

PHYLOGEOGRAPHICAL PATTERNS OF
NEOBALANOCARPUS HEIMII (DIPTEROCARPACEAE)
FOR THE EVOLUTIONARY HISTORY
AND CHAIN OF CUSTODY CERTIFICATION

TNAH LEE HONG

THESIS SUBMITTED IN FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2010

CORAK FILOGEOGRAFI *NEOBALANOCARPUS HEIMII*
(DIPTEROCARPACEAE) UNTUK SEJARAH EVOLUSI DAN
RANGKAIAN PENSIJILAN KUSTODI

TNAH LEE HONG

TESIS YANG DIKEMUKAKAN UNTUK MEMENUHI
SYARAT MEMPEROLEHI IJAZAH
DOKTOR FALSAFAH

FAKULTI SAINS
UNIVERSITI MALAYA
KUALA LUMPUR

2010

UNIVERSITI MALAYA
ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Tnah Lee Hong (I.C No: 810709-10-6094)

Registration/Matric No: SHC070080

Name of Degree: Doctor of Philosophy

Title of Thesis: Phylogeographical patterns of *Neobalanocarpus heimii* (Dipterocarpaceae) for the evolutionary history and chain of custody certification

Field of Study:

I do solemnly and sincerely declare that:

- (1) I am the sole author/writer of this Work;
- (2) This Work is original;
- (3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
- (4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
- (5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
- (6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature



Date: 27 October 2010

Subscribed and solemnly declared before,

Witness's Signature

Date:

Name:

Designation:

Specially dedicated to my loving husband, daughter and family members...

ABSTRACT

Tectonic movement and climatic oscillations during the Cenozoic have had dramatic effect on the biota of the tropical rain forest. This study aims to reveal the phylogeography and evolutionary history of a Peninsular Malaysian endemic tropical timber, *Neobalanocarpus heimii* (Dipterocarpaceae), based on chloroplast DNA (cpDNA) variation. Fifteen haplotypes were identified from 10 intraspecific variable sites of five non-coding cpDNA regions: *trnL* intron, *trnS-trnG* spacer, *trnG* intron, *trnK* intron and *psbK-trnS* spacer. Two major genealogical cpDNA lineages of *N. heimii* were elucidated: a widespread southern and a northern region. The species is predicted to survive in multiple refugia during climatic oscillation: the northwestern region (R1: Sungkop), the northeastern region (R2: Gunung Basur), and the southern region (R3: Panti compartment 16). Recolonization of refugia R1 and R2 could have first expanded into the northern region and migrated both northeastwardly and northwestwardly. Meanwhile, recolonization of *N. heimii* throughout the southern region could have commenced from refugia R3, and migrated toward northeast and northwest respectively. The populations of Tersang, Pasir Raja and Rotan Tunggal exhibited remarkably high haplotype diversity, which could have been the contact zones that received an admixture of organisms from the northerly and also southerly regions. As a whole, understanding the past history of the extant populations is of the utmost importance when developing sound conservation policies or sustainable management strategies.

The inbuilt unique properties of DNA within the timber could serve as tracking and monitoring tools to verify the legality of a suspected timber in the context of illegal logging, forest certification and chain of custody certification. By using *N. heimii* as an example, a population identification database and a haplotype distribution map in Peninsular Malaysia were generated for authenticity testing based on four cpDNA markers (*trnL* intron, *trnG* intron, *trnK* intron and *psbK-trnS* spacer). Twenty-one haplotypes were identified from 10 significant intraspecific variable sites. The results

clearly revealed that only the northern and southern regions of Peninsular Malaysia were distinguishable. Thus, this database could only be used to determine the wood lot of unknown origin at the regional level. Statistical procedure based on the composition of the wood lot was used to test whether a suspected timber conforms to a given regional origin. Overall, the observed types I and II errors of the database showed good concordance with the predicted 5% threshold, which might indicate that the database is useful to reveal provenance and establish conformity of wood lot from the northern and southern regions of Peninsular Malaysia. In terms of application, this database could be applied to traceability in two different circumstances: (1) to verify the provenance of a wood lot in the context of forest certification and chain of custody certification and (2) to identify the potential population of origin of the suspected illegal harvested wood lot.

Wood can be a good source of DNA for various applications in forensic forestry and timber trade if high quality DNA can be retrieved from the dry wood. In order to provide a general guideline for DNA authenticity testing established for *N. heimii*, this study was designed to evaluate the potential for extracting DNA from the dry wood of *N. heimii* using the Qiagen kit, CTAB, and CTAB with PTB protocols. Overall, the efficacy of DNA extraction was higher for the cambium and sapwood than for the heartwood tissues. In terms of tissue types, the Qiagen kit yielded higher PCR amplification rates from the cambium tissue, while the CTAB with PTB protocol showed higher amplification rates in the sapwood and heartwood tissues. In order to safeguard the intactness of the DNA, it is recommended that DNA extraction from the wood should be carried out within six weeks after felling for logs and six months after felling for stumps. The results also showed that the amplicon size might not account for the PCR amplification success rate and chloroplast genome yielded higher amplification success rate compared with nuclear genome. Additionally, the PCR amplifications also showed that both the nuclear and chloroplast regions can be retrieved from lumber that was heat-treated at 40 °C to 100 °C, although the phenomena of allelic dropout and inconsistency of genotyping were noted for some of the nuclear regions. In short, the guideline obtained from this study are ready to be used together with the population and

individual identification databases developed for the timber tracking system of *N. heimii* in Peninsular Malaysia.

ABSTRAK

Pergerakan tektonik dan perubahan iklim semasa Senozoik memberi kesan yang dramatis ke atas biota dalam hutan hujan tropikal. Kajian ini bertujuan untuk mendedahkan filogeografi dan sejarah evolusi bagi salah satu balak tropikal yang endemis di Semenanjung Malaysia, iaitu *Neobalanocarpus heimii* (Dipterocarpaceae) dengan berdasarkan variasi DNA kloroplas (cpDNA). Daripada lima kawasan bukan pengekodan cpDNA: intron *trnL*, penjarak *trnS-trnG*, intron *trnG*, intron *trnK* dan penjarak *psbK-trnS*, 15 haplotip telah dikenalpasti daripada 10 kawasan jujukan berubah yang intraspesifik. Dua genealogi cpDNA yang utama bagi *N. heimii* telah ditemui di sebelah kawasan utara dan selatan. Spesies ini dijangka hidup dalam pelbagai refugia semasa perubahan iklim, iaitu di kawasan utara-barat (R1: Sungkop), kawasan utara-timur (R2: Gunung Basur) dan kawasan selatan (R3: Panti bahagian 16). Penghijrahan *N. heimii* dari refugia R1 dan R2 mungkin diperluaskan ke kawasan utara pada permulaan, dan kemudian berpindah secara beransur-ansur ke sebelah utara-timur dan utara-barat. Pada masa yang sama, penghijrahan *N. heimii* ke kawasan selatan mungkin berpunca daripada refugia R3, dan kemudian berhijrah ke kawasan utara-timur dan utara-barat. Antaranya, populasi Tersang, Pasir Raja dan Rotan Tunggal menunjukkan kepelbagaian haplotip yang tinggi. Ini mungkin disebabkan oleh populasi tersebut merupakan zon pertemuan yang menerima campuran organisma daripada kawasan utara dan selatan. Secara keseluruhan, memahami sejarah lepas untuk populasi yang masih wujud ini adalah amat penting apabila menyediakan polisi pemuliharaan atau strategi pengurusan secara berkekalan.

Dalam konteks pembalakan haram, pensijilan hutan dan rangkaian pensijilan kustodi, kandungan DNA yang unik dalam kayu balak boleh dijadikan sebagai alat pengesanan dan pengawas untuk menguji kesahan kayu balak. Dengan menggunakan *N. heimii* sebagai contoh, pangkalan data untuk identifikasi populasi dan peta taburan haplotip di Semenanjung Malaysia telah dijana untuk ujian kesahan berdasarkan empat penanda cpDNA (intron *trnL*, intron *trnG*, intron *trnK* dan penjarak *psbK-trnS*).

Antaranya, 21 haplotip telah dikenalpasti daripada 10 kawasan jujukan berubah yang penting dari segi intraspesifik. Keputusan dengan jelasnya menunjukkan terdapat perbezaan antara kawasan utara dan selatan Semenanjung Malaysia. Maka, pangkalan data ini hanya dapat digunakan untuk menentusahkan punca sesuatu kayu balak sama ada berasal daripada kawasan utara atau selatan sahaja. Prosedur statistik yang berdasarkan komposisi kayu telah digunakan untuk menguji sama ada kayu balak yang dicurigai akur dengan kawasan asal yang diisytihar. Secara keseluruhan, ralat cerapan jenis I dan II untuk pangkalan data menunjukkan keselarasan yang baik dengan jangkaan ambang sebanyak 5%. Ini juga menunjukkan bahawa pangkalan data ini adalah sesuai digunakan untuk menentukan asal-usul dan konformasi kayu balak sama ada berasal daripada kawasan utara atau selatan Semenanjung Malaysia. Pendek kata, pangkalan data ini boleh digunakan untuk sistem pengesanan balak dalam dua situasi, (1) menentusahkan asal-usul kayu balak dalam konteks pensijilan hutan dan rangkaian pensijilan kustodi, dan (2) mengenal pasti populasi asal untuk balak yang ditebang secara haram.

Kayu merupakan sumber DNA yang sesuai untuk pelbagai aplikasi forensik perhutanan dan perdagangan kayu balak jika DNA yang berkualiti tinggi dapat diekstrak daripada kayu. Dengan tujuan menghasilkan satu panduan untuk ujian kesahan DNA yang telah dijana bagi *N. heimii*, kajian ini direka bentuk untuk menilai kebolehan mengekstrak DNA daripada kayu dengan menggunakan protokol kit Qiagen, CTAB dan CTAB dengan PTB. Secara keseluruhan, keberkesanan pengekstrakkan DNA adalah lebih tinggi untuk tisu kambium dan sapwood berbanding dengan tisu heartwood. Dari segi jenis tisu, protokol kit Qiagen menghasilkan kadar amplifikasi PCR yang lebih tinggi untuk tisu kambium, manakala protokol CTAB dengan PTB menunjukkan kadar amplifikasi yang tinggi dalam tisu sapwood dan heartwood. Untuk memastikan kesempurnaan DNA, pengekstrakkan DNA daripada bahagian balak perlulah dilakukan dalam masa enam minggu selepas ditebang, manakala enam bulan untuk bahagian tunggul. Keputusan ini juga menunjukkan saiz amplicon tidak mempengaruhi kadar amplifikasi PCR dan secara keseluruhannya genom kloroplas menghasilkan kadar amplifikasi yang lebih tinggi berbanding dengan genom nukleus.

Selain itu, amplifikasi PCR juga menunjukkan kedua-dua DNA nukleus dan kloroplas dapat diekstrak daripada kayu yang dipanaskan daripada 40 °C ke 100 °C, walaupun berlakunya fenomena keciciran alel dan ketidaktekan penentuan untuk sesetengah lokus nukleus. Pendek kata, keputusan yang diperolehi daripada kajian ini boleh digunakan bersama dengan pangkalan data identifikasi populasi dan individu yang dijanakan bagi sistem pengesanan balak *N. heimii* di Semenanjung Malaysia.

ACKNOWLEDGEMENTS

First and foremost, I would like express my deepest thanks and appreciation to my supervisor, Dr. Lee Soon Leong, from the Forest Research Institute Malaysia (FRIM). His generous advice, encouragement and support, which extended far beyond the call of duty and academic relationships, are highly appreciated. My deepest gratitude is also extended to Professor Dr. Rofina Yasmin Othman and Dr. Subha Bhasu from the University of Malaya for their huge contributions, guidance and keen interest in my research. Their criticisms, support and valuable suggestions were most helpful throughout the process of completing this thesis.

My special thanks go to the Director General of FRIM, Dato' Dr. Abdul Latif Mohmod, for granting me a scholarship to pursue the study. I also thank my Divisional Director, Dr. Norwati Muhammad, for her encouragement and unending support. The Forest Departments of Kedah, Perak, Selangor, Negeri Sembilan, Johor, Pahang, Terengganu and Kelantan are acknowledged for granting me permission to access the forest reserves. I thank the District Forest Officers and the staffs of the Renjer Offices who provided assistance and logistic support during the field trips. Special thanks and profound gratitude are due to Dr. Naoki Tani, who guided and assisted me throughout the statistical analyses.

I am deeply indebted to my Genetics Laboratory colleagues, Dr. Lee Chai Ting, Dr. Kevin Ng Kit Siong and Dr. Ng Chin Hong who guided me and coached me throughout. I also thank Dr. Norwati Adnan, Dr. Norlia Basherudin, Dr. Siti Salwana Hashim and Mr. Mohd Rosli Haron who gave their help directly or indirectly. My sincerest thanks go to Mariam Din, Ghazali Jaafar, Yahya Marhani, Ramli Ponyoh, Sharifah Talib, Suryani Che Seman, Nurul Hudaini Mamat and Nor Salwah Abdul Wahid for their excellent assistance in the laboratory and the field. All research group members were helpful with their information. All my friends at FRIM, especially Dr. Ho Wai Mun, Ms. Sun Wan Fong, and Mr. Brian Yap Jing Wei, gave much support and encouragement.

This study was supported in part by the e-Science Research Grant (02-03-10-SF0009) entitled “Development of DNA barcode of *Neobalanocarpus heimii* (chengal) as a tool for forensics and chain of custody certification” and the Bioversity International Agreement No. APO 05/016.

Last but not least, I owe my deepest appreciation and thanks to my parents, Mr. Tnah Boon Keat and Madam Low Soo Chow, and parents-in-law, Mr. Tan Seng Wah and Madam Tan Siok Gern and all the family members for their concern, care and encouragement throughout the study. To my beloved husband Kay Win, I cannot express how much his love, support and understanding have meant to me over the years and I look forward to their continuity in the years to come. My lovely daughter Kyanne Tan, who has been my wonderful companion throughout the study, has provided the incentive for the successful completion of this study. I dedicate this thesis to them and to all those who are always inspired by values but not discouraged by their high prices.

TABLE OF CONTENTS

| | Page |
|---|-------------|
| ABSTRACT | v |
| ABSTRAK | viii |
| ACKNOWLEDGEMENTS | xi |
| LIST OF TABLES | xv |
| LIST OF FIGURES | xvii |
| LIST OF SYMBOLS AND ABBREVIATIONS | xx |
| CHAPTER 1: INTRODUCTION | 1 |
| CHAPTER 2: LITERATURE REVIEW | 7 |
| 2.1 Phylogeography | 7 |
| 2.1.1 Geological time scales | 8 |
| 2.1.2 Geological events and climatic changes in Southeast Asia | 8 |
| 2.1.3 Origin of tropical rain forest | 11 |
| 2.1.4 Evidence of climate change and areas of continuing rain forest | 13 |
| 2.1.5 The persistence of rain forest refugia in Peninsular Malaysia | 14 |
| 2.2 Tracing the geographic origin | 15 |
| 2.2.1 Issues of illegal logging, forest certification, eco-labelling, and chain of custody certification | 15 |
| 2.2.2 Authenticity testing and databases for traceability | 17 |
| 2.3 DNA extraction from dry wood | 19 |
| 2.3.1 General consideration in plant DNA extraction | 19 |
| 2.3.2 Structure of wood | 22 |
| 2.4 Chloroplast DNA markers | 24 |
| 2.5 Description of <i>Neobalanocarpus heimii</i> (King) Ashton | 28 |
| CHAPTER 3: PHYLOGEOGRAPHICAL PATTERN AND EVOLUTIONARY HISTORY OF AN IMPORTANT PENINSULAR MALAYSIA TIMBER SPECIES, <i>NEOBALANOCARPUS HEIMII</i> (DIPTEROCARPACEAE) | |
| 3.1 Introduction | 32 |
| 3.2 Materials and methods | 36 |
| 3.2.1 Sample collection and DNA extraction | 36 |
| 3.2.2 PCR amplifications and sequencing | 36 |
| 3.2.3 Statistical analyses | 39 |
| 3.3 Results | 40 |
| 3.3.1 Chloroplast DNA variability and genetic diversity | 40 |

| | | |
|---|--|-----|
| 3.3.2 | Genetic differentiation | 41 |
| 3.3.3 | Haplotype distribution and relationship inferred from NCPA | 45 |
| 3.4 | Discussion | 52 |
| 3.4.1 | Hypothetical phylogeographical history | 52 |
| 3.4.2 | Genealogical lineages | 54 |
| 3.4.3 | Three potential refugia recognized in Peninsular Malaysia | 56 |
| 3.4.4 | Post-glacial recolonization routes | 57 |
| 3.4.5 | Implications for conservation | 58 |
| | | |
| CHAPTER 4: GEOGRAPHICAL TRACEABILITY OF AN IMPORTANT TROPICAL TIMBER (<i>NEOBALANOCARPUS HEIMII</i>) INFERRED FROM CHLOROPLAST DNA | | |
| 4.1 | Introduction | 60 |
| 4.2 | Materials and methods | 63 |
| 4.2.1 | Sample collection and DNA extraction | 63 |
| 4.2.2 | PCR amplifications and sequencing | 63 |
| 4.2.3 | Population identification database and test of conformity of origin | 65 |
| 4.3 | Results | 66 |
| 4.3.1 | Population identification database and haplotype distribution | 66 |
| 4.3.2 | Test of conformity of origin | 68 |
| 4.4 | Discussion | 71 |
| | | |
| CHAPTER 5: DNA EXTRACTION FROM DRY WOOD OF <i>NEOBALANOCARPUS HEIMII</i> FOR FORENSIC DNA PROFILING AND TIMBER TRACKING | | |
| 5.1 | Introduction | 74 |
| 5.2 | Materials and methods | 76 |
| 5.2.1 | Plant material | 76 |
| 5.2.2 | DNA extraction protocol | 77 |
| 5.2.3 | DNA quantification | 78 |
| 5.2.4 | PCR amplification, genotyping and sequencing | 79 |
| 5.3 | Results | 80 |
| 5.3.1 | DNA extraction protocols for cambium, sapwood and heartwood | 80 |
| 5.3.2 | Optimal preservation period of wood for DNA extraction | 84 |
| 5.3.3 | Effect of amplicon size and genome's copy number | 84 |
| 5.3.4 | Effect on heat-treated lumber | 89 |
| 5.4 | Discussion | 89 |
| | | |
| CHAPTER 6: CONCLUSION | | 96 |
| | | |
| REFERENCES | | 102 |
| | | |
| APPENDIX: PUBLISHED MANUSCRIPT | | 122 |

LIST OF TABLES

| Table | | Page |
|-------|---|------|
| 3.1 | Sampling localities chosen for the population of the <i>Neobalanocarpus heimii</i> in Peninsular Malaysia accompanied with the population code and their haplotypes. Eight samples per population were used in this study. | 37 |
| 3.2 | Five universal chloroplast DNA primer pairs used in this study. | 38 |
| 3.3 | Distribution of intraspecific variable sites in the five noncoding region of chloroplast DNA (<i>trnL</i> intron, <i>trnS-trnG</i> spacer, <i>trnG</i> intron, <i>trnK</i> intron and <i>psbK-trnS</i> spacer) of <i>Neobalanocarpus heimii</i> in Peninsular Malaysia. | 42 |
| 3.4 | Population of <i>Neobalanocarpus heimii</i> with haplotype and nucleotide diversity associated with the results of Tajima's D neutrality test. | 43 |
| 3.5 | Hierarchical analysis of molecular variance (AMOVA) of <i>Neobalanocarpus heimii</i> based on two different grouping schemes. | 44 |
| 3.6 | Population structure of <i>Neobalanocarpus heimii</i> for the northern region, southern region and the whole Peninsular Malaysia. Values in parenthesis are standard deviation. | 46 |
| 3.7 | Nested contingency analysis of geographic associations and their interpretations according to the inference key of Templeton <i>et al.</i> , (2005) for 32 populations of <i>Neobalanocarpus heimii</i> in Peninsular Malaysia. Clade numbers refer to numbers reported in Figure 3.5. | 53 |
| 4.1 | Sampling localities chosen for the population of the <i>Neobalanocarpus heimii</i> in Peninsular Malaysia accompanied by the population codes. Eight samples per population were used in this study. | 64 |
| 4.2 | Distribution of significant intraspecific variable sites in the non-coding region of chloroplast DNA of <i>Neobalanocarpus heimii</i> in the population identification database. The positions of the variable sites in the <i>trnL</i> intron, <i>trnG</i> intron, <i>trnK</i> intron and <i>psbK-trnS</i> spacer, and (-) deletion are indicated. | 67 |
| 5.1 | Results of PCR amplification comparing three DNA extraction methods: the Qiagen kit, CTAB, and CTAB with PTB protocols for logs. Logs are labelled as 1–8. Number 1 corresponds to logs immediately felled, numbers 2–8 correspond to logs preserved for two, four, six weeks and | 82 |

three, six, nine and 12 months, respectively (numbers indicate amplification and dashes (-) indicate no amplification).

- 5.2 Results of PCR amplification comparing three DNA extraction methods: the Qiagen kit, CTAB and CTAB, with PTB protocols for stumps. Stumps are labelled as 1–8. Number 1 corresponds to stumps of trees immediately felled, numbers 2–8 correspond to stumps preserved for two, four, six weeks and three, six, nine and 12 months, respectively (numbers indicate amplification and dashes (-) indicate no amplification). 83
- 5.3 PCR amplification tested on DNA extracted from lumber that had undergone drying process from 40 °C to 100 °C using the CTAB with PTB method. Symbol ‘+’ indicates amplification. 90

LIST OF FIGURES

| Figure | | Page |
|--------|---|------|
| 2.1 | The International Stratigraphic Chart summarizing the set of chronostratigraphic units (geologic stages, periods) and their computed ages, which are the main framework for Geologic Time Scale 2004 (Grastein <i>et al.</i> , 2004). | 9 |
| 2.2 | Map of Sundaland at the Last Glacial Maximum (adapted from Bird <i>et al.</i> , 2005), showing “savanna corridor” proposed by Heaney (1991). Glacial refugia sites inferred from palynology, geomorphology and biogeography data are denoted by circles (Emmel & Curray, 1982, Bird <i>et al.</i> , 2005; Quek <i>et al.</i> , 2007). | 12 |
| 2.3 | Cross-section of a <i>Neobalanocarpus heimii</i> trunk: outer bark, inner bark, cambium, sapwood, heartwood and pith. | 23 |
| 2.4 | Gene map of the chloroplast genome from <i>Nicotiana tabacum</i> . Genes shown on the inside of the circle are transcribed clockwise, and those on the outside are transcribed counter-clockwise. Genes for transfer RNAs are represented by 1-letter code of amino acids with anticodons. Asterisks denote split genes. Open-reading frames are shown by orf plus codon number (≥ 70 codons). When two genes overlap, the one that is located downstream or inside the other gene is displayed with a lower-height box (by Yukawa <i>et al.</i> , 2005). | 26 |
| 2.5 | Morphological characteristics of <i>Neobalanocarpus heimii</i> . (A) the mature tree; (B) leaf; (C) fruits of the mature tree; (D) intact seeds; (E) seedlings; (F) stipules and (G) the largest tree of <i>N. heimii</i> in Pasir Raja Forest Reserve, Peninsular Malaysia. | 29 |
| 3.1 | (a) Postulated distribution of land and sea in Southeast Asia at 15 Ma (Middle Miocene, adapted from Hall, 1998), everwet tropical rain forests were widespread across the region (Morley, 2000). Land areas are shaded in green colour and shallow sea in light grey; (b) Sundaland at the Last Glacial Maximum (adapted from Bird <i>et al.</i> , 2005), showing “savanna corridor” proposed by Heaney (1991). Glacial refugia sites inferred from palynology, geomorphology and biogeography data are denoted by circles (Emmel & Curray, 1982; Bird <i>et al.</i> , 2005; Quek <i>et al.</i> , 2007). | 33 |
| 3.2 | Relationship between pairwise population F_{ST} and geographical distance of <i>Neobalanocarpus heimii</i> for the (a) whole 32 populations of Peninsular Malaysia (Mantel test of correlation, $r = 0.619$, $P = 0.0000$), | 47 |

(b) southern region (Mantel test of correlation, $r = 0.288$, $P = 0.0082$), and (c) northern region (Mantel test of correlation, $r = -0.027$, $P = 0.5720$).

- 3.3 Contribution of each population of *Neobalanocarpus heimii* to the total diversity, as described by the haplotype richness (*CTR*). Circles represent total diversity; solid and open bars represent contributions of differentiation and diversity to the total diversity, respectively. 48
- 3.4 Locations of 32 populations of *Neobalanocarpus heimii* in 29 forest reserves of Peninsular Malaysia. The geographical distribution of cpDNA haplotypes (h1–h15) in each population is shown and haplotypes are colour coded and their frequencies are indicated by sectors of pies. 49
- 3.5 The resolved cpDNA haplotype network and resulting set of nested clades for *Neobalanocarpus heimii*. Each line connecting one haplotype to another indicates one mutational step. The relative sizes of the circles represent the frequency of these haplotypes and unsampled haplotypes are represented by square box. 51
- 3.6 (a) Distribution of potential refugia sites (R1, R2 and R3) inferred for *Neobalanocarpus heimii* and special phytogeographical subprovinces recognized in Peninsular Malaysia include (1) Perak subprovince, (2) rough extent of core of seasonal Asiantic intrusion, and (3) the Riau Pocket (Corner, 1960; Ashton, 1992); (b) Hypothetical phylogeographical history of *N. heimii* in Peninsular Malaysia. Region in black indicates mountain range and arrows indicate putative recolonization routes from the R1, R2 and R3. 55
- 4.1 Locations of 32 populations of *Neobalanocarpus heimii* from 29 forest reserves of Peninsular Malaysia. The distributions of 13 haplotypes in the population identification database that occurred in more than two individuals are shown in different colours. The remaining eight unique haplotypes that occurred in only one individual are each shown in black. 69
- 4.2 Simulation of the number of wood samples/lots considered as non-conformity with the size of lot ranging from 1–100, originating from the southern and northern of Peninsular Malaysia were tested against the southern and northern regions of population identification database. 70
- 5.1 Agarose gel (0.85%) showing total DNA extracted from the cambium, sapwood and heartwood tissues using three extraction methods performed immediately after felling: the Qiagen kit (lane 81

2: cambium, lane 3: sapwood and lane 4: heartwood); the CTAB method (lane 5: cambium, lane 6: sapwood and lane 7: heartwood); the CTAB with PTB method (lane 8: cambium, lane 9: sapwood and lane 10: heartwood) and after 12 months preservation: the Qiagen kit (lane 12: cambium, lane 13: sapwood and lane 14: heartwood); the CTAB method (lane 15: cambium, lane 16: sapwood and lane 17: heartwood); the CTAB with PTB method (lane 18: cambium, lane 19: sapwood and lane 20: heartwood). Lane 1 and 11 denote for MassRuler DNA Ladder (Fermentas).

- | | | |
|-----|--|----|
| 5.2 | Three different DNA extraction methods were compared for logs and stumps using the cambium, sapwood and heartwood tissues. | 85 |
| 5.3 | PCR amplification success rates of DNA extracted from the cambium, sapwood and heartwood tissues for logs using the Qiagen kit. The amplifications were performed for nuclear and chloroplast regions immediately after felling (start) and two (2w), four (4w), six weeks (6w) and three (3m), six (6m), nine (9m) and 12 months (12m) of preservation. | 86 |
| 5.4 | PCR amplification success rates of DNA extracted from the cambium, sapwood and heartwood tissues for stumps using the CTAB with PTB method. The amplifications were performed for nuclear and chloroplast regions immediately after felling (start) and two (2w), four (4w), six weeks (6w) and three (3m), six (6m), nine (9m) and 12 months (12m) of preservation. | 87 |
| 5.5 | PCR amplification success rate related to amplified fragment length and genome's copy number for logs and stumps (open squares indicate nuclear STR and black triangles indicate chloroplast DNA). | 88 |
| 5.6 | The problems of allelic dropout and inconsistency of genotyping were observed for DNA amplified from heat-treated lumber using nuclear STR. Genotypes of loci <i>Nhe011</i> , <i>Hbi161</i> , <i>Sle392</i> and <i>Shc07</i> for control and heat-treated lumber are shown. | 91 |

LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|-------------------|--|
| AFP | Asia Forest Partnership |
| AFLP | Amplified fragment length polymorphism |
| AMOVA | Analysis of molecular variance |
| bp | Base pair |
| BSA | Bovine serum albumin |
| CAPS | Cleaved amplified polymorphic sequences (CAPS) |
| cpDNA | Chloroplast DNA |
| cpSSR | Chloroplast microsatellites |
| CSA | Canadian Standards Association |
| CTAB | Hexadecyltrimethyl-ammonium bromide |
| DIECA | Diethyldithiocarbamic acid |
| DNA | Deoxyribonucleic acid |
| dNTP | 2'-deoxynucleoside 5'-triphosphate |
| EDTA | Diaminoethanetetra-acetic acid |
| FSC | Forest Stewardship Council |
| FLEGT | Forest Law Enforcement, Governance and Trade |
| FR | Forest Reserve |
| FRIM | Forest Research Institute Malaysia |
| HCl | Hydrochloric acid |
| Indel | Insertion and deletion |
| IUCN | International Union for Conservation of Nature and Natural Resources |
| kbp | Kilobase pair |
| KCl | Potassium chloride |
| LGM | Last glacial maximum |
| MgCl ₂ | Magnesium chloride |
| min | Minute |
| MTC | Malaysian Timber Council |
| MTCC | Malaysian Timber Certification Council |
| Ma | Million years ago |

| | |
|---------------------|---|
| NaCl | Sodium chloride |
| NCPA | Nested Clade Phylogeographic Analysis |
| NH ₄ OAc | Ammonium acetate |
| nSTR | Nuclear Short Tandem Repeat |
| PCR | Polymerase chain reaction |
| PEFC | Programme for the Endorsement of Forest Certification Schemes |
| PTB | <i>N</i> -phenacylthiazolium bromide |
| PVP | Polyvinylpyrrolidone |
| RFLP | Restriction fragment length polymorphism |
| SDS | Sodium dodecyl sulphate |
| s | Second |
| SFI | Sustainable Forestry Initiative |
| STR | Short tandem repeat |
| TAE | Tris-acetate EDTA |
| TBE | Tris-borate EDTA |
| TE | Tris-EDTA |
| Tris | Trishydroxymethylaminomethane |