Molecular Phylogenetic and Evolution of Malaysian Primates with an Emphasis on the Phylogeography of Malaysian Bornean Proboscis Monkey

(Nasalis larvatus)

Ho Licia

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(Molecular Ecology)
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MOLECULAR PHYLOGENETIC AND EVOLUTION OF MALAYSIAN PRIMATES
WITH AN EMPHASIS ON THE PHYLOGEOGRAPHY OF MALAYSIAN
BORNEAN PROBOSCIS MONKEY (NASALIS LARVATUS)

HO LICIA

A thesis submitted
in fulfilment of the requirement for the degree of
Master of Science
(Molecular Ecology)

Faculty Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2015
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declared that this thesis has not been previously or concurrently submitted for any other degree or qualification to this or any other university or institutions of higher learning.

..........................

(Ho Licia)
I dedicate my work to my beloved parents, family, and my loving friends
ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere gratitude to my principle supervisor, Dr. Faisal Ali Bin Anwarali Khan and co-supervisor, Professor Dato’ Dr. Mohd Tajuddin Abdullah and Associate Professor Dr. Yuzine Bin Esa for everything that I have achieved today. I am greatly indebted for their thoughtful opinions, constructive comments and invaluable motivation throughout this study. Thank you to three of you for being a father figure and for having faith in me to complete my master’s degree. Next, I would like to express my heartfelt thanks to all Department Zoology staffs, Mr. Wahap Marni, Mr. Huzal Irwan Husin, Mr. Trevor Allen Nyaseng, Mr. Mohamad Jalani Mortada, Mr. Nasron Ahmad, and Mr. Isa Sait for their field assistance throughout my study.

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ABSTRACT

This thesis comprised of two major studies including, 1) molecular phylogeny of Malaysian primates and 2) phylogeography of proboscis monkey (*Nasalis larvatus*) inferred from mitochondrial (mtDNA) gene. In Malaysia, there are a total of 21 described primate species, from five families and nine genera namely: *Hylobates, Macaca, Nasalis, Nycticebus, Pongo, Presbytis, Cephalopachus, Trachypithecus*, and *Symphalangus*. A total of 79 sequences with 1044 base pairs (bp) of mitochondrial NADH dehydrogenase subunit 2 (ND2) gene from 15 species of Malaysian Primates were used in the first study. Phylogenetic analyses based on NJ, MP, ML, and Bayesian Inference (BI) provided support for similar topology, confirming the monophyly for all the major primate clades, with five major clades identified. The divergence date analysis support for an origin of crown primates at 76.1 Mya during the Late Cretaceous period, whereby the crown haplorhines are estimated to have originated 69.5 Mya shortly after the occurrence of crown primates. The most recent common ancestor of the living catarrhines was estimated to have lived 29.2 Mya, whereas crown Cercopithecoida and Hominoidea were estimated to lived approximately 21.7 Mya and 16.4 Mya, respectively. The diversification of primate major lineages in Malaysia and the geographical variation among the primate species are correspond to the sea level changes during Pleistocene period. Proboscis monkey is an example of such primate diversification in Southeast Asia and are endemic to Borneo. This species are highly restricted to the lowland coastal areas which include mangrove and peat swamp forests. However, their population are threaten due to their habitats that are gradually fragmented due to land conversion and oil palm plantations. Therefore in the second study, patterns of genetic variation among isolated
populations of proboscis monkey from selected localities from Malaysian Borneo (Sarawak and Sabah) were explored. A total of 70 proboscis monkeys faecal samples were collected from six sampling sites include Bako National Park, Kuching Wetland National Park., Maludam National Park, Klias Wetland, Kinabatangan River, and Labuk Bay Proboscis monkey Sanctuary. The genetic structure of *N. larvatus* was inferred using 471 bp of the mitochondrial DNA D-loop control region segment. Result shows that the *N. larvatus* populations in Malaysian Borneo had retained high genetic diversity and was separated into two haplogroups, namely, Haplo-group 1 (containing Sarawak populations) and Haplo-group 2 (containing Sarawak and Sabah populations) by high genetic divergence (6.83%). The divergence analysis showed that separation between Sarawak and Sabah populations occur during the early Pleistocene (1.1 Mya), suggesting that historical events during the last glacial maximum may be the main factor that led to this separation. Besides, sharing of haplotypes and high gene flow (Nm= 12.56) were detected between Maludam NP and Labuk Bay populations indicating the presence of gene flow which might be a result of the secondary contact among isolated populations when the rainforest expansion during the last glacial cycle. Furthermore, high genetic diversity and low gene flow (Nm= 0.24) were found between Bako NP and Labuk Bay populations, suggesting that there may be another forest refugia for *N. larvatus* located in the south-western part of Borneo. In conclusion, both studies had profoundly emphasised on phylogeny, phylogeography relationship and the diversification of primates within Malaysia region, especially on the Bornean endemic *N. larvatus*.

**Keywords:** Malaysian Borneo, Malaysian Primates, mtDNA, *N. larvatus*, phylogeny, phylogeography
ABSTRAK

Filogenetik Molekul dan Evolusi Primat di Malaysia dengan Kajian Spesifik dalam Filogeografi Monyet Proboscis \((\text{Nasalis larvatus})\) dari Borneo, Malaysia.

Tesis ini merangkumi dua hasil penyelidikan yang utama. Ini termasuk, 1) filogeni molekul bagi primat di Malaysia dan 2) filogeografi bagi monyet proboscis \((\text{Nasalis larvatus})\) dengan menggunakan molekul DNA mitokondria (mtDNA). Di Malaysia, terdapat sebanyak 21 spesies primat yang terdiri daripada lima Family dan sembilan genera iaitu: \text{Hylobates, Macaca, Nasalis, Nycticebus, Pongo, Presbytis, Cephalopachus, Trachypithecus, dan Symphalangus.} Jumlah 79 urutan dengan 1044 jujukan bes gen NADH dehydrogenase subunit 2 \((\text{ND2})\) bagi setiap satu daripada 15 spesies primat telah digunakan dalam kajian pertama. Analisis filogenetik berdasarkan NJ, MP, ML, dan Bayesian Inference (BI) menyokong secara statistik struktur pokok filogeni yang sama. Pokok filogeni ini juga telah mengesahkan monofili bagi semua kumpulan primat yang utama daripada lima kumpulan \text{Family} primat yang telah dikenal pasti. Analisis penentuan masa bagi moyang yang dikongsi bersama, menyokong asal usul primat yang bermula 76.1 juta tahun yang lampau (ketika zaman Cretaceous), manakala moyang haplorhines dianggarkan telah muncul lebih kurang 69.5 juta tahun yang lepas iaitu tidak lama selepas munculnya moyang primat amnya. Moyang yang paling muda bagi catarrhines telah dianggarkan telah hidup sejak 29.2 juta tahun yang lepas, manakala moyang cercopithecoidea dan Hominoidea dianggarkan telah hidup sekurang-kurangnya 21.7 juta tahun dan 16.4 juta tahun yang lepas masing-masing. Kepelbagaian keturunan utama primat di Malaysia dan variasi geografi antara spesies primat adalah sesuai
yang tinggi dan pengaliran gen yang rendah (Nm= 0.24) pula direkodkan di antara populasi Bako NP dan Labuk Bay, yang mencadangkan kawasan sekitar Bako NP (Barat Borneo) merupakan kawasan hutan yang telah terpisah lebih lama daripada hutan-hutan lain dalam kajian ini. Kesimpulannya, hasil kajian tesis ini mengurai hubungan filogeni, filogeografi dan kepelbagaian bagi primat di Malaysia dan bagi monyet probosis endemik di Borneo.

Kata kunci: Borneo Malaysia, filogeni, filogeografi, mtDNA, N. larvatus, primat Malaysia
TABLE OF CONTENTS

Declaration.......................................................................................................................... ii
Dedication .......................................................................................................................... iii
Acknowledgement ............................................................................................................ iv
Abstract ............................................................................................................................ vi
Abstrak ............................................................................................................................. viii
Table of Contents .............................................................................................................. xi
List of Figures .................................................................................................................... xv
List of Tables ...................................................................................................................... xvii
Abbreviation ..................................................................................................................... xix
Thesis Outline .................................................................................................................. xxii

CHAPTER ONE
General Introduction .......................................................................................................... 1
  1.1 Primates..................................................................................................................... 1
  1.2 Strepsirrhini............................................................................................................. 3
  1.3 Haplorrhini ............................................................................................................. 4
  1.4 Playrrhini................................................................................................................ 4
  1.5 Catarrhini ................................................................................................................ 5
  1.6 Malaysian Primates ............................................................................................... 7
  1.7 Study Species: Proboscis Monkey ........................................................................ 10
  1.8 Rationale ................................................................................................................ 12
  1.9 Research Objectives .............................................................................................. 13

CHAPTER TWO
Literature Reviews ............................................................................................................ 14
  2.1 Phylogenetics ......................................................................................................... 14
  2.2 Population Genetic Variation ................................................................................. 15
  2.3 Mitochondrial Genome (mt-genome) .................................................................... 19
  2.4 NADH dehydrogenase subunit 2 (ND2).................................................................. 21
  2.5 Displacement Loop (D-loop) Control Region ......................................................... 21
  2.6 Molecular Phylogenetic Studies ............................................................................. 22
  2.7 Population Genetic Studies .................................................................................... 24
# CHAPTER THREE

Molecular Phylogeny of Malaysian Primates Inferred from mitochondrial ND2 (MT-ND2) Gene

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Introduction</td>
<td>27</td>
</tr>
<tr>
<td>3.2 Materials and Methods</td>
<td>28</td>
</tr>
<tr>
<td>3.2.1 Field Techniques and Samples Collection</td>
<td>28</td>
</tr>
<tr>
<td>3.2.2 Molecular and Laboratory Methods</td>
<td>29</td>
</tr>
<tr>
<td>3.2.2.1 Blood/Blood Spot Extraction</td>
<td>29</td>
</tr>
<tr>
<td>3.2.2.2 Tissues Samples Extraction</td>
<td>30</td>
</tr>
<tr>
<td>3.2.2.3 Gel Electrophoresis and Visualisation</td>
<td>31</td>
</tr>
<tr>
<td>3.2.2.4 Polymerase Chain Reaction (PCR)</td>
<td>32</td>
</tr>
<tr>
<td>3.2.2.5 DNA Purification and DNA Sequencing</td>
<td>34</td>
</tr>
<tr>
<td>3.2.2.6 Sequence Data Analysis</td>
<td>35</td>
</tr>
<tr>
<td>3.2.2.7 Time Scale Divergence Analysis</td>
<td>37</td>
</tr>
<tr>
<td>3.3 Result</td>
<td>40</td>
</tr>
<tr>
<td>3.3.1 Analysis of Sequences</td>
<td>40</td>
</tr>
<tr>
<td>3.3.2 Nucleotide Divergence</td>
<td>43</td>
</tr>
<tr>
<td>3.3.3 Phylogenetic Analysis</td>
<td>45</td>
</tr>
<tr>
<td>3.3.4 Age of Divergence Estimation</td>
<td>51</td>
</tr>
<tr>
<td>3.4 Discussion</td>
<td>54</td>
</tr>
<tr>
<td>3.4.1 Genetic Relationships of Malaysian Primates</td>
<td>54</td>
</tr>
<tr>
<td>3.4.2 Divergence Dates of Malaysian Primates</td>
<td>60</td>
</tr>
<tr>
<td>3.5 Conclusion</td>
<td>62</td>
</tr>
</tbody>
</table>

# CHAPTER FOUR

Phylogeography of Proboscis monkey (*Nasalis larvatus*) in Malaysian Borneo inferred from D-loop control region

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Introduction</td>
<td>63</td>
</tr>
<tr>
<td>4.2 Materials and Methods</td>
<td>66</td>
</tr>
<tr>
<td>4.2.1 Sampling Sites</td>
<td>66</td>
</tr>
<tr>
<td>4.2.1.1 Bako National Park</td>
<td>69</td>
</tr>
<tr>
<td>4.2.1.2 Kuching Wetland National Park</td>
<td>70</td>
</tr>
</tbody>
</table>
5.2 Hypothetical Route of the Diversification and Distribution of *N. larvatus* in Malaysian Borneo ................................................................. 129
5.3 General Conclusion ........................................................................ 132
5.4 Challenges of Present Study ............................................................ 134
5.5 Future Directions ........................................................................... 136
References .......................................................................................... 138
Appendix A .......................................................................................... 173
Appendix B .......................................................................................... 175
Appendix C .......................................................................................... 177
Appendix D .......................................................................................... 187
Appendix E .......................................................................................... 188
Appendix F .......................................................................................... 217
Appendix G .......................................................................................... 219
Appendix H .......................................................................................... 221
Appendix I .......................................................................................... 229
Appendix J .......................................................................................... 230
Appendix K .......................................................................................... 239
Appendix L .......................................................................................... 244
LIST OF FIGURES

Figure 1.1 Geographic distribution of proboscis monkey, *Nasalis larvatus* in Borneo. ................................................................. 11

Figure 2.1 The mammalian mtDNA genome. ................................................................. 20

Figure 3.1 Saturation plot of ND2 gene on transition, (X) and transversion, (∆), against sequence divergence based on Kimura-2-parameter model. ................................................................. 42

Figure 3:2 A neighbor-joining 50% majority rule consensus tree of 15 species of Malaysian primates under study based on mtDNA ND2 gene sequences. ................................................................. 47

Figure 3.3 A maximum-parsimony 50% majority rule consensus tree of 15 species of Malaysian primates under study based on mtDNA ND2 gene sequences. ................................................................. 48

Figure 3.4 A maximum likelihood 50% majority rule consensus tree of Malaysian primates under study based on 1044 bp ND2 gene sequences................................................................. 49

Figure 3.5 A Bayesian inference with 50% majority rule consensus tree of Malaysian primates under study based on 1044 bp ND2 gene sequences................................................................. 50

Figure 3.6 Single chronogram showed the divergence date estimates from dataset mtDNA ND2 gene sequences from 15 species of Malaysian primates. ................................................................. 53

Figure 4.1 The location of *N. larvatus* populations sampled in this study. ............... 67

Figure 4.2 Trails and sites in Bako National Park............................................................... 70

Figure 4.3 Neighbor-joining (NJ) 50% majority rule consensus tree of D-loop control region mtDNA of all haplotypes from six *N. larvatus* populations in Malaysian Borneo. ............................................................... 91
Figure 4.4  Maximum parsimony (MP) 50% majority rule consensus tree of D-loop control region mtDNA of all haplotypes from six *N. larvatus* populations in Malaysian Borneo................................................................. 92

Figure 4.5  Maximum-likelihood 50% majority rule consensus tree of D-loop control region mtDNA of all haplotypes from six *N. larvatus* populations in Malaysian Borneo................................................................. 93

Figure 4.6  Bayesian inference of 50% majority rule consensus tree of D-loop control region mtDNA of all haplotypes from six *N. larvatus* populations in Malaysian Borneo................................................................. 94

Figure 4.7  Haplotypes mapping of 33 assigned haplo-nodes for six populations of *N. larvatus* from Malaysian Borneo................................................................. 97

Figure 4.8  Scatter plots with 95% linear regression fit of geographical distance versus percentage of net nucleotide divergence (Da) between six populations of *N. larvatus* in Malaysian Borneo. ......................... 98

Figure 4.9  Chronogram showing the divergence date estimates from mtDNA D-loop control region sequences from 70 samples of *N. larvatus*. ................................................................................................................. 101

Figure 4.10  Population expansion signatures in mtDNA D-loop control region sequences data of *N. larvatus*. a) Mismatch distribution of observed frequencies of pairwise differences among D-loop control region and expected frequencies. b) Allele frequency spectrum of segregating sites among six populations of *N. larvatus* in Malaysian Borneo under the null hypothesis of no population change. .......................................................................................... 106

Figure 4.11  Mismatch distribution of *N. larvatus* within populations in Sarawak and Sabah (a–f) and mismatch distribution within grouped populations (g–h). ................................................................................................................. 107

Figure 4.12  The hypothetical Pleistocene refugia for proboscis monkey in Malaysian Borneo. ............................................................................................................................ 121

Figure 5.1  The hypothetical routes of proboscis monkeys in Malaysian Borneo. ............................................................................................................................ 130
LIST OF TABLES

Table 1.1 Checklist of Malaysian Primates................................................................. 9
Table 3.1 List of sequences downloaded from GenBank............................................. 29
Table 3.2 Mitochondrial ND2 gene primers used in this study................................. 32
Table 3.3 Master-mix preparation for one reaction of ND2 gene............................... 33
Table 3.4 PCR amplification parameter for ND2 gene.............................................. 33
Table 3.5 Nucleotide composition based on mtDNA ND2 gene sequences among Malaysian primate, *Tu. belangeri* and *G. variegatus* are as outgroups................................................................. 41
Table 3.6 Test of substitution saturation of third codon position of ND2 sequences........................................................................................................................... 42
Table 3.7 Average percentage of genetic distance values within (in boldface type along diagonal) and among species in Malaysian primates (*Tu. belangeri* and *G. variegatus* as outgroups) based on Kimura-2-parameter.................................................. 44
Table 3.8 Bayesian divergence time estimates for major nodes of Malaysian primates based on mtDNA ND2 gene sequences.............................. 52
Table 4.1 List of sampling sites with sampling periods, GPS coordinates and forest types.................................................................................................................. 68
Table 4.2 Geographical distance in kilometers (km) estimates between each sampling sites.............................................................................................................. 68
Table 4.3 Control region sequence primers used in PCR and DNA sequencing.................................77
Table 4.4 The PCR reaction mixtures ............................................................................ 77
Table 4.5 PCR amplification parameter for D-loop control region ............................ 78
Table 4.6  Nucleotide positions of 63 segregating sites of mtDNA D-loop control region segment discovered from 33 haploptyes within six *N. larvatus* populations in Malaysian Borneo. .................................................. 84

Table 4.7  Haplotype distribution of 70 mtDNA D-loop control region sequences within and among six proboscis monkey populations in Malaysian Borneo. ................................................................. 86

Table 4.8  Number of haplotypes and nucleotide diversity within each population of *N. larvatus*. .................................................................................................................. 88

Table 4.9  Analysis of nucleotide diversity (π), net nucleotide divergence among six populations of *N. larvatus*. ........................................................................................................ 88

Table 4.10  Summary analysis of mtDNA D-loop control region sequence variation in six populations of *N. larvatus* from Malaysian Borneo. ................................................................. 104

Table 4.11  Geographical population differentiation in *N. larvatus* based on analysis of molecular variance (AMOVA) using D-loop control region........................................................................ 110

Table 4.12  Genetic differentiation matrix of six *N. larvatus* populations calculated by $\Phi_{st}$ and $p$ values are shown in parenthesis................................. 110

Table 4.13  Statistical estimates of nucleotides subdivision ($N_{st}$), population subdivision ($F_{st}$) and gene flow/number of migrants ($Nm$) among six populations of *N. larvatus* in Malaysian Borneo. ...................... 112
## ABBREVIATION

### Symbol

<table>
<thead>
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<th>Symbol</th>
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<tbody>
<tr>
<td>°C</td>
<td>degree Celsius (temperature)</td>
</tr>
<tr>
<td>µl</td>
<td>microliter</td>
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<tr>
<td>%</td>
<td>percentage</td>
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<tr>
<td>&gt;</td>
<td>more than</td>
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<tr>
<td>&lt;</td>
<td>less than</td>
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<tr>
<td>-</td>
<td>negative</td>
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<td>/</td>
<td>per</td>
</tr>
<tr>
<td>=</td>
<td>equal</td>
</tr>
<tr>
<td>≈</td>
<td>approximately</td>
</tr>
<tr>
<td>X</td>
<td>multiply or times</td>
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### A

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<thead>
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<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
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<tr>
<td>AIC</td>
<td>Akaike Information Criterion</td>
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### B

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<tbody>
<tr>
<td>BNP</td>
<td>Bako National Park</td>
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<tr>
<td>bp</td>
<td>base pair</td>
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<tr>
<td>BPP</td>
<td>Bayesian posterior probability</td>
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<tbody>
<tr>
<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>CI</td>
<td>Consistency index</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>COI</td>
<td>Cytochrome oxidase I</td>
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<tr>
<td>CTAB</td>
<td>Cetyltrimethyl ammonium bromide</td>
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### D

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<tbody>
<tr>
<td>ddH₂O</td>
<td>deionised distilled water</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTPs</td>
<td>deoxyribonucleotide triphosphate</td>
</tr>
<tr>
<td>DWNP</td>
<td>Department of Wildlife and National Park</td>
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### E

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<td>e.g.</td>
<td>example</td>
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<tr>
<td>EtBr</td>
<td>ethidium bromide</td>
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<tr>
<td>EDTA</td>
<td>ethelene diamine tetra acetic acid</td>
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</tbody>
</table>
G
G Guanine
g gram
GTR+I+G General Time Reversible+ Invariant Proportion+ Gamma

I
IUCN International Union for Conservation of Nature

K
km kilometer
kb kilo base pair
K2P Kimura- 2 parameter
KLS Klias Wetland
KWP Kuching Wetland National Park

L
LBB Labuk bay

M
MLD Maludam National Park
MEGA Molecular Evolutionary Genetics Analysis
MgCl₂ magnesium chloride
ml mililiter
mM millimolar
ML Maximum Likelihood
MP Maximum Parsimony
mtDNA mitochondrial deoxyribonucleic acid
Myr million years
Mya million years ago
MCMC Markov chain Monte Carlo

N
N number of samples
NA not available
NaCl sodium chloride
NJ neighbour-joining
NADH 2 nicotinamide adenine dinucleotide (reduced)
P
PCR  Polymerase chain reaction
PRP  Probosics Research Project

R
RI    Retention index
rpm   Rotation per minute

S
SFD   Sarawak Forestry Department
SFC   Sarawak Forestry Corporation
SKK   Sukau, Kinabatangan
sp.   Species

T
T     Thymine
TAE   tris-acetate-ethylenediaminetetraacetic
TE    tris EDTA

U
UNIMAS Universiti Malaysia Sarawak
UV    ultraviolet

V
v     transversion
V     voltage

W
WPL   Wilayah Persekutuan Labuan

Z
ZM    Zoo Melaka
There are five chapters in this thesis. Chapter one is the general introduction and objectives of this study. Chapter two presents the literature review of this study and previous works which had done related with this study. Chapter three discusses the finding on phylogenetic relationships of Malaysian Primates inferred from mtDNA ND2 gene. Chapter Four reviews the genetic variation based on D-loop control region of selected proboscis monkey populations in Malaysian Borneo. Lastly, chapter five general discussions provide summary in this study and conclude the main results, challenges faced in this thesis and recommendation for future study.
CHAPTER ONE

GENERAL INTRODUCTION

1.1 Primates

The name Primates was derived from Latin word, *primat*, which means prime or first rank. Lemurs, monkeys, and apes are the best-known primates, but this order also includes tarsiers, lorises, bushbabies, pottos, and angwantibos. Primates are thought to be the “highest” order in Class Mammalia because primates exhibit a wide range of the original mammalian features. There are several features that can be distinguished between primates and other mammals. One of the main distinctive characteristics of primates is their brain size is larger as compared to other mammals. Their eyes that face forward allow them to perceive a stereoscopic image and able to judge distance accurately. They also have eye sockets where the eyeball sits inside a protective ring of bone called the orbit. Some primate species have even developed three-colour vision features. All primates possess nails and grasping hands which enable them to climb trees. Besides, all primates have large caecum, and their cheek teeth tend to be low variability and low crowned, often with secondary development of surface wrinkling and upper molar hypocones (Hunter and Jernvall, 1995). Primates usually exhibit sexual dimorphism. The dimorphism can be attributed to and affected by mating system, size, habitat and diet (Leutenegger and Cheverud, 1982).