

## ABSTRACT

The title of this thesis is “Ranging Patterns and Group Kinship Composition of Wild Malaysian Slow Loris (*Nycticebus coucang coucang*): Using Radio Telemetry and Mitochondrial DNA Analysis.” This study focused on the ranging patterns of five groups of Loris and analyzed their home range size and kinship relatedness. Initially, the triangulation method was used, followed by visual monitoring and radio-telemetry studies whereby 12 individuals were tracked from June 2006 to January 2007.

Results obtained for the mean daily path lengths of resident animals was 1,562.48 m. The three estimators used to analyze home ranges were the MCP 100% estimator, the KDE  $h_{ref}$  (Kernel 95 %) estimators and the KDE LCSV estimator, which produced variable results for all categories of age-groups and contrasting males and females ranging patterns as well as core area sizes. It is concluded that KDE 95% ( $h_{ref}$ ) performed better than MCP which portrayed in almost 80% of the cases biologically unrealistic ranges. The biotopes covered included Belukar (secondary forests), Forest-High Orchards, Rubber Plantation, Scrub Grass Land, Grass, Main/Small Roads, Cemetery, Mosque, Shrubs and Open Areas. The Belukar forest was the most common biotope type, having an average observed use of 47% by all study individuals. Individuals used structural types such as main wires, house electrical wires, trees along ponds and bamboos as their movement corridors. Based on the observations for five groups within the two study areas, the group size was 3-4 individuals, and the population density was 13 individuals /km<sup>2</sup>. *Nycticebus c. coucang* mainly (49%) utilized the lower strata (0-10m) of the forest while fruit feeding occurred at all heights.

Kinship or relatedness was confirmed by mitochondrial DNA D-loop haplotype analyses. A total of 466bp nucleotide sequences in the polymorphic D-loop region of matrilineal inherited mitochondrial DNA were compared in samples from 26 individuals. From the 30 polymorphic sites, 18 haplotypes were detected within 26 individuals. Five cases were presented in order to demonstrate the inheritance of maternal mitochondrial DNA. More interestingly, this method also managed to prove a unique case of infant adoption from another locality by an adult female.

## ABSTRAK

Tajuk tesis ini ialah "Ranging Patterns and Group Kinship Composition of Wild Malaysian Slow Loris (*Nycticebus coucang coucang*): Using Radio Telemetry and Mitochondrial DNA Analysis." Penyelidikan ini memfokus kepada corak pergerakan lima kumpulan kongkang dan perkaitan kerabat (kin). Permulaannya, kaedah triangulasi digunapakai, diikuti dengan pengawasan visual dan kajian radio-telemetri di mana 12 individu dijejak daripada bulan Jun 2006 sehingga bulan Januari 2007.

Keputusan yang didapati dari purata jarak jejak harian haiwan residen adalah 1,562.48 m. Tiga estimator yang diguna untuk analisa julat kawasan kediaman adalah MCP 100% estimator, KDE  $h_{ref}$  (Kernel 95 %) dan KDE LCSV yang menghasilkan keputusan pelbagai dalam kategori kumpulan umur dan ada perbezaan di antara jantan dan betina untuk corak pergerakan dan saiz kawasan utama. Dapat dirumuskan bahawa KDE 95% ( $h_{ref}$ ) adalah perkiraan lebih baik daripada MCP yang mempamerkan sehingga 80% kes, julat yang tidak tepat mengikut pengamatan biologi.

Biotop meliputi Belukar (hutan sekunder), Ladang Buah, Ladang Getah, Kawasan Renek, Rumput, Jalan Utama/Jejak, Tanah Perkuburan, Masjid dan Kawasan Lapang. Jenis biotop Belukar adalah paling digemari (47%). Individu haiwan telah menggunakan jenis infrastruktur seperti dawai utama, tali letrik rumah, pokok dipinggiran kolam dan pokok buluh sebagai koridor gerakalih. Berdasarkan pengamatan keatas lima kumpulan dalam dua tapak kajian, saiz kumpulan adalah 3-4 individu dan kepadatan populasi adalah 13 individu/km<sup>2</sup>. *Nycticebus c. coucang* banyak (49%) menggunakan strata bawah hutan (0-10 m), manakala semua strata dieksploitasi untuk memakan buah.

Perkaitan kerabat telah dibuktikan dengan analisis mitokondria DNA D-loop haplotip. Sejumlah 466bp sekuen nukleotid didalam kawasan polimorfik D-loop dari mitokondria DNA warisan bersaudara dibandingkan untuk sampel dari 26 individu. Dari 30 lokasi polimorfik, 18 haplotip telah dikesan dalam 26 individu. Lima kes dipaparkan bagi menjelaskan warisan bersaudara mitokondria DNA. Lebih menarik lagi, kaedah ini telah dapat membuktikan satu kes unik yang mana betina dewasa memperagakan aksi mengambil anak angkat, yang hidup dilokasi berbeza.

## ACKNOWLEDGEMENT

I have been fascinated with the combination of field work and cutting age technology to study primates, and I thank the Lord for the passion and providence that allowed me to do this work. I would here like to express my appreciation and gratitude to the many individuals and organizations that contributed to undertake my project thesis. First and foremost, I would like to express my gratitude and greatest appreciation to my supervisors Professor Norma-Rashid Yusoff and Professor Richard Francis Dorall for their insights, unwavering support in giving me unlimited intellectual freedom, and a lot of patience. Most of all, I thank Professor Norma for the wonderful opportunity to work on this project while experiencing the culture and gracious people of Peninsular of Malaysia. Furthermore, I thank my supervisor Richard Dorall for many stimulating discussions about this project. They inspired me to think critically, and have been extremely influential in shaping my understanding and appreciation in the field of modeling and mapping software and technology due to his vast experience on these fields. I am deeply grateful to Professor Zulqarnain Mohamed and his students from the Institute of Biological Sciences (Genetics), Faculty of Science, who were very helpful in the technical aspects and for kindly allowing me to undertake several biomolecular techniques in his laboratory which were crucial for the molecular chapter of this thesis. In particular, I would like thank Ms Siti Sarah Daud who acted as my sounding board for most of my outrageous ideas when working in the molecular field. Sarah did a lot to both keep me grounded in reality and encourage novel ideas. *Nycticebus c. coucang* is not easy to work on and it is simply not possible to catch them without assistance. Therefore, I am greatly indebted to the entire community of the Kampong Cempaka and Kampong Bukit Boloh, Temeroh, Pahang, for their broadmindedness and trust in helping me with priceless numerous forms of assistance to further the objectives of an unknown foreigner. Last, but not the least, it is a pleasure to acknowledge the generous financial support of WWF Malaysia and the WildAsia Team (seed grant), The Malaysian Star Newspaper which helped me to raise public awareness about *Nycticecus c. coucang*, the Malaysian Centre for Remote Sensing (MACRES) for facilitating satellite images of the study areas, Malaysian Federal Land Development Authority (FELDA) for facilitating the Digital Lot Map of Temerloh District, Pahang, Malacca Zoo for collaborating with *Nycticebus c. coucang* DNA samples, and for permission given by the Malaysian Department of Wildlife and National Parks (Jabatan Perhilitan) to undertake the field study of Slow Loris. To anyone I have inadvertently omitted, I sincerely apologize.

## TABLE CONTENTS

CHAPTER 1 – Introduction, Literature Review and Thesis Objectives	
<b>1.1 Introduction</b> .....	<b>1</b>
1.1.1 Literature Review.....	1
1.1.2 Natural History of the Family <i>Lorisidae</i> .....	1
1.1.3 Classification, Distribution, Evolution and Systematic .....	1
1.1.4 General Anatomy of the Family <i>Lorisidae</i> .....	5
1.1.5 Genus within the Family <i>Lorisidae</i> .....	6
1.1.5.1 In Africa: “ <i>Arctocebus</i> and <i>Perodicticus</i> ”.....	6
1.1.6 In Asia: “ <i>Loris</i> and <i>Nycticebus</i> ” .....	7
1.1.6.1 Description .....	7
1.1.6.2 Classification and Systematics.....	9
1.1.6.3 Ecology and Behavior of <i>Loris</i> and <i>Nycticebus</i> .....	11
1.1.6.4 Social Grouping .....	15
1.1.6.5 Evolution and Phylogenetics .....	16
1.1.7 <i>Nycticebus c. coucang</i> in Peninsular Malaysia: Literature Review .....	17
1.1.8 Discussion of the Present Status of Research on the Malaysian Slow Loris .....	21
<b>1.2 Review of Methodologies Used to Study the Slow Loris</b> .....	<b>23</b>
1.2.1 (Radio-telemetry) Radio-Tracking Techniques Overview .....	23
<b>1.3 Mapping Techniques</b> .....	<b>29</b>
1.3.1 Biotope Mapping and Cartalinx .....	29
1.3.2 Cartalinx Overview .....	30
1.3.3 Features.....	30
1.3.4 GPS and GIS.....	31
<b>1.4 Molecular Techniques</b> .....	<b>33</b>
1.4.1 Integration of Field Methods and Molecular Biology .....	33
1.4.2 Molecular Markers in Behavioral, Social Structure and Genetic Structure in Non-human Primates .....	35
1.4.3 Mitochondria.....	37
1.4.4 D-Loop - mtDNA Control Region (CR) .....	41
1.4.5 Microsatellites.....	44
1.4.6 How to Obtain a Microsatellite Marker?.....	49
1.4.6.1 Isolation and Development of Microsatellite DNA .....	49
1.4.6.2 The Application of Microsatellites Isolated From Various Species in Order to Amplify the Corresponding Polymorphic Loci in Related Species .....	50
1.4.6.3 Multiplex STR System Information .....	51

1.4.6.4 Allelic Ladder.....	52
1.4.6.5 Cross-species Microsatellites .....	53
<b>1.5 The Polymerase Chain Reaction PCR.....</b>	<b>53</b>
1.5.1 The Principle of PCR.....	53
1.5.2 The Standard Thermal Cycling Parameters for PCR .....	55
1.5.3 The Important Components of PCR.....	56
1.5.4 Analysis of PCR Products: Gel Electrophoresis as a Tool to View DNA Results Obtained From the PCR.....	58
<b>1.6 DNA Sequencing.....</b>	<b>60</b>
1.6.1 Radioactivity .....	62
1.6.2 Fluorescence .....	62
<b>1.7 DNA (Collection, Extraction) .....</b>	<b>64</b>
1.7.1 DNA Collection and Preservation .....	64
1.7.2 DNA Sample Sources .....	65
1.7.3 DNA Extraction Protocols .....	66
1.7.3.1 Organic (Phenol-Chloroform) Extraction .....	66
1.7.3.2 Non-Organic (PROTEINASE K AND SALTING OUT).....	67
1.7.3.3 CHELEX (Ion Exchange Resin) EXTRACTION.....	68
1.7.3.4 FTA™ Paper.....	68
<b>1.8 Thesis Research Objectives .....</b>	<b>70</b>
<b>1.9 Conclusion .....</b>	<b>71</b>

## CHAPTER 2 – Research Materials and Methods

<b>2.1 Introduction .....</b>	<b>73</b>
<b>2.2 Objective 1.....</b>	<b>73</b>
2.2.1 Material and Methods for Biotope Map of the Studies Areas .....	73
<b>2.3 Objective 2. ....</b>	<b>76</b>
2.3.1 Material and Methods for Studying the Ranging Patterns of <i>Nycticebus c. coucang</i> .....	76
2.3.2 Capture, Handling, and Radio Collars .....	76
2.3.3 Finding Animals with Radio Tracking .....	81
2.3.4 Finding Animal before Radio Failure .....	83
2.3.5 Mapping Techniques: Definitions and Analysis .....	84
<b>2.4 Objective 3. ....</b>	<b>90</b>
<b>2.4.1 Material and Methods for Evaluation of the Paternal Genetic Inheritance Using Cross-Species Microsatellites Markers.....</b>	<b>90</b>
<b>2.4.2 Chemicals / Reagents .....</b>	<b>90</b>
2.4.2.1 DNA Extraction .....	90

2.4.2.2 Amplification –Polymerase Chain Reaction .....	90
2.4.2.3 Agarose Gel Electrophoresis .....	91
2.4.2.4 Polyacrylamide Gel Electrophoresis .....	91
2.4.2.5 Silver Staining .....	92
<b>2.4.3 Instruments.....</b>	<b>92</b>
2.4.3.1 DNA Extraction .....	92
2.4.3.2 Microsatellites Amplification –Polymerase Chain Reaction .....	93
2.4.3.3 Agarose Gel Electrophoresis .....	93
2.4.3.4 Polyacrylamide Gel Electrophoresis .....	93
2.4.3.5 Silver Staining .....	94
<b>2.5 Study Subjects .....</b>	<b>94</b>
2.5.1 Sample Collection .....	94
<b>2.6.2 Methods.....</b>	<b>97</b>
<b>2.6.2.1 Extraction of Genomic DNA.....</b>	<b>97</b>
2.6.2.2 Chelex ® Extraction (FTA Cards -Blood DNA Samples) .....	97
2.6.2.3 Measurement of DNA Concentration and Purity .....	97
2.6.2.4 Polymerase Chain Reaction (PCR) Set Up for Microsatellites Genotyping .....	98
2.6.2.5 DNA Amplification for Microsatellites Genotyping .....	101
2.6.2.6 Negative Control .....	102
<b>2.6.3 Agarose Gel Electrophoresis and Photography .....</b>	<b>103</b>
<b>2.6.4 Denaturing Polyacrylamide Gel Electrophoresis- Fragments Resolution .....</b>	<b>103</b>
2.6.4.1 Preparation of Glass Plates .....	104
2.6.4.2 Preparation of the Polyacrylamide Gel .....	104
2.6.4.3 Samples Loading and Electrophoresis Parameters .....	105
<b>2.6.5 Silver Staining Procedure. ....</b>	<b>106</b>
<b>2.6.6 Polyacrylamide Gel Visualization and Data Analysis.....</b>	<b>107</b>
<b>2.7 Materials: Evaluation of the Maternal Genetically Inheritance by Using D-loop Mitochondrial Sequences.....</b>	<b>107</b>
<b>2.7.1 Chemicals / Reagents .....</b>	<b>107</b>
2.7.1.1 DNA Extraction.....	107
2.7.1.2 Amplification –Polymerase Chain Reaction .....	108
2.7.1.3 Agarose Gel Electrophoresis .....	108
2.7.1.4 D-loop Mitochondrial DNA Sequences Amplification –Polymerase Chain Reaction ....	108
2.7.1.5 Polymerase Chain Reaction (PCR) Set Up for D-loop Mitochondrial DNA Sequences .....	109
2.7.1.6 DNA Amplification for the Hypervariable Region of D-loop Mitochondrial DNA .....	109
2.7.1.7 PCR Product Purification .....	111

2.7.1.8 Mitochondrial DNA Sequencing .....	112
2.7.1.9 D-loop Mitochondrial Sequences Alignment and Data Analysis .....	112
2.8 Statistical Analysis for Data Collected for Ranging Patterns.....	114

### **CHAPTER 3 – Study Sites**

<b>3.1 Introduction .....</b>	<b>115</b>
3.2 Study Sites .....	115

### **CHAPTER 4 – Biotope Map of the Study Areas**

<b>4.1 Biotopes of Cempaka and Bukit Boloh Study Areas. ....</b>	<b>125</b>
4.1.1 Biotope Map Statistics of the Areas Bukit Boloh and Cempaka .....	135

### **Chapter 5 – Ranging Patterns, Analysis and Modeling**

<b>5.1 Ranging Patterns of <i>Nycticebus c. coucang</i> .....</b>	<b>139</b>
<b>5.1.1 Introduction .....</b>	<b>139</b>
5.1.2 Causes of Mortality and Interruption .....	140
5.1.3 Home Range Elevation Analysis .....	142
5.1.4 Summary of Spatial Statistics .....	144
5.1.5 Analysis of Activity Patterns .....	145
5.1.6 Analysis of Daily Movement .....	148
5.1.7 Sleeping Associations .....	151
5.1.8 Traveling Corridors Analysis .....	159
<b>5.2 Analysis of Group Home Ranges .....</b>	<b>164</b>
5.2.1 Home Range Estimators: 1) MCP % 2) KDE $h_{ref}$ 95 % Fixed Contours and Core Areas Using 50 % of Fixes.....	166
5.2.2 Home Range by Type, Shape and Distribution .....	175
5.2.3 Home Range Estimators: KDE $h_{ref}$ 50 % Core Areas Contours .....	177
<b>5.3 Analysis of Overlapping Home Ranges.....</b>	<b>179</b>
5.3.1 Spatial Overlap of Individual Home Ranges .....	179
5.3.1.2 Intra-Group Overlap Estimated Using MCP % and KDE 95 % .....	179
5.3.1.3 Inter-Group Overlap Estimated Using MCP %.....	185
<b>5.4 Analysis of Three Home Range Estimators Employed to Estimate <i>Nycticebus c. coucang</i> Home Ranges (MCP %, KDE (<math>h_{ref}</math>) and KDE <math>h_{lcsv}</math> .....</b>	<b>189</b>
<b>5.5. Analysis of Biotope/ Habitat Association .....</b>	<b>194</b>

<b>5.5.1 Habitat Selection Indicating Sex and Age</b> .....	<b>197</b>
5.5.1.1 Biotopes Used by Adult Males .....	197
5.5.1.2 Biotopes Used by Adult Females .....	198
5.5.1.3 Biotopes Used by Sub-adults .....	202
5.5.1.4 Biotopes Used by Infants .....	204
5.6 Analysis of Each Individual .....	204

## **Chapter 6 – Identification of Paternal and Maternal Genetic Inheritance of the *Nycticebus c. coucang***

<b>6.1 Introduction</b> .....	<b>205</b>
6.1.1 Relatedness or Kinship Observed through Radio Telemetry Data Pedigree .....	205
6.1.2 Group A .....	205
6.1.3 Group B .....	207
6.1.4 Group C .....	207
6.1.5 Group D .....	208
6.1.6 Group E .....	209
6.1.7 Zoo Malacca .....	209
<b>6.2 Microsatellite Analysis</b> .....	<b>212</b>
6.2.1 DNA Extraction Yield .....	212
6.2.2 Cross-Amplification .....	212
<b>6.3 D-Loop Mitochondrial DNA (mtDNA) Sequences Analysis</b> .....	<b>214</b>
6.3.1 Finding D-loop Mitochondrial Sequence .....	214
6.3.2 Genetic Variation .....	220
<b>6.3.3 Observation of the Genetically Maternal Inheritance</b> .....	<b>224</b>
6.3.3.1 Presence of Close Kinship: Intra-groups and Inter-groups.....	224
6.3.3.2 Case of Group “D” Cempaka Comprising “Bonita”, “Baby-Born” and Group C “Cop”.....	225
6.3.3.3 Case of Group “A” Bukit Boloh Comprising Male-Boloh”, “Linda”, “Yuri”, and “Adopted”.....	227
6.3.3.4 Case of Group “B” Cempaka Comprising “Aggressive”, “Timida”, “Hermosa”, and “Little” .....	229
6.3.3.5 Case of Group “E” in Cempaka Comprising “Eca”, “Kro”, “IAB” .....	231
6.3.3.6 Case of Zoo Malacca Individuals “2Malacca” and “5Malacca “.....	232

<b>6.4 Pedigrees Demonstrating the Inheritance of Maternal Mitochondrial DNA among Eight Groups of Slow Loris.....</b>	<b>234</b>
6.4.1 Parent and Offspring.....	234
6.4.2 Siblings.....	234
<b>6.5 Phylogenetic Trees.....</b>	<b>237</b>
6.5.1 Maximum Parsimony .....	237
6.5.2 UPGMA (Unweighted Pair Group Method with Arithmetic Mean) Computed with (ML).....	239
6.5.3 NJ Computed with (ML) .....	241
 <b>Chapter 7 – Discussions and Conclusions</b>	
<b>7.1 Introduction .....</b>	<b>243</b>
<b>7.2 Biotope Mapping and Analysis .....</b>	<b>243</b>
<b>7.3 Ranging Patterns .....</b>	<b>244</b>
7.3.1 Forest Vertical Use.....	244
7.3.2 Sleeping Sites.....	244
7.3.3 Density .....	248
7.3.4 Reproduction .....	249
7.3.5 Daily Movement Traveling Crossing-Structures Indicating Spatial Cognition .....	249
7.3.6 Home Range Estimators .....	252
7.3.7 Spatial Overlap of Individual Home Ranges.....	255
<b>7.4 Molecular Investigation .....</b>	<b>256</b>
7.4.1 Limitations of the Microsatellite Marker for Paternity Analysis .....	256
7.4.2 Matrilineal Inheritance by D-loop DNA.....	257
7.4.3 Phylogenetics and Ancestry Inheritance of the Slow Loris from D-Loop Analysis.....	258
7.4.4 Comparison of Kinship Analysis from Field and Genetic Data. ....	259
7.4.5 The Adoption Case Observed In the Wild .....	260
7.4.6 Radio-Telemetry, Genetics, and GIS Technologies .....	262
<b>7.5 Conclusions .....</b>	<b>263</b>
 <b>References.....</b>	 <b>267</b>

## APPENDICES

### Appendix 1

In Chapter 2, Section 2.3.2: *Nycticebus c. coucang* Data Sheet for use in the field .....306

### Appendix 2a

In Chapter 5, Section 5.1.4: Appendix 2a Definitions of the Summary Spatial Statistics Movements Recorded for Each of the *Nycticebus c. coucang* Tracked .....310

### Appendix 2b

In Chapter 5, Section 5.1.7; 5.1.8; 5.2.3; 5.6: Further Ranging Patterns Analysis in illustrations carried out for Individuals (Aggressive, Timida, Hermosa, Little, Linda) .....311

### Appendix 2c

In Chapter 5, Section 5.1.7; 5.1.8; 5.2.3; 5.6: Further Ranging Patterns Analysis in illustrations carried out for Individuals (Adopt, Cop, Gent, Fala, Bonita, Eca, Kro) .....333

### Appendix 3

In Chapter 6, Section 6.3.3.2: Offspring Infant named “Baby-Born” delivered by Bonita, after 3 days of being born B) Later at the age of 6 months photo taken (17-01-07).....362

### Appendix 4 Scientific Meeting Abstract, Award and Related Publication. ....364

**4.1 Jaime C. G.** Badrul Munir Md. Zain, Zainal Zahari Zainuddin Polymerase Chain Reaction (PCR) Optimization for Mitochondria Cyt-b and D-loop Gene Fragment of Malaysian Slow Loris (*Nycticebus c. coucang*).Poster presentation; Expo-science and Innovation University Malaya competition (IPS). 2006 (Bronze Medal Winner) .....364

**4.2 Jaime C. G.**, Norma Yussof, Mohamed Z, et al. Cross-Species Amplification of 12 Primates Microsatellites Markers Previously Developed Using Samples from the Pro-primate Slow Loris (*Nycticebus c. coucang*). Poster Presentation; Expo-science and Innovation University Malaya June- 2007. Kuala Lumpur .....365

**4.3 Jaime C.G** Conservation and Awareness of Slow Loris. Trailing the Slow Loris, The Star newspaper Kuala Lumpur Malaysia (17October 2006).....366

**4.4 Jaime C. G.** Wild Asia Seed Grant Recipients and Awareness, The Star newspaper Kuala Lumpur Malaysia (17 October 2006) .....369

**4.5 Jaime C.G.** Department of Veterinary Services of Perak, Malaysia, Raising Awareness of Slow Loris (Trapping One) ..... 370

**Jaime C. G.** A Manual Review of How to Handle Slow Loris in Captivity [A copy submitted to Malaysian Wildlife Department for their Reference-not Enclosed in this Thesis Owing to the Number of Pages.

## ABBREVIATIONS

Most of the abbreviations used are standard. However, attention is drawn to the following:-

°C	degree Celsius
%	percent
[ $\alpha$ - <sup>33</sup> P]dATP	deoxyadenosine 5'-[ $\alpha$ - <sup>33</sup> P]-triphosphate
[ $\gamma$ - <sup>33</sup> P]ATP	adenosine 5'-[ $\gamma$ - <sup>33</sup> P]-triphosphate
bp	base-pair
cm <sup>2</sup>	centimeter square
cM	centi-Morgan
CsCl	cesium chloride
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
dH <sub>2</sub> O	distilled water
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dsDNA	double-stranded DNA
dTTP	2'-deoxythymidine 5'-triphosphate
EDTA	ethylenediamine-tetraacetic acid
e.g.	for example
EtBr	ethidium bromide
g	gram
i. e.	that is
kbp	kilo-base pair
L	liter (litre)
M	molar
µg	microgram
mg	milligram
MgCl <sub>2</sub>	magnesium chloride
min	minute
µl	microliter
ml	milliliter

µm	micrometer
mm	millimeter
mM	millimolar
N	normal
N/A	not available
ng	nanogram
nm	nanometer
rpm	revolution per minute
ssDNA	single-stranded DNA
SDS	sodium dodecyl sulphate
TBE	Tris-borate-EDTA
TE	Tris-EDTA
UV	ultraviolet
V	volt
V/cm	volt per centimeter
v/v	volume per volume
w/v	weight per volume
GPS	Global Positioning System (GPS)
ARCVIEW	Software for visualizing and analyzing geographic data
GIS	Geographic Information System.
MCP %	Minimum Convex Polygon
KDE	Kernel Density Estimation
<i>h</i>	smoothing parameter
lcsv	least squares cross validation
AMAE	Animal Movement Analyst Extension program
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
ML	Maximum Composite Likelihood
nj	neighbor-joining
PAUP	(Phylogenetic Analysis Using Parsimony)
MEGA	Molecular Evolutionary Genetics Analysis
BLAST	Basic Local Alignment Search Tool
Chelex	Chelating material to purify compounds via ion exchange
GenBank	Genetic Sequence Data Bank
D-loop	Displacement Loop

## LIST OF FIGURES

### Chapter 1

**Figure 1.1** Classification of Family *Lorisidae*.

**Figure 1.2** Family *Lorisidae* are two Subfamilies (*Lorinae* and *Perodicticinae*).

**Figure 1.3** Map showing recent distribution of Common and Pygmy Slow Lorises in Southeast Asia.

**Figure 1.4** Distribution of (*Nycticebus coucang coucang*) in Peninsular Malaysian based on distribution map.

**Figure 1.5** Showing parts of the Cell and Location of the Mitochondria within it.

**Figure 1.6** Showing Mammalian Mitochondrial Genome.

**Figure 1.7** Steps involved in performing Mitochondrial Sequence Comparisons.

**Figure 1.8** Showing Chromosomes Karyotype Analysis.

**Figure 1.9 (A)** Showing the Short Tandem Repeat AATG. **(B)** The Repeat Sequence is aaga – This particular individual has 14 repeats.

**Figure 1.10** Polymorphism in Microsatellites.

**Figure 1.11** Schematic representation of Microsatellite Isolation.

**Figure 1.12** DNA Amplification Process with the Polymerase Chain Reaction.

**Figure 1.13** Thermal Cycling Temperature Profile for PCR.

**Figure 1.14** Diagram and digital image of agarose gel stained with ethidium bromide: The ladder is a commercially obtained DNA size marker, number of samples loaded and band estimating different number of base pair. The position of the wells and direction of DNA migration is noted.

**Figure 1.15** Showing DNA and its Developments.

**Figure 1.16** Broken lines describing the use of DNA-based techniques after the DNA sample has been collected in the field.

**Figure 1.17** Schematic representation of Phenol-Chloroform Extraction.

**Figure 1.18** Schematic representation of Chelex DNA Extraction.

**Figure 1.19** Image shows FTA Cards for DNA Collection and Shipment and Storage.

### Chapter 2

**Figure 2.1A** Satellite SPOT5 Pansharp Supermode PNC 2007 (Lat/Lon, WGS84).

**Figure 2.1B** Digital Lot Map of Temerloh District, Pahang State.

**Figure 2.2** Example of utilization distribution for an individual *Nycticebus* (Adult female Linda) tracked in Bukit Boloh area, generated from a fixed kernel density estimator. Dots in yellow

represent the location collected for this individual during the sampling period. The outermost line represents the 95<sup>th</sup> density isopleth (values that describe the likelihood of encountering this individual in a particular area) and territory boundary. The grey area represents the core area, which resolved at the 50th density isopleth for this particular individual.

**Figure 2.3** Map of Peninsular of Malaysia showing Sampling site States in Color, the number of individuals sampled and the names given to each Slow Loris to facilitate the study.

**Figure 2.4** Map showing Ecological Distribution of the Cross-Species Primates used.

### Chapter 3

**Figure 3.1** Map showing the Study Areas Bukit Boloh and Cempaka, Temerloh District, State of Pahang, Peninsular Malaysia.

**Figure 3.2** Current human settlements with 4 km buffer and 1 km bands inside Krau Wild Reserve Boundary (Perhilitan & Danced 2005) Malay names; Bukit= hill; Kuala= river or stream mouth; Ulu= head waters; Kg “kampung”= Village or settlement; Sungei= river, stream; Orang Asli = aboriginals.

**Figure 3.3 A** Elevation Values obtained from Google Earth for the Area of Cempaka.

**Figure 3.3 B** Elevation Values obtained from Google Earth for the Area of Bukit Boloh.

### Chapter 4

**Figure 4.1** Areas of Bukit Boloh and Cempaka (Map A) at the scale of 1:55870 and Temerloh District (Map B) at the scale of 1:487900.

**Figure 4.2A** Digitized biotope map and legend of Bukit Boloh study area, graphically illustrating in some cases features that are linear and representing areas done through the use of color. The number of sites digitally design and evaluated with field work were 147 with a total area of 201.53 ha and total perimeter of 99320.2 m, at the scale of 1:1555.

**Figure 4.2B** Digitized biotope map of Bukit Boloh highlighting, paths, small roads and main road (black color) within the area.

**Figure 4.2C** Digitized biotope map and legend of Cempaka study area, graphically illustrating in some cases features that are linear and representing areas done through the use of color. The number of sites digitally designed and evaluated with field work were 245 with a total area of 642.94 ha and perimeter of 226453.81 m, at the scale of 1:1555.

**Figure 4.2D** Digitized biotope map of Cempaka highlighting Rubber Plantation, Belukar and Orchards with a range of dark green color (green 64;hue 80;sat 240; lum:30) LIGHT (green 128;hue 80;sat 240;lum 60) Lighter ( green 255;hue 80;sat 240; lum;175).

**Figure 4.3 A** Area (ha) by Class for Bukit Boloh Biotope map.

**Figure 4.3 B** Area (ha) by Class for Cempaka Biotope Map.

**Figure 4.4 A** Perimeter (m) by Class for Bukit Boloh Biotope Map.

**Figure 4.4 B** Perimeter (m) by Class for Cempaka Biotope Map.

**Figure 4.5 A** Percentage Area by Class for Bukit Boloh Biotope Map.

**Figure 4.5 B** Percentage Area by Class for Cempaka Biotope Map.

## Chapter 5

**Figure 5.1** Study areas and MCP % for individual *Nycticebus c. coucang*.

**Figure 5.2** Elevations plotted on Satellite Images of the Study Areas. Yellow color represents elevations where tracked individuals moved. Red color represents land marks around the study areas: **(A)** Bukit Boloh, and **(B)** Cempaka.

**Figure 5.3** Vertical use of the Forest shown by the Distribution of Main Activities (%) across Height Ranges.

**Figure 5.4** Shown here are examples of Individual Nightly Movements of **(A)** One adult male “Aggressive”, **(B)** Adult female “Timida”, **(C)** Adult female “Bonita”, **(D)** Sub-adult female “Hermosa,” and **(E)** the Sub-adult male “Gent”.

**Figure 5.5** Longest Horizontal Movement in One Day recorded by the adult male “Aggressive” , from point A to point B.

**Figure 5.6 A** Individual Sleeping Sites within the Bukit Boloh Area superimposed onto the Biotope-Map of the Area (Right Brace represents defined group). Area enclosed by black line legend in a form of a geometric shape represents a group.

**Figure 5.6 B** Groups Individual Sleeping Sites within the Cempaka Area superimposed onto the Biotope-Map of the area (Right Brace shows defined groups). Areas enclosed by black line legend in a form of a geometric shape represent a group.

**Figure 5.7** Schematic representation of Sleeping Proximity between individuals from the Same Group.

**Table 5.8** Superimposed Corridors Structures (black color) and Biotopes in the Cempaka Area (left) and the Bukit Boloh Area (right).

**Figures 5.9 A and B.** Showing the Structure of Traveling Corridors used in both Study Areas.

**Figure 5.10** Comparison of Traveling Corridors of two females with, and without, neonatal born in the Cempaka study area. A1: Red color represents traveling corridor of the adult female Timida and its actual movement within its home range. A2: Shows superimposed tracking corridor (red) with satellite image of the study area. B1: Black color represents traveling corridors of the adult female Bonita within its home range. B2: Showing superimposed traveling corridor (white) and satellite image.

**Figure 5.11** (A) Superimposed biotopes and Traveling Corridors (light grey) of an individual adult male "Aggressive" and satellite image. (B) Showing Traveling Corridors (dark grey) and Corridors Structures of adult male "Aggressive", light green color represents sleeping sites.

**Figure 5.12** Example of locations collected for the individual adult male AG (Aggressive) during the sampling period. Note that the locations occur over a consistent area and showed obvious overlap during the study period, suggesting fidelity to this area during the sampling period.

**Figure 5.13 A.** Spatial Distribution and Distance among Group A (Bukit Boloh) and Cempaka Groups (B, C, D and E) using Minimum Convex Polygons of individuals. Dash lines describe adult Male-Boloh, adult Female Yuri and sub-adult male IAB captured but not tracked whereas individual, GIA, ROU was never captured, but several locations were obtained when tracking those closer individuals within the group, hence, it is proposed as a home range for those individuals.

**Figure 5.13 B.** Representing the Home Ranges of those individuals tracked superimposed with the satellite image obtained from MACRES. The distance between the study areas is 1320 meters.

**Figure 5.14** Mean (Minimum, Maximum) Size of *Nycticebus c. coucang* Adult, Subadult and Infant individual Home Ranges for 5 different Spatial Groups, (A) Minimum Convex Polygon MCP % (B) 95 % Kernel Density Estimation (KDE  $h_{ref}$ ).

**Figure 5.15** Showing Home Range Analysis results obtained in (ha) by using two analysis techniques, MCP % and Kernel Density Estimation (KDE  $h_{ref}$ ).

**Figure 5.16** Correlation Plots between Analysis Techniques MCP % and Kernel Density Estimation (KDE  $h_{ref}$ ).

**Figure 5.17** Home Range color illustrations for ADULT M/F, SUB-ADULTS, and INFANTS tracked using Kernel Density Estimation (KDE  $h_{ref}$ ) Home range with smoothing factor (HDE  $h_{ref}$ ). Concentric contours indicate intensity of use, ranging from 95% (most external contours) to 50 % (most internal contour line). Complementary information in Table 5.9.

**Figure 5.18** Graph representing the average home range in adults combining by groups Males, Females, Sub-adults and infants with the method of MCP % and KDE 95 %.

**Figure 5.19** Home Ranges of (A) MALES and (B) FEMALE (C) SUB-ADULTS and (D) INFANTS. The continuous black line denotes home range estimation calculated using Fixed Kernel method 95% and 50 % (grey color). Areas bordered by dot lines represent Minimum Convex Polygon method 100%.

**Figure 5.20** The Core Areas (ha) of the Individuals Tracked.

**Figure 5.21** Represents the Spatial Home Ranges Overlap of 12 individuals tracked using two estimators MCP % and KDE (ref) 95 %.

**Figure 5.22 A** Represents Intra-group overlap using MCP % for groups A, B, C, D and E, superimposed with its biotope. The square shaped dotted line shows possible home range for those animals seen but not caught.

**Figure 5.22 B** Showing 6 types (A, B, C, D, E, F) of Inter-group overlap using MCP % superimposed with its biotope. The square shaped dotted line shows possible home range for those animals seen but not caught.

**Figure 5.23 A** Shows Intra-group overlap using Kernel Density Estimation KDE ( $h_{ref}$ ) 95 % and 50 % for groups A, B, C, D and E, superimposed with its biotope.

**Figure 5.23 B** Showing 4 types (A, B, C, D) of Inter-group overlap using KDE ( $h_{ref}$ ) 95 % and 50 % superimposed with its biotope.

**Figure 5.24** Territory boundaries for two individual males generated by the KDE 95 % ( $h_{ref}$ ) and the MCP red outline. A. Areas enclosed by the MCP % do not overlap with the kernel boundaries but the area obtained by the estimator KDE 95 % produced biologically unrealistic range contours in this case. B. Areas enclosed by the minimum convex polygon that do not overlap with the kernel boundaries for this individual adult male ECA were in Orchards, and Fresh Water, and certainly the individual was never recorded there.

**Figure 25 A.** Individuals Number of Fixes vs. Home Range Estimator Comparison (ha).

**Figure 25 B.** Number of Fixes for Individuals vs. Core area (ha) using Different Estimators.

**Figure 5.26** Examples of comparisons of two Individuals (Aggressive & Linda) home range values in (ha) using three Home-range estimators (MPC % ; KDE ( $h_{ref}$ ) ; KDE ( $h_{csv}$ ), and different smoothing factors.

**Figure 5.27A** Biotopes visited by the 12 individuals in the Cempaka Area, superimposed with the Home Range estimator Minimum Convex Polygon (MCP %). The thick black color line legend represents individuals tracked.

**Figure 5.27B** Biotopes visited by the two individuals *Nycticebus c. coucang* in the Bukit Boloh area, superimposed with Home Range estimator Minimum Convex Polygon MCP %. The thick black color line legend represents individual tracked.

**Figure 5.28** Percentage GPS readings / observed locations and use of habitat types by *Nycticebus c. coucang* in Bukit Boloh and Cempaka area.

**Figure 5.29** Represents Habitat Usage compared with Individual Locations.

**Figure 5.30 A.** Percentage of Biotope/Habitat used by each adult male *Nycticebus c. coucang* tracked in Cempaka area.

**Figure 5.30 B.** Biotopes visited by adult males in Cempaka Area, superimposed with Home range estimator Minimum Convex Polygon (MCP %). The thick black color line legend represents individual tracked.

**Figure 5.31 A.** Percentage of Biotope/Habitat used by each adult female *Nycticebus c. coucang* tracked in the Bukit Boloh and Cempaka areas.

**Figure 5.31 B.** Biotope visited by adult females in Bukit Boloh and Cempaka Areas, superimposed with home range estimator Minimum Convex Polygon (MCP %). The thick black color line legend represents individual tracked.

**Figure 5.32 A.** Percentage of Biotope/Habitat used by each sub-adult tracked in Cempaka area, six biotopes were used by sub-adult female “Hermosa,” and four biotopes by sub-adult male “Gent”.

**Figure 5.32 B.** Biotope visited by Sub-adult female and Sub-adult male Cempaka Area.

**Figure 5.33 A.** Biotope visited by Infant Adopt in Bukit Boloh and Infant Little in Cempaka Area.

**Figure 5.33 B.** Percentage of biotope/habitats used by each infant tracked in Bukit Boloh and Cempaka area.

## Chapter 6

**Figure 6.1** Matrilineal Pedigree of *Nycticebus c. coucang* based on Radio-Telemetry data observations. Open square- male; filled circle-female with progeny; slashed circle-female without progeny; red square-individual (male) was not caught but locations trapped was recorded; blue square-individual caught but was not tagged. (X) The X in the group “A”, “D” and Malacca Zoo represents that there was no seen another offspring with these putative parents.

**Figure 6.2** The Electrophoresis Pattern of PCR products using Microsatellites Markers for the Amplification of DNA Lanes from 1, 2, 4,5,8,9 and 12 mtDNA; NC: Template Control Lane 3, 6, and the Ladder Lanes 7, 10 and 11.

**Figure 6.3** Sample of the Electrophoresis Pattern of mtDNA PCR Products using Primers (L15996) (H16498) as Template.

**Figure 6.4** Shows the size of PCR product (in base pairs) for 12 *Nycticebus c. coucang* individuals analyzed using DNA 1000 Labchip, Agilent Bioanalyzer.

**Figure 6.5** Complete Sequence Alignment 391bp of D-loop mtDNA gene, employing: Complete *Nycticebus* Mitochondrial Genome “Genbank Accession Number AJ309867”, “AY875955 *Nycticebus c. coucang* D-loop” and *Nycticebus c. coucang* Malaysian samples obtained in different localities in Peninsular Malaysia. Note: The two vertical black squares show where the first hypervariable region of the D-loop starts (nucleotide-15485) and where it ends (nucleotide-15876).

**Figure 6.6** The Matrilineal Sequence of 3 individuals in Cempaka Group C and D.

**Figure 6.7** The Matrilineal Sequence of 2 individuals in Bukit Boloh Group A.

**Figure 6.8** The Matrilineal Sequence of 2 individuals in Bukit Boloh Group A.

**Figure 6.9** H–Strand DNA Sequence Plot in the first Hypervariable D-loop of Mitochondrial DNA with 5 different base variations as compared to the *Nycticebus c. coucang* Mitochondrial genome (GenBank accession No. AJ309867). This fragment corresponds to position 15520 to 15688 according to access number mentioned above. (a) Sequence plot for male adult father aggressive; (b) Sequence plot for the adult mother Timida; (c) sequence plot for the sub-adult

female Hermosa; **(d)** Sequence plot for the infant male “Little”. Different Nucleotide A,G,C or T and degree nucleotide confidence are represented by color. The horizontal arrow represents the direction of the strand.

**Figure 6.10** The Matrilineal Sequence of 3 individuals in Cempaka Group B.

**Figure 6.11** The Matrilineal Sequence of 3 individuals in Cempaka Group E.

**Figure 6.12** Matrilineal Sequence of 2 individuals in the Zoo Malacca.

**Figure 6.13** Pedigrees for Group A – Group E were drawn for the same selected animals as in Figure 6.1 based on haplotype uniformity within the mtDNA D-Loop region. Open square - male; filled circle - female with progeny; slashed circle - female without progeny; red square - individual (male) was not caught but locations trapped was recorded; blue square - individual caught but was not tracked. (X) The X in group A, D and Malacca Zoo represents that there was no seen another offspring with these putative parents.

**Figure 6.14** Unrooted Maximum Parsimony tree with bootstrap values, showing relationships among 27 partial mtDNA D-loop sequences from *Nycticebus c. coucang* within several localities of Peninsular Malaysia: **(1)** Bukit Boloh (Group A); **(2)** Cempaka (Group B); **(3)** Cempaka (Group C); **(4)** Cempaka (Group D); **(5)** Cempaka (Group E); **(6)** Kampung Pasu (Pahang); **(7)** Zoo Malacca; **(8)** Seremban Perhilitan Sanctuary, and *Nycticebus c. coucang* haplotype (GenBank No. AY875955).

**Figure 6.15** Rooted UPGMA (ML) Phylogenetic Tree showing Distance Relationships between 27 partial mtDNA D-loop sequences from *Nycticebus c. coucang* from several localities in Peninsular Malaysia, and an outgroup (*Nycticebus c. coucang* GenBank accession No. AY875955).

**Figure 6.16** Rooted NJ Tree showing Evolutionary Distances among 27 partial mtDNA D-loop sequences from *Nycticebus c. coucang* from several localities in Peninsular Malaysia, and an outgroup (*Nycticebus c. coucang* GenBank accession No. AY875955).

## LIST OF TABLES

### Chapter 1

**Table 1.1** Adapted from (Merrill, 2002); Collar weight varies by Species and Collar Manufacturer; Depending on Frequency of Data Downloading.

**Table 1.2** Appropriate molecular techniques for studies involving relationships (maternal and paternity) in primates.

**Table 1.3** Library cost, time investment, and yield compared among the different protocols of microsatellite isolation.

**Table 1.4** Typical Components for PCR Amplification.

### Chapter 2

**Table 2.1** Materials and Software used to undertake Objective 1.

**Table 2.2** Technology and Items used for Radio-Tracking.

**Table 2.3** Radio-Transmitter Unit used with its Programmed Channel and Frequencies.

**Table 2.4** Radio-Tracking Data for 12 *Nycticebus c. coucang* in two Localities in Pahang, Temerloh District within the Buffer of the KWR (Krau Wildlife Reserve).

**Table 2.5** Materials for DNA extraction.

**Table 2.6** Materials for Cross-Species Microsatellites Amplification.

**Table 2.7** Materials for Agarose Gel Electrophoresis.

**Table 2.8** Materials for Polyacrylamide Gel Electrophoresis.

**Table 2.9** Materials for Silver Staining.

**Table 2.10** Instruments for DNA Extraction.

**Table 2.11** Instruments for Cross – Amplification.

**Table 2.12** Instruments for Agarose Gel Electrophoresis.

**Table 2.13** Instruments for Polyacrylamide Gel Electrophoresis.

**Table 2.14** Instruments for Silver Staining.

**Table 2.15** Collection Sites and Sample Sizes of *Nycticebus c. coucang* by Locality.

**Table 2.16** Sequences and Amplicon Sizes for those Isolated Microsatellites.

**Table 2.17A** Parameters of PCR conditions (1) (33 cycles) for amplifying Cross-Species Microsatellite Loci species the Woolly Monkey (311 and 1118).

**Table 2.17B** Parameters of PCR conditions (2) (33 cycles) for amplifying Cross-Species Microsatellite Loci species *Cheirogalous medius* and *Microcebus murinus* (3328 and Mm22) respectively.

**Table 2.18** PCR Master Mix Preparation for Microsatellite Genotyping.

**Table 2.19** Preparation of the 4% Polyacrylamide Gel.

**Table 2.20** Silver Stain Protocol.

**Table 2.21** Materials for D-loop Mitochondrial DNA Sequences.

**Table 2.22** Instruments for D-loop Mitochondrial Amplification.

**Table 2.23** Primer Sequences for the mtDNA Amplification, Hypervariable Region of the D-loop.

**Table 2.24** PCR Master Mix Preparation for D-loop Mitochondrial Sequences Amplification.

**Table 2.25** Parameters of PCR Conditions (35 cycles) for Amplifying HV-1 Region of D-Loop Mitochondrial.

### Chapter 3

**Table 3.1** Types of Trees Surveyed within the Study Areas (Scientific and Local Malay Names Equivalent).

### Chapter 4

**Table 4.1A** Characteristic Plant Species Groups for Bukit Boloh Biotopes.

**Table 4.1B** Characteristic Plant Species Groups for Cempaka Biotopes.

**Table 4.2A** Biotopes Types in Cempaka.

**Table 4.2B** Biotopes Types in Bukit Boloh.

**Table 4.3** Statistical Summary of the Total Area and Perimeter for the Study Areas.

### Chapter 5

**Table 5.1** Number of GPS Readings (Fixes) recorded and the Monitoring Period for twelve radio-tracked *Nycticebus c. coucang* in Bukit Boloh and Cempaka, Pahang, Malaysia.

**Table 5.2** Elevations (in meters above mean sea level) detected for those individuals tracked in Chempaka and Bukit Boloh.

**Table 5.3** Spatial Statistics.

**Table 5.4** Feeding Trees from where *Nycticebus c. coucang* consumed Fruits, Flowers or Young Tree Leaves.

**Table 5.5** Summary and Average Daily Distances Traveled/Night of 12 radio tracked *Nycticebus c. coucang* in Bukit Boloh and Cempaka.

**Table 5.6** Averaged Leaving and Entering Nest Times during the Study Period.

**Table 5.7** Sleeping Trees and their Malay Names for the Focal Groups in the Cempaka and Bukit Boloh Study Areas.

**Table 5.8** Distances between Sleeping Trees on Consecutive Nights.

**Table 5.9** *Nycticebus coucang coucang* Home Range Estimates calculated for Minimum Convex Polygons (MCP), Kernel Density Estimation probability from 95 % to 50 % ( $KDE_{ref}$ ) determined by radio-telemetry locations.

**Table 5.10** Number of Core Areas visited by *Nycticebus c. coucang* and Biotopes located within these.

**Table 5.11 A** Showing Home Range Overlapping using MCP % of Groups Tracked in Bukit Boloh and Cempaka.

**Table 5.11 B** Showing Home Range Overlapping using KDE 95% of Groups Tracked in Bukit Boloh and Cempaka.

**Table 5.12** Kernel Density Estimation (KDE) 95 % and 50 % with smoothing factor KDE ( $h_{ref}$ ), Least Squares Cross-Validation KDE( $h_{scv}$ ) and Minimum Convex Polygon (MCP %) for the *Nycticebus c. coucang* individuals tracked.

## Chapter 6

**Table 6.1** Parameters used from Data Collected in the Field to Infer Relatedness.

**Table 6.2 A** Comparison of Two Methods used for DNA extraction from Blood on FTA Paper.

**Table 6.3** Four Cross-Species Amplification of Microsatellites Markers used for *Nycticebus c. coucang* DNA (namely, “33228”, “Mm22”, “311”, “1118”) that were originally identified to amplify continent primate DNA.

**Table 6.4** Polymorphic Sites within the *Nycticebus c. coucang* mtDNA D-loop Sequence (Nucleotides 15 485- 15 876), according to GenBank accession number AY875955).

**Table 6.5** Computational Analyses carried out in 26 mtDNA sequences (Haplotypic Data).

**Table 6.6** Inter-haplotypic Pairwise Distance Matrix between each haplotype of *Nycticebus c. coucang* used in this study (S.D. above the diagonal).

**Table 6.7** Haplotypes, Frequencies and Nucleotide Composition of each Individuals coming from Different Locations.

## LIST OF PICTURES

### Chapter 1

**Photo 1.1** Showing *Nycticebus coucang coucang*.

### Chapter 2

**Photo 2.1 A) & B)** showing one *Nycticebus c. coucang* golden-yellow eye reflection detected by the use of torching white light. **C)** Expert tree climbers from the local community. **D)** Adult Female Bonita with her born infant.

**Photo 2.2** Showing an Individual Tagged for Tracking Purposes.

**Photo 2.3A** TR-4 Telonics Receiver.

**Photo 2.3B** RA-2AK"H" Antenna Yagi.

### Chapter 3

**Photo 3.1** These pictures show typical landscape and vegetation within the study areas, **A.** Kampung (Village) small road surrounded by orchards and patches of Belukar; **B.** Active Rubber Plantation; **C.** Fresh water surrounded by Belukar Forest and Rubber Plantations, **D.** Orchards ; **E.** Secondary Forest; **F.** Fresh Water Swamp, surrounded by Belukar ; **G.** Abandoned Rubber Plantation mixed with Belukar; **H.** High Secondary Forest; **I.** Banana Plantation surrounded by Belukar and Orchards; **J.** Abandoned Rubber Plantation covered by Rattan Palm.

### Chapter 5

**Photo 5.1** Minutes after a Rain Event "Hermosa" is getting Warmth and Drying out while sleeping underneath a Street Light. This shows adaptation to the physical surroundings.

**Photo 5.2** Showing adult "Timida" sleeping near the orchards covered by foliage shrubs.

**Photo 5.3** Use of the Electrical Wires as Traveling Corridors. **A.** Individual about to reach the electrical wire while still on the tree. **B, C, E** Individual Aggressive, moving along the electrical wire; sometimes this was done at low speed but sometimes at high speed. **D.** "Hermosa" and "Timida" walking along the electrical wire.

## Chapter 6

**Photos 6.1 (A)** Temporary captivity place where “Bonita” and the baby were brought for few days to being observed before days later being release in the exact place where “Bonita” was trapped; **(B)** Sample comparison after hours later the baby was born, using soft cotton in order not to harm the fragile neonatal; **(C)** Comparison individual hand size, **(D)** and **(E)** Showing the size and indication of the cordon umbilical; **(F)** “Bonita” and “Baby-born” resting after few days before being released in the wild. Refer to Appendix 6.1 to see the baby after 6 months old.

**Photos 6.2:** Photos **(A)** and **(B)** showing Infant “Adopted” (Adopt) by the female “Linda” Color Comparison and Size. **(C)** and **(D)**: “Adopt” infant being held by “Linda” before being released in the wild.

**Photos 6.3 (A)** and **(B)** Infant female (“5Malacca”); **(C)** Mother (“2Malacca”) and its baby (“5 Malacca”) mother protecting the baby before blood sample being taken for genetics evaluation; **(D)** Infant looking for mother’s protection in the cage.