

PHYLOGENETIC STUDY OF *Nephelium* SPECIES BASED ON CHLOROPLAST AND NUCLEAR DNA SEQUENCES

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INTRODUCTION

The genus *Nephelium* (Sapindaceae) contains 22 species, with 13 species occurring in Malaysia and three of these are endemic species. This genus is distributed in Indo-Malaya, the Philippines and Borneo (Verheij & Coronel 1991). Information on *Nephelium* species and their relationships are useful in exploiting genes for crop improvement. Wild relatives are often potential sources of useful genes for genetic improvement of cultivated species. Buerki et al. (2009) carried out phylogenetic analysis among genera in Sapindaceae family using plastid and nuclear markers and showed the relationships of the genera. Nevertheless, the molecular phylogenetic relationships of *Nephelium* species have not been investigated, and this study is to examine the species relationships within the genus *Nephelium* using sequence data from the chloroplast (*trnL-trnF*, *trnT-trnL* and *trnD-trnT*) and nuclear (ITS and UFGT) genomes.

MATERIALS AND METHODS

Thirteen species of *Nephelium* and two outgroup species were used in this study (Table 1). The samples were obtained from MARDI, Serdang and Hutan Simpan Pasoh, Negeri Sembilan. Most DNA samples were extracted based on the method of Doyle and Doyle (1990) with some modifications using the CTAB extraction buffer. DNA for some samples was extracted by using Promega Wizard DNA Extraction Kit.

Taxon	Location
<i>Nephelium ramboutan-ake</i> (Pulasan)	MARDI Serdang
<i>Nephelium</i> sp. (Linang)	MARDI Serdang
<i>N. cuspidatum</i> var. <i>eripetalum</i> (Lotong)	MARDI Serdang
<i>N. cuspidatum robustum</i> (Rambutan Gergasi)	MARDI Serdang
<i>N. lappaceum</i> (Rambutan klon Anak Sekolah)	MARDI Serdang
<i>N. lappaceum</i> (Rambutan klon Gula Batu)	MARDI Serdang
<i>N. lappaceum</i> (Rambutan klon Gading Muar)	MARDI Serdang
<i>N. maingayi</i> (Redan)	MARDI Serdang
<i>N. costatum</i> (Rambutan Pasek)	H.S. Pasoh, N. Sembilan
<i>N. lappaceum</i> var. <i>pallens</i> (Rambutan Hutan)	H.S. Pasoh, N. Sembilan
<i>N. eripetalum</i> (Rambutan Kabung)	H.S. Pasoh, N. Sembilan
<i>N. ophiodes</i> (Rambutan Siamang)	H.S. Pasoh, N. Sembilan
<i>N. hamulatum</i> (Sangal Lotong)	H.S. Pasoh, N. Sembilan
<i>Litchi chinensis</i> (Lychee)	MARDI Serdang
<i>Xerospermum noronhanium</i> (Rambutan Pacat)	MARDI Serdang

Table 1. The taxa used in phylogenetic analyses

Five DNA regions (*trnL-trnF*, *trnT-trnL*, *trnD-trnT*, ITS and UFGT) were analysed for sequence variation. The *trnL-trnF* spacer (500 bp) and the *trnT-trnL* spacer (800 bp) were amplified using primers designed by Taberlet et al. (1991) while the *trnD-trnT* spacer (1200 bp) was amplified by using primers designed by Demesure et al. (1995). The ITS region (600 bp) was amplified by using primers designed by White et al. (1990) while primers for UFGT region (415 bp) was designed by using *Litchi chinensis* (FJ172529) sequence from the GenBank. Standard PCR procedure was used to amplify these DNA regions. The sequence data of the five regions were combined for phylogenetic analyses. Ambiguous regions in the DNA alignment were excluded from the phylogenetic analyses. Insertions and deletions were treated as gaps. Pairwise distances based on HKY85 evolution model were obtained using PAUP beta ver.4.0 b10 (Swafford 2000) to generate phylogenetic trees based on Neighbour-Joining (NJ) approach. PAUP was also employed to construct Maximum Parsimony (MP) and Maximum Likelihood (ML) trees. In the ML analysis, the HKY85 evolution model was selected. Bootstrap analyses with 1000 replicates were used to assess the internal support of all the phylogenetic trees.

RESULTS AND DISCUSSION

The MP analysis yielded 10 most parsimonious trees with a tree length of 303 steps, CI = 0.68 (excluding uninformative characters) and RI = 0.50. One of the MP trees is shown in Figure 1. The topologies of the MP, ML and NJ trees differed slightly in one or two small clades, and these clades did not receive bootstrap support. Only the MP tree is presented here for phylogenetic inferences.

The MP tree showed that all the *Nephelium* taxa were clustered together to form a monophyletic group with 100% bootstrap support (Figure 1). *N. costatum* and *N. hamulatum* formed one main clade with high bootstrap support. On the other hand, *N. cuspidatum* var. *robustum*, *N. cuspidatum* var. *cuspidatum*, *N. cuspidatum* var. *ophiodes*, *N. cuspidatum* var. *eriopetalum*, *N. lappaceum* var. *pallens*, *N. ramboutan-ake*, *N. maingayi*, *Nephelium* sp. and three *N. lappaceum* clones formed another main clade with moderate bootstrap support. The three *N. lappaceum* clones formed one subclade with moderate bootstrap support. Not all the four *N. cuspidatum* varieties in this study were clustered together. *N. cuspidatum* var. *ophiodes* was grouped with *N. lappaceum* var. *pallens* while *N. cuspidatum* var. *eriopetalum* was grouped with *Nephelium* sp. The molecular data did not seem to support the varietal status of *N. cuspidatum*. It is of much interest to look into the varietal status of *N. cuspidatum* more comprehensively by including the other two varieties which are absent in this study i.e. *N. cuspidatum* var. *bassacense* and *N. cuspidatum* var. *multinerve*. Overall, species which clustered together in clade are from the same regions, such as *N. costatum* and *N. hamulatum* which can be found in Perak, Pahang, Negeri Sembilan and Melaka. *Nephelium cuspidatum* var. *ophiodes* and *N. lappaceum* var. *pallens* can be found in Sumatera and Peninsular Malaysia, whereas *N. cuspidatum* var. *robustum* and *N. cuspidatum* var. *cuspidatum* have the same origin in Borneo. *N. ramboutan-ake* and *N. maingayi* were clustered together but lacked bootstrap support. These two species having the same origins occur in Peninsular Malaysia, Borneo and Sumatera.

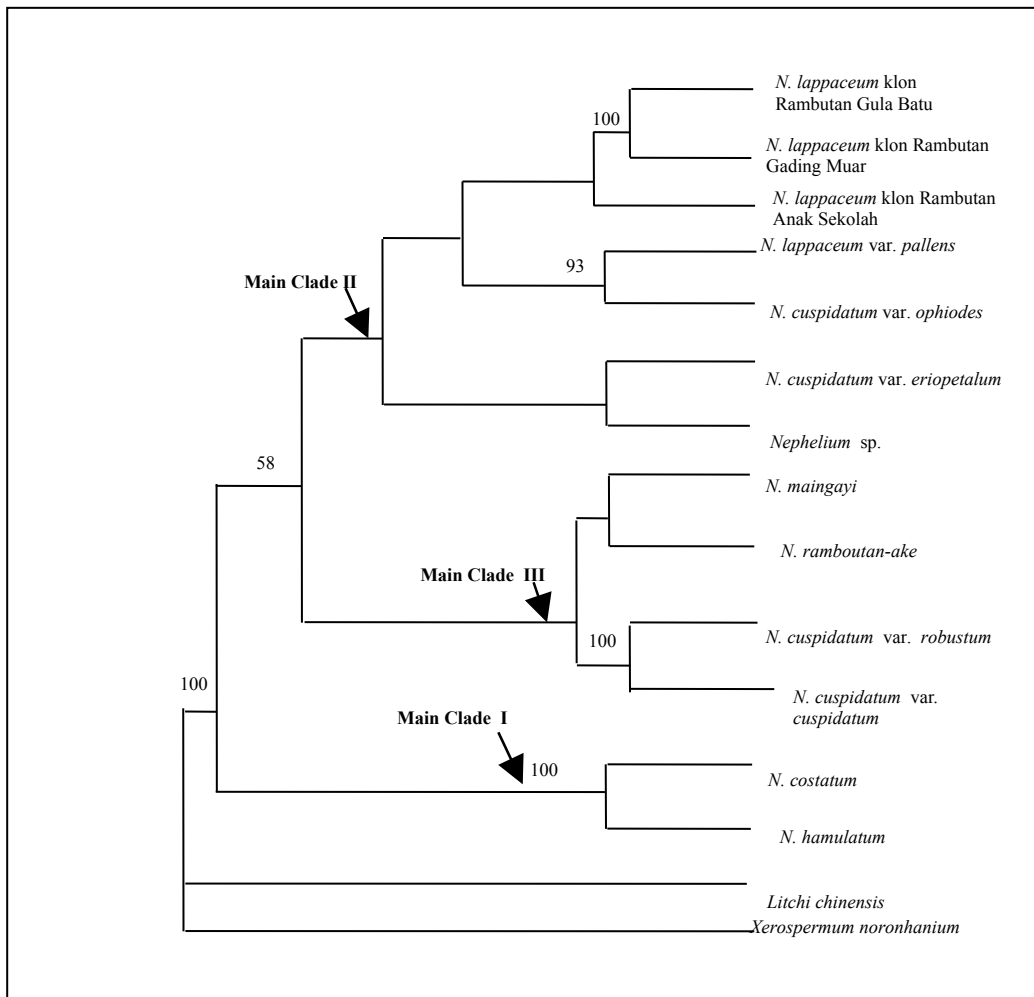


Figure 1. The MP tree of *Nephelium* species. Number above branch indicates bootstrap support.

CONCLUSION

The molecular phylogenetic analyses showed the *Nephelium* taxa were clustered together to form a monophyletic group. The *Nephelium* taxa which clustered together in a clade have the same geographical origins. Generally, the molecular phylogenetic study of *Nephelium* has generated useful information for a more comprehensive study on the genus and also the other genera in the family Sapindaceae.

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REFERENCES

- Buerki, S., Félix, F., Pedro, A.R., Martin, W.C., Johan, A.A.N., Mark, H., Isabel, S., Philippe, K. & Nadir, A. (2009). Plastid and nuclear DNA markers reveal intricate relationships at subfamilial and tribal levels in the soapberry family (Sapindaceae). *Molecular Phylogenetics and Evolution* **51**: 238–258.
- Demesure, B., Sodzi, N. & Petit, R.J. (1995). A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* **4**: 129-131.
- Doyle, J.J. & Doyle, J.L. (1990). Isolation of plant DNA from fresh tissues. *Focus* **12(1)**: 13-15.
- Swofford, D.L. (2000). *PAUP: Phylogenetic Analysis Using Parsimony (and Other Methods)*, Version 4.0. Slunderland, Sinauer Associates Inc.
- Taberlet, P., Gieley, L., Pautou, G & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Systematics and Evolution* **170**:97-106.
- Verheij, E.W.M. & Coronel, R.E. (1991). Plants Resources of South-East Asia No. 2: Edible Fruits and Nuts. Pudoc Wagenigen.
- White, T.J., Burns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Snisky, J.J. & White, T.J. (eds.). *PCR Protocols: a Guide to Methods and Applications*, 315-322. San Diego: Academic Press.