem loop* 1 (SL1) was in loop P6, locations of stem loop 2, 3, dan 4 (SL 2, 3, 4) were in loop P8 (after Yulita 2007).

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 *trn*L 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 genebank 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.

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