

BANDED LEAF MONKEYS IN SINGAPORE:  
PRELIMINARY DATA ON TAXONOMY, FEEDING ECOLOGY,  
REPRODUCTION, AND POPULATION SIZE

ANG HUI FANG ANDIE

NATIONAL UNIVERSITY OF SINGAPORE

2010

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*(B.Sc. (Hons.), NUS)*

**A THESIS SUBMITTED  
FOR THE DEGREE OF MASTER OF SCIENCE  
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## TABLE OF CONTENTS

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Acknowledgements	i
Table of Contents	iii
Summary	vi
List of Tables	ix
List of Figures	x
List of Publications	xi

### **Chapter 1: Population estimate and diet of the banded leaf monkey in Singapore**

<i>1.1 Introduction</i>	2
1.1.1 Feeding ecology	4
1.1.2 Vocalization	6
<i>1.2 Materials and Methods</i>	7
1.2.1 Study site	7
1.2.2 Data collection and analysis	8
1.2.3. Feeding observations and plant phenology	11
1.2.4. Habitat sampling	12
<i>1.3 Results</i>	14
1.3.1 Population estimate	14
1.3.2 Dietary profile	20
1.3.3 Habitat	23
1.3.4 Vocalization	26

<i>1.4 Discussion</i>	29
1.4.1 Banded leaf monkey populations in Singapore	29
1.4.2 Plant food selection and availability	31
1.4.3 Vocalization	38
<i>1.5 Conclusion</i>	40

**Chapter 2: Reproduction and infant pelage coloration of the banded leaf monkey in Singapore (Raffles Bulletin of Zoology 58, 237-241)**

<i>2.1 Introduction</i>	41
<i>2.2 Materials and Methods</i>	44
<i>2.3 Results</i>	44
<i>2.4 Discussion</i>	50
<i>2.5 Conclusion</i>	52

**Chapter 3: Mitochondrial sequences and the taxonomy of Singapore's banded leaf monkey**

<i>3.1 Introduction</i>	55
3.1.1 Asian colobines and genus <i>Presbytis</i>	55
3.1.2 Taxonomy of banded leaf monkey	56
3.1.3 Conservation of banded leaf monkey in Singapore	59
3.1.4 Genetic markers	59
<i>3.2 Materials and Methods</i>	61
3.2.1 Fecal collection and DNA extraction	61

3.2.2 DNA amplification and sequencing	61
3.2.3 Primer design	64
3.2.4 Sequence alignment and phylogenetic analysis	64
<i>3.3 Results</i>	65
3.3.1 Fecal samples	65
3.3.2 Molecular data	66
<i>3.4 Discussion</i>	69
3.4.1 Conservation of banded leaf monkey in Singapore	69
3.4.2 Asian colobines	73
3.4.3 Shortcomings of this study and suggested improvements	75
<i>3.5 Conclusion</i>	77
<b>References</b>	78
<b>Appendices</b>	91

## SUMMARY

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The banded leaf monkey (*Presbytis femoralis femoralis*) is critically endangered in Singapore. This is mainly due to the small estimated population size of approximately 15-20 in the 1990s with little else being known about the ecology of this species. In my first chapter, I provide new information on population size and feeding ecology based on 22 months of field observations. I estimate that there are at least 40 banded leaf monkeys in six social groups, comprising of 2-14 individuals per group. I also use feeding observations for preparing a list of plant species and plant parts consumed, and examine the phenology of food species. My observations are compared to the results of a preliminary vegetation sampling that was carried out in eight 75m x 20m plots within the Central Catchment Nature Reserve. I identify 23 plant species that are consumed by banded leaf monkeys, of which more than half are locally threatened. The diet consists mainly of fruits, a preference uncommon among leaf-eating Asian colobines. Food plant species are not consumed in accordance with their abundance; instead, the banded leaf monkeys prefer uncommon species. Fourteen species of climbers previously presumed to be nationally extinct were rediscovered, demonstrating the need to regularly survey the habitat in order to monitor the availability of food resources.

Prior to this study it was uncertain whether the species is reproducing in Singapore and there were conflicting reports about infant coloration, casting doubts on whether the subspecies in Singapore is different from that in Johor, Malaysia. In my second chapter, which has been published in Raffles Bulletin of Zoology, I present the



first report on reproduction, infant pelage coloration and development of the banded leaf monkeys in Singapore. I report at least six births from 2008 to 2010, and present evidence that there is at least one birth season (June-July) observed for each of three consecutive years. Moreover infants have survived beyond seven months, indicating low infant mortality. The infants are born white, with a distinctive cruciform black pattern on the dorsum. This natal pelage pattern is consistent with that of the infants in Johor, suggesting no differentiation between the two populations based on infant coloration.

At least three subspecies of banded leaf monkeys are currently recognized: *P. f. femoralis* occurs in Singapore and Johor, *P. f. robinsoni* in the Northern Malay Peninsula and *P. f. percura* in Eastern Sumatra. The taxonomic status of *P. f. femoralis* in Singapore and Johor is in dispute based on alleged difference in adult pelage coloration. In the third chapter, I describe the genetic analyses carried out in order to contribute to the taxonomic status of the Singapore population. I extracted genomic DNA from five fecal samples that were collected from the wild and amplified three genes. I compare the 12S rRNA (346 bp) and cytochrome *b* (453 bp) with reference sequences from banded leaf monkeys in Kluang, Kota Tinggi, Mersing and Pontian in Johor. The 12S rRNA sequences are identical and *cyt-b* near identical (<0.51%) for the Singapore and Johor populations, which most likely belong to the same subspecies, while the Northern Malay Peninsula population probably belongs to a different species (*cyt-b*: 9.8%). An examination of the genetic distance between *P. f. femoralis* and the other subspecies, *P. f. percura*, will be necessary to reevaluate the taxonomy of the species and the IUCN conservation status of banded leaf monkeys.

My study provides preliminary information on the population size, reproduction, feeding ecology, and taxonomy of the banded leaf monkeys in Singapore. Further research is necessary to complement these data in order to assist in the conservation of this native species of Singapore.

## LIST OF TABLES

---

<b>Table</b>	<b>Description</b>	<b>Page</b>
1.1	Comparison of diet in percentages in Asian colobines	5
1.2	Age categories of the banded leaf monkeys	9
1.3	Number of groups and group size of banded leaf monkeys in the CCNR	14
1.4	Number of sighting days, and details of observations	16
1.5	Identified plant species consumed by banded leaf monkey in Singapore	21
1.6	Conservation status of the food plant species	22
1.7	The 10 most common tree species based on basal area	25
1.8	The 10 most common climber species based on number of individuals	26
1.9	Number of tree and climber species in each sampling plot	26
3.1	Primer sequences utilized in analysis of DNA from banded leaf monkey feces	63
3.2	Fecal sample collections	65
3.3	Pairwise distances between Singapore population and other <i>Presbytis</i> species for cytochrome <i>b</i> sequences	67

## LIST OF FIGURES

Figure	Description	Page
1.1	Adult pelage of banded leaf monkey	2
1.2	Central Catchment Nature Reserve and surrounding reservoirs	8
1.3	A sample power spectrum of the loud call of banded leaf monkey showing the dominant frequency of 2112.89 Hz	11
1.4	A map of nine forest plots in the CCNR (Ismail, 2010). Plot 4 was only partially sampled and was thus removed from analysis. The line in light blue demarcates the approximate area focused under the study	13
1.5	Number of food species with the availability of preferred plant parts of banded leaf monkeys. YL: young leaves; FL: flowers; FR: fruit	23
1.6a	The most common tree families at the CCNR based on basal area	24
1.6b	The most common tree families at the CCNR based on number of stems	24
1.7	The most common climber families at the CCNR based on number of individuals	25
1.8	Spectrogram of a loud call of banded leaf monkey	27
1.9a	Timing (x-axis) and number of nocturnal loud calls (y-axis) on 2 February 2010. The group at Nee Soon began the calling round first at all times, immediately followed by calls from Seletar group	28
1.9b	Timing (x-axis) and number of nocturnal loud calls (y-axis) on 2 March 2010. Nee Soon group initiated the calling rounds except the second calling round at 04:52am	29
2.1	Distribution of <i>Presbytis femoralis</i> . <i>Presbytis f. robinsoni</i> is found in north Malay Peninsula, <i>P. f. percura</i> in east-central Sumatra, and <i>P. f. femoralis</i> in Johor and Singapore	42
2.2	Natal pelage pattern of an infant banded leaf monkey from Pantii Forest Reserve (Johor). Photo: D. L. Yong	46
2.3	Infant banded leaf monkey (red arrow) from Singapore	47
2.4	(A) Infant banded leaf monkey from Singapore (red arrow) separated from carrying adult (pink arrow); (B) Banded leaf monkey infant being carried by an adult	48
2.5	Black infant banded leaf monkey feeding on vegetation	49
3.1	Maximum parsimony heuristic bootstrap mtDNA tree for <i>Presbytis</i> and <i>Trachypithecus</i> species (Figure 4 in Md-Zain et al., 2008). The bootstrap support values are shown below the branches of the tree	57
3.2	Consensus tree of 25 taxa (93 sequences) of colobines using maximum parsimony. Bootstrap support values are shown on the branches	68
3.3	Map of Singapore with reference to four localities in Johor where genetic data of banded leaf monkeys are available. Percentages in parentheses indicate <i>cyt-b</i> pairwise distances as compared to Singapore population	70

## LIST OF PUBLICATIONS

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1. **Ang, A.**, Ismail, M.R.B., Meier, R., 2010. Reproduction and infant pelage colouration of the banded leaf monkey (Mammalia: Primates: Cercopithecidae) in Singapore. *The Raffles Bulletin of Zoology* 58, 411-415.

## **CHAPTER 1**

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# **POPULATION ESTIMATE AND DIET OF THE BANDED LEAF MONKEY IN SINGAPORE**

## 1.1 INTRODUCTION

The banded leaf monkey (*Presbytis femoralis femoralis*) is the largest extant non-human primate in Singapore. This native species is one of the key representatives of Singapore's national and natural heritage and hence has a high priority in conservation. The banded leaf monkey is a member of the family Cercopithecidae (Old World monkeys); is small-bodied (6.0-6.5 kg) with no sexual dimorphism and covered in black fur with a white zone ventrally and on the inside of the thighs (Fig. 1.1).



Figure 1.1: Adult pelage of banded leaf monkey

The banded leaf monkey was first described from Singapore in 1838, making Singapore the type locality of the species. Widespread on the island in the last century (Chasen, 1924), they were still reported to be common in various areas including Bukit Timah and Changi (Chasen, 1940) up till the 1920s. Unfortunately, deforestation for urban

development continued to reduce their habitat, confining them to the remaining spaces in the Bukit Timah Nature Reserve (BTNR) and the Central Catchment Nature Reserve (CCNR). On October 1987, an elderly female believed to be the last banded leaf monkey in the BTNR was killed by dogs as she descended from a tree (Yang and Lua, 1988). With the subsequent construction of the Bukit Timah Expressway right across the two reserves, habitat availability for the banded leaf monkeys further decreased as the two green lungs of Singapore are now disconnected, leaving the CCNR as the last refuge for the banded leaf monkeys.

The ecology of colobine monkeys (subfamily Colobinae: e.g. langurs and odd-nosed leaf monkeys) including the banded leaf monkeys have received less attention than most other primates (Davies and Oates, 1994). This is partly due to their shy and indolent dispositions (Fashing, 2006), traits that make them particularly challenging study subjects: habituation can take years or even decades (Doran-Sheehy et al., 2007). In addition to the elusive nature of the banded leaf monkeys in Singapore, they are also mainly found in secondary and freshwater swamp forests in the CCNR which consists of scattered patches of extensive rattans and pandans which are difficult and dangerous for observers to access. These conditions make following the highly mobile, arboreal banded leaf monkeys challenging (Hüttche, 1994). Consequently there were very few studies on the banded leaf monkeys in Singapore: Chasen (1940) examined museum specimens and documented its morphology while Yang et al. (1992) provided brief information on their distribution. Between 1982 and 1987, ecological research at Nee Soon swamp forest within the CCNR by Lucas et al. (1988) yielded one sighting, leading to their conclusion



that it was unlikely that there were more than one or two troops in the swamp forest. The only study that focused solely on the banded leaf monkeys was carried out by Hüttche (1994). Based on six months of field research, Hüttche (1994) recorded 13 sighting days (excluding those instances where only loud calls were registered) and presented preliminary data on the distribution of the banded leaf monkey; he also compared their morphology with conspecifics in Malaysia and found no significant differences in adult pelage coloration. Since then, additional information only came from sporadic nature surveys carried out by wildlife groups, which provided locality information. Based on this information, the population was estimated to be 10 individuals in the 1980s and approximately 20 in the 1990s (Corlett and Lucas, 1995; Teo and Rajathurai, 1999). Ng and Lim (1992) concluded that the banded leaf monkeys' population size was not self-sustaining and other studies also commented on the dire situation faced by banded leaf monkeys and highlighted the urgency for study. Here, I report the results of field observations addressing population size, troop numbers, and feeding ecology.

### **1.1.1 Feeding ecology**

Banded leaf monkeys possess the fore-stomach fermentation digestive system characteristic of all colobine monkeys. Unlike the cercopithecines (subfamily Cercopithecinae: e.g. baboons, macaques, and guenons), colobines have large, multi-chambered stomachs that contain a vast and diverse array of microflora needed to process and ferment plant material (Bauchop and Martucci, 1968; Strasser and Delson, 1987). Such ruminant-like stomachs confer advantages for the monkeys: the bacteria can assist in the breakdown of plant cell walls, thereby releasing cell contents for the host to digest

and absorb (Kay et al., 1976); the bacteria may also detoxify alkaloid defense chemicals in plant tissues, allowing colobines to eat plants containing toxins which would otherwise be harmful (Oates et al., 1977). Therefore leaf monkeys have some generalized pattern of leaf preference in their diet (Kirkpatrick, 1999; see Table 1.1).

Table 1.1: Comparison of diet in percentages in Asian colobines

Species	Leaf	Fruit	Seed	Flower	Others	Sources
<i>Nasalis larvatus</i>	41	41	15	3	-	Bennett and Sebastian, 1988
<i>Presbytis potenziani</i>	55	32 (fruit and seed)		13 (flower, bark, sap)		Fuentes, 1996
<i>Pygathrix nigripes</i>	39.9	11.4	39.7	8.8	0.2	Rawson, 2006
<i>P. nemaesus</i>	82	14 (fruit and seed)		4	-	Lippold, 1998
<i>P. nemaesus</i>	62	13	-	25	-	Otto, 2005
<i>Rhinopithecus brelichi</i>	74	13	-	12.4	-	Bleisch et al. 1993
<i>Semnopithecus entellus</i>	49.1	24.4 (fruit and seed)		9.5	17	Newton, 1992
<i>Trachypithecus delacouri</i>	78	9	<1	5	-	Workman, 2010
<i>T. francoisi</i>	52.8	17.2	14.2	7.5	7.4	Zhou et al., 2006
<b>Mean colobines</b>	52	15	16	-	-	Kirkpatrick, 1999

The availability of plant parts may change depending on seasons, forcing colobines to switch dietary preference during different months of the year. For example, capped langurs (*Trachypithecus pileatus*) subsist on perennial plant parts like mature leaves during the cool dry season (80% of diet from November to March), and select seasonal foods like new leaves and fruit when they become available during the hot dry (20% to 60% from March to May) and the monsoon (50% from May to September) periods respectively (Stanford, 1991). Similarly, western purple-faced langurs (*T. vetulus nestor*) maintain a consistently high proportion of seasonal foods in the diet by exploiting a large number of species with asynchronous phenological cycles, particularly for fruits (Dela, 2007). These data suggest that feeding strategy of some colobines is adapted to cope with seasonal food availability.

Feeding is one of the most basic aspects of an animal's ecology (Hohmann et al., 2007). Knowledge of a species' diet is essential for understanding its place in a biological community and for structuring effective management plans for its conservation. Conservation measures such as quantifying suitable habitat, choosing areas for protection or species to be planted for remediation of degraded habitat are possible only if food plants are known. Obtaining this information however, is especially complicated for primates because diet varies throughout the year and the geographical range (Barnett, 1995). Moreover, the total number of food plant species consumed by leaf monkeys can vary tremendously. While Phayre's leaf monkeys (*Trachypithecus phayrei*) fed on only 18 species (Gupta and Kumar, 1994), Hoang et al. (2009) demonstrated that black-shanked douc langurs (*Pygathrix nigripes*) feed on 152 species of plants. Therefore a long study period is needed before one can provide a comprehensive overview of a leaf monkey's food plants. Prior to my study, there were no data on the plant species consumed by banded leaf monkeys in Singapore. In this chapter, the diet of *P. f. femoralis* will be described and discussed in relation to the seasonality of plant parts and the abundance of food plant species in the forest.

### **1.1.2 Vocalization**

Primates have the capacity to produce referential vocalizations (Marler et al., 1992; Seyfarth et al., 1980; Zuberbühler et al., 1997; Zuberbühler, 2001), i.e. different types of calls that carry different meanings. Vocalizations can serve to maintain territory (e.g. in gibbons, Carpenter, 1940; siamangs, Chivers, 1974), attract mates (Wrangham, 1979),

promote group cohesion, and alarm group members of predators (diana monkeys, Zuberbühler et al., 1997), among others. Hence the structural identification of different vocal units constitutes a crucial step for an effective comparison of vocal repertoires and its respective semantic functions (Pozzi et al., 2010). Here I present preliminary analysis of the structural characteristics of loud calls of banded leaf monkeys, and document their nocturnal calling behavior.

## **1.2 MATERIALS AND METHODS**

### **1.2.1 Study site**

The Central Catchment Nature Reserve (CCNR) is the largest nature reserve in Singapore, comprising 455 ha (National Parks Board, 2007) of mostly young and mature secondary forest with patches of primary forest (Wong et al., 1994). It acts as a catchment area for the reservoirs within, namely MacRitchie, Upper Seletar, Upper Peirce and Lower Peirce (Fig. 1.2). It receives an average annual rainfall of 2191.5 mm. The mean daily maximum temperature is 31.5°C and the mean daily minimum is 24.7 (Data from 1982-2008; National Environmental Agency, 2009).



Figure 1.2: Central Catchment Nature Reserve and surrounding reservoirs (Google Earth)

### 1.2.2 Data collection and analysis

Fieldwork was carried out from September 2008 to August 2010. In a team of two, we walked on existing trails from dawn to dusk and stopped for an average of 15s every 20m. Once the banded leaf monkeys were found, they were followed for as long as possible. However, because the leaf monkeys often hid in dense vegetation during midday or moved to places where observers could not follow them, we could not conduct

full-day consecutive behavior sampling on many observation days (see Zhou et al., 2006). All observations were made using Nikon Monarch 10x42 DCF binoculars. If possible, I took photographs of the monkeys using Nikon D700 with Sigma AF 150-500mm F/5-6.3 DG OS HSM, and video-taped their behavior using Sony Handycam HDD HDR-SR12E. The location of the group was tracked using Global Positioning System (GPS; Garmin GPSMAP 60CSx) whenever it moved in order to document ranging behavior. Additional information came from video recordings which were analyzed using FinalCut Pro and iMovie '09 (ver. 8.0.2 Apple Inc.).

Data were recorded using *ad libitum* sampling (Altmann, 1974) particularly for animals which were difficult to see and identify (Supriatna et al., 1986). Whenever possible, individuals were counted and classified as adult males, adult females, subadults, juveniles and infants based on the age categories and distinguishing criteria (Table 1.2) described by Bennett (1983) for *Presbytis melalophos* [*P. femoralis* was previously recognized as a subspecies of *P. melalophos* by Chasen (1940) and Oates et al. (1994)]. Additional distinguishing characteristics were used for the 'Infant' category to classify the local leaf monkeys based on field observations as shown in Table 1.2.

Table 1.2: Age categories of the banded leaf monkeys (after Bennett, 1983)

Category	Distinguishing features
Infant – 1	From birth until pattern at head changes to adult color. This occurs at about six months of age. *Infant is <b>still white</b> . (0-1 year)
Infant – 2	From the end of Infant – 1 until the <b>tail is more than three-quarters</b> of the adult length. This occurs at about one year of age. *Infant is fully black. (1-2 year)
Juvenile – 1	From the end of Infant – 2 until the animal is <b>more than half of the adult body</b> size. This occurs at about two years of age. (2-3 year)
Juvenile – 2	From the end of Juvenile – 1 until the animal is more than three-quarters

	of the adult body size. This occurs at about three to four years of age. (3-5 year: 4 <sup>th</sup> & 5 <sup>th</sup> year)
Subadult	From the end of Juvenile – 2 until the animal is of fully adult size.
Adult	Full sized animal (Sexual maturity could not be determined in the field).

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\*Additional distinguishing characteristics based on field observations

Group and population estimates are based on several methods. Group composition and demographics provide essential evidence to confirm the identities of various groups (e.g. number of adult, subadult, juvenile, and infant; sex of individual). Sightings of multiple groups at the same time also verified the number of groups in separate home areas. Individuals which travelled towards different directions were considered to belong to separate groups. On the other hand, individuals which were spread out as they foraged or travelled were considered to belong to a single group.

Vocalizations from the monkeys were recorded opportunistically during day surveys using Panasonic IC recorder (RR-US395). I inspected the loud calls and sampled the ones that exhibited little disturbance by background noise. These analyses employed PRAAT (Boersma and Weenink, 2009). I passed the vocalizations through a high pass filter at 200Hz using an inbuilt algorithm in PRAAT. By visually analyzing the spectrogram as well as the playbacks of the vocalization, after setting a dynamic range of 40 decibels (dB) as recommended by Beckers et al. (2003), I characterized the duration of the long calls. In addition, I characterized the dominant frequency of vocalizations by using Fast Fourier Transform of the spectrogram which will yield the power spectrum for each long call (Fig. 1.3). Vocalizations were also recorded for two nights to estimate the number of groups through triangulation (Brockelman and Ali, 1987; Estrada et al., 2004). Two listening points were selected to provide coverage of the main study area. One observer

was assigned to each listening point to record the time of calls and estimate the distance of the calling troop from the observers and its compass direction (Estrada et al., 2004). Resulting information was placed on map to approximate the location of sleeping sites. While daytime calls are thought to aid in the defense of home ranges, nocturnal calls are believed to space troops within sleeping areas (Ripley, 1967; Hohmann, 1990).

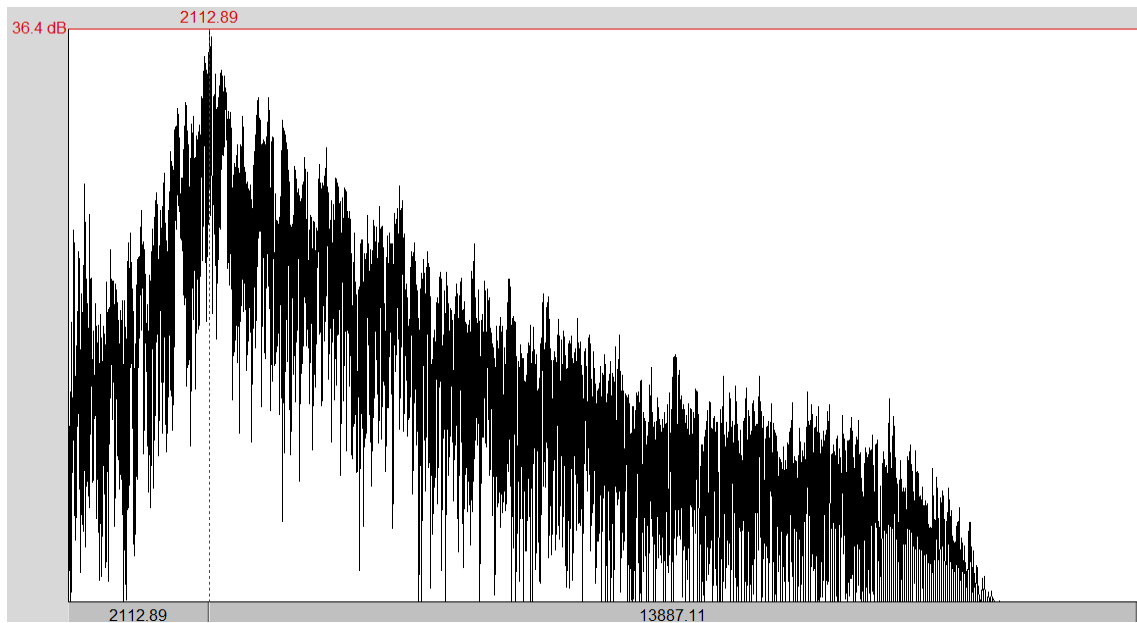


Figure 1.3: A sample power spectrum of the loud call of banded leaf monkey showing the dominant frequency of 2112.89 Hz

### 1.2.3 Feeding observations and plant phenology

A feeding record was used whenever a monkey manually or orally handled a food item, and brought it into the mouth (Davies, 1991; Fashing, 2001; Zhou et al., 2006). I recorded plant species and parts eaten, including leaves, fruit, flowers, and other plant items. However, it was often difficult to distinguish between age classes of leaves, leaves and leaf buds and therefore I recorded leaves as a single class (Hoang et al., 2009). Leaves were classified as new if they were paler in color and smaller than mature leaves of the



same species (Davies, 1984; Stanford, 1991). Due to difficulties in distinguishing between flowers and floral buds, I classified both as flowers. Similarly, I combined fruits and seeds because it was difficult to tell which parts of the fruits were eaten (Fuentes, 1996).

When a banded leaf monkey was seen feeding on a particular tree, the tree was mapped using GPS and I collected plant samples. Whenever possible, leaves, flowers and/or fruits were collected from the ground or the tree and kept in zip-lock bags. The samples were then identified by staff at the National Parks Board Herbarium. After identification, I recorded the conservation status of all identified food species in Singapore in accordance with Chong et al. (2009). In order to examine the availability of favored plant parts during different months, phenology of all recorded food plant species was examined based on herbarium specimens and plant samples to determine whether different plant parts were available (e.g. young leaves and shoots, flowers, fruits). For this study, only samples from Singapore and West Malaysia were included because of the relatively similar climatic conditions. Herbarium samples from fallen or dead trees were excluded.

#### **1.2.4 Habitat sampling**

I examined food resource abundance by investigating the composition and structure of forest in the study area. Within the range of banded leaf monkeys, nine 20 x 75m plots were established (Fig. 1.4). Within each plot, all trees with girths  $\geq 40$ cm at approximately 1.3m from the ground were recorded. Each stem was measured and recorded as a separate tree for trees with multiple stems or with coppice shoots as long as

the bifurcation occurred <1.3m (Wong et al. 1994). I computed the basal area of each tree from its girth ( $g$ ) using the following formula (Hédli et al. 2009):

$$\text{Basal area (cm}^2\text{)} = \frac{g^2}{4\pi}$$

Some plant species were individually identified in the field by experts from National Biodiversity Centre, National Parks Board. For unidentified species, leaves, flowers and/or fruits were collected from the ground or the plants and stored in zip-lock bags for later identification by the National Parks Board Herbarium. Plants from families *Areaceae* (palms) and *Pandanaceae* (pandans) were not included in the study because of their low likelihood of being selected as food plants by leaf monkeys.

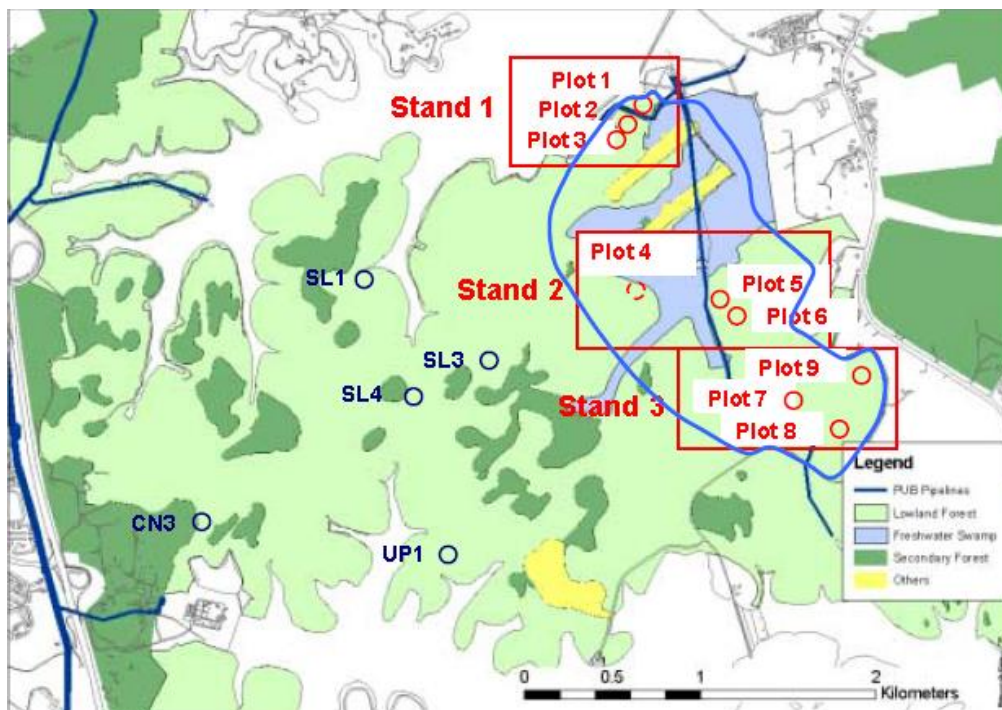


Figure 1.4: A map of nine forest plots in CCNR (Ismail, 2010). Plot 4 was only partially sampled and was thus removed from analysis. The line in light blue demarcates the approximate area focused under the study

## 1.3 RESULTS

### 1.3.1 Population estimate

The total time in the study area was 52,762 minutes (879 hours; including vegetation sampling). Two hundred field trips were made to the CCNR, yielding 93 encounter-days where banded leaf monkey(s) were sighted (47% sighting success; occasions when only vocalizations were heard not included). I estimate that there are at least 40 banded leaf monkeys in the CCNR. There are at least six social groups with two to 14 individuals in each group (Table 1.3). To ensure that these data will only be used for research purposes, the exact locations of study sites and home ranges of the banded leaf monkeys are not revealed, but can be available upon request.

Table 1.3: Number of groups and group size of banded leaf monkeys in the CCNR

Group	No. of individuals	Location
A	8	Upper Seletar
B	5	Nee Soon West
C	10	Nee Soon East
D	3	Old Upper Thomson Road
E	2	Old Upper Thomson Road
F	14	Old Upper Thomson Road

There were 17 occasions when more than one troop of banded leaf monkeys were encountered (Table 1.4). In addition, night surveys that recorded vocalizations of the monkeys located the presence and confirmed the number of groups. All the sightings from this study are shown in Table 1.4. Information and results are also available on my website on the banded leaf monkey at <http://evolution.science.nus.edu.sg/monkey.html>.

In addition, Appendix I includes the older sightings (past 1986) as compiled by the Raffles Museum of Biodiversity Research (RMBR) and the study by Hüttche (1994).

Table 1.4: Number of sighting days, and details of observations

No.	Date	Time	Quantity	Group	Composition	Location	Observation
1	03-Oct-08	07:45	2		2 A	S.R.	Calls; Traveling
2	04-Oct-08	11:30	2 to 3		-	S.R.	Crossing; Calls
3	25-Oct-08	09:30	1 to 3		1 A	S.R.	Calls; Traveling
4	17-Nov-08	07:40	2 to 3		1 A	S.R.	Crossing; Feeding
5	19-Nov-08	07:30	5		1 A, 1 black I-2	S.R.	Crossing; Feeding
6	21-Nov-08	09:40	5		1 A, 1 black I-2	S.R.	Crossing; Calls
7	27-Nov-08	17:30	≥2		-	S.R.	Crossing; Feeding; Conflict with macaques
8	05-Feb-09	09:30	2		2 SA	S.R.	Feeding yellow fruit
9	24-Feb-09	08:00	8		-	S.R.	Feeding on rough tree
10	26-Feb-09	09:40	≥5		-	S.R.	Calls; Traveling
11	27-Feb-09	10:02	≥6		-	S.R.	Calls; Feeding
12	01-Mar-09	09:05	7		-	S.R.	Crossing; Feeding
13	04-Mar-09	09:32	≥7	A	-	S.R.	Calls
14	12-Mar-09	10:14	≥5	B/C?	1 A, 1 black I-2	N.S.	Calls; Moving
15	15-Mar-09	09:15	≥7	C?	1 A	N.S.	Crossing; Calls
16	20-Mar-09	10:20	9	C?	1 A, 1 black I-2	N.S.	Crossing; Traveling on the ground
17	22-Mar-09	12:00	≥5	B?	-	N.S.	Calls; Feeding
18	01-Apr-09	08:30	≥3		1 A	N.S.	Feeding; Calls
19	03-Apr-09	09:00	3 to 5		1 A	N.S.	Feeding
20	05-Apr-09	08:20	≥4	B?	1 A	N.S.	Defecating; Crossing; Calls
21	10-Apr-09	11:45	≥4		3 A, 1 J-2/J-1	N.S.	Calls; Possibly conflict with macaques
22	12-Apr-09	09:00	≥7	C?	3 A	N.S.	Grooming?; Calls; Traveling
23	16-Apr-09	09:20	≥5		-	N.S.	1 <sup>st</sup> group; Calls; Traveling East
		10:35	≥2	E?	1 A	N.S.	2 <sup>nd</sup> group: Calls; Traveling West
		11:10	≥6	F?	-	M.D.	3 <sup>rd</sup> group: With macaques
24	24-Apr-09	16:50	2 to 3	E?	-	N.S.	Crossing; Calls
25	06-May-09	09:45	≥2		-	N.S.	Possibly feeding; Calls
26	10-May-09	09:30	1 to 2	E?	1 A	N.S.	Traveling
27	12-May-09	17:19	≥6		2 A, 2 SA, 1 A/SA, 1 J-1	N.S.	1 <sup>st</sup> group; Gave chase; Calls; Feeding
		17:49	1 to 2	E?	-	N.S.	2 <sup>nd</sup> group; Being chased; Crossing; Calls
28	14-May-09	17:37	≥4	B?	3 A, 1 J-1	N.S.	Crossing; Calls
29	13-Jul-09	07:10	6		No juveniles	N.S.	1 <sup>st</sup> group; Feeding on sleeping tree; Calls
		08:15	1 to 2	E?	-	N.S.	2 <sup>nd</sup> group; Calls
30	16-Jul-09	08:15	≥4		-	N.S.	Feeding

31	17-Jul-09	07:25	6	-	-	N.S.	Traveling; Calls; With macaques
32	22-Jul-09	17:00	≥4		1 A	N.S.	Traveling; Feeding; Calls; With macaques
33	23-Jul-09	09:00	≥4		-	N.S.	Traveling; Calls
34	28-Jul-09	07:00	10 to 12	F		M.D.	Traveling; Calls; With macaques
35	30-Jul-09	08:45	≥5	B	2 A, 1SA, 1 J-1, 1 white I-1	N.S.	Play between all individuals
36	01-Aug-09	07:15	13 to 15	F	7 A, 2 white I-1	M.D.	Crossing; Calls
37	05-Aug-09	07:35	≥3		1 A	N.S.	Feeding
38	12-Aug-09	17:12	>5		-	N.S.	Feeding
39	15-Aug-09	08:10	≥3		1 J	N.S.	Traveling; Calls
40	17-Aug-09	07:50	≥1		-	N.S.	Calls
41	23-Aug-09	12:00	≥13	F	1 A, 1 J-1, 1 white I-1	M.D.	Crossing; Calls
42	24-Aug-09	08:25	≥2	E?	1 A	N.S.	1 BLM called another back
		11:40	≥6	F?	4 A, 1 SA, 1 white I-1(f?)	M.D.	Defecating; Sleeping 40mins
43	28-Aug-09	18:00	2	E	2 A (1m)	M.D.	Crossing; Feeding
44	29-Aug-09	08:15	2	E	-	M.D.	Traveling
		11:04	≥10	F	1 A, 1 white I-1	M.D.	Defecating; Crossing; Feeding; Calls
45	07-Sep-09	07:00	6 to 7			M.D.	Feeding; Calls; With macaques
46	08-Sep-09	16:40	≥6			M.D.	Crossing; Feeding; Calls
47	09-Sep-09	09:00	2		1 A	M.D.	Traveling
48	11-Sep-09	07:07	≥6		1 A, 1 SA	M.D.	Crossing; Feeding; Calls
		17:51	1		1 J-2	N.S.	Crossing; Actively approach
49	17-Sep-09	1630	2		2 SA	N.S.	Feeding
50	20-Sep-09	10:10	-		-	N.S.	1 <sup>st</sup> group: Calls
		10:15	-	A	-	S.R.	2 <sup>nd</sup> group: Calls
		10:59	≥6		1 J-2	N.S.	3 <sup>rd</sup> group: Calls
51	22-Sep-09	17:06	1		1 A	M.D.	1 <sup>st</sup> group: Traveling
		18:30	6 to 7		3 A, 1 J-2 (5 <sup>th</sup> )	N.S.	2 <sup>nd</sup> group: Traveling; Going to sleeping tree
		19:03	-		-	N.S.	3 <sup>rd</sup> group: Calls
52	23-Sep-09	06:59	≥4		3 A, 1 J-2 (5 <sup>th</sup> )	N.S.	1 <sup>st</sup> group: Waking up from sleeping tree; Calls
		07:30	-		-	N.S.	2 <sup>nd</sup> group: Calls in response to 1 <sup>st</sup> group
53	09-Oct-09	16:30	≥5			N.S.	1 <sup>st</sup> group: Traveling
		16:35				N.S.	2 <sup>nd</sup> group: Calls
54	10-Oct-09	07:30	-		-	N.S.	1 <sup>st</sup> group: Calls
		07:36	5 to 6		-	N.S.	2 <sup>nd</sup> group: Feeding; Calls
55	13-Oct-09	07:47	9		3 A, 1 J-2 (5 <sup>th</sup> ), 1 white I-1	N.S.	1 <sup>st</sup> group: Feeding; Calls
		08:45			-	N.S.	2 <sup>nd</sup> group: Calls

		1835	3	D	2 A, 1 J-2 (4 <sup>th</sup> )	M.D.	3 <sup>rd</sup> group: Traveling
56	14-Oct-09	07:00	3	D	2 A, 1 J-2 (4 <sup>th</sup> )	M.D.	Feeding; Calls
57	22-Oct-09	07:23	≥7		2 A, 3 A/SA, 1 J, 1 white I-1	N.S.	Traveling; Calls
58	23-Oct-09	07:30	≥5		-	N.S.	Calls
59	26-Oct-09	07:21	11 to 12	C	3 A, 2 white I-1	N.S.	Calls; Feeding; Crossing
60	27-Oct-09	16:16	6		2 A, 1 white I-1	N.S.	Feeding; Calls
61	30-Oct-09	10:35	8	A	2 A, 1 SA, 1 J-2 (4 <sup>th</sup> ), 1 black I-2, 1 white I-1	S.R.	Traveling; Play between I-2 and J-2
62	10-Nov-09	08:10	11-12	C	3 A, 2-J, 1 black I-2, 1 white I-1	N.S.	Crossing; Calls
		08:10	5			N.S.	Calls
63	12-Nov-09	08:00	8		1 A, 1 white I-1, 1 black I-2	N.S.	Feeding
64	13-Nov-09	07:51	≥2	B?	-	N.S.	Calls; Feeding; Traveling
65	18-Nov-09	08:40	≥4	B?	2 A	N.S.	Calls; Traveling
66	23-Nov-09	06:45	7	A	4 A, 2 J-1, 1 black I-2	S.R.	Different sleeping sites; Crossing; Feeding
67	02-Dec-09	06:55	≥7	A	3 A, 1 SA, 1 black I-2	S.R.	Different sleeping sites; Traveling; Feeding
68	05-Dec-09	17:00	≥4	A	-	S.R.	Traveling
69	19-Jan-10	16:25	7	A	1 A, 2 SA, 2 J-2, 1 J-1, 1 black I-2	S.R.	With macaques
70	20-Jan-10	17:15	≥8	C	3 A, 2 SA, 1 J-2, 1 J-1	N.S.	Crossing; Feeding; Different sleeping sites
71	21-Jan-10	09:07	7	C	2 A, 3 SA, 2 J-1	N.S.	With 1 macaque
72	27-Jan-10	06:58	≥5	A	1 A, 1 J-2, 1 J-2/J-1, 1 black I-2	S.R.	Different sleeping sites; Crossing; Defecating
73	10-Feb-10	09:28	≥5	B?	1 A(m)	N.S.	Calls; With 1 macaque
74	19-Feb-10	08:05	3 to 5	B?	2 A, 1 J-2	N.S.	Defecating; Feeding
		11:40	6	A	4 A, 1 SA/J-2, 1 black I-2	N.S.	Crossing; Calls
75	25-Feb-10	10:13	5	B	-	N.S.	1 <sup>st</sup> group; Gave chase; Calls
		10:13	1		1 A	N.S.	2 <sup>nd</sup> group; Being chased
76	02-Mar-10	17:35	≥1	C?	(1 small)	N.S.	Calls
77	12-Mar-10	09:51	≥2	D	1 A, 1 J-2	M.D.	Moving
78	17-Mar-10	10:30	≥1	E	1 SA	M.D.	Moving
79	24-Mar-10	09:00	≥4	B?	3 A, 1 J-2	N.S.	Feeding, traveling
80	08-Apr-10	09:20	3	D	2 A, 1 J-2	M.D.	Calls, moving
		10:50	2	E	2 A	M.D.	Traveling on the ground
81	21-Apr-10	09:49	1		1 A/SA	N.S.	Lone individual with macaques
82	07-May-10	07:25	≥3	A	(1 small)	N.S.	Moving
83	16-May-10	15:48	≥4	A	2 A, 1 J-2 (4 <sup>th</sup> ), 1 black I-2	N.S.	Crossing; Feeding
84	21-May-10	10:49	≥5	A	2 A, 2 SA, 1 J-1	S.R.	Defecating; Calls
85	25-May-10	14:10	≥7	A	1 A(f), 1 SA, 1 J-1	S.R.	Feeding
86	26-May-10	08:05	≥6	A	3 A, 2 SA, 1 black I-2	S.R.	Crossing; Feeding

87	10-Jun-10	17:21	2 to 3	A	-	N.S.	Calls, moving quickly
88	14-Jun-10	16:50	8	A	5 A, 1 J-2 (5 <sup>th</sup> ), 1 black I-2, 1 white I-1	S.R.	Crossing; Feeding; With macaques
89	15-Jun-10	15:05	≥6	A	1 black I-2, 1 white I-1	S.R.	Crossing; Calls; Macaques left
90	13-Jul-10	17:32	≥1		1 A	N.S.	After rain; Calls
91	16-Jul-10	07:45	3	D	1 A (m), 1 A (f), 1 SA	M.D.	Crossing
92	27-Jul-10	10:55	≥3	D	2 A, 1 SA	M.D.	Feeding
		16:50	1		-	M.D.	Calls
93	29-Jul-10	07:47	≥5	A	3 A, 1 black I-2, 1 white I-1	S.R.	Feeding; Calls

S.R.: Seletar Reservoir Park; N.S.: Nee Soon; M. D.: Mousedeer Trail along Old Upper Thomson Road  
Crossing: Crossing forest fragments or disconnectivity; (Age in years)



### 1.3.2 Dietary profile

Twenty-three species of plants from 17 families were identified as food of banded leaf monkeys based on feeding observations (Table 1.5). Lauraceae contributed the highest number of food plant species (four), followed by Fabaceae (two), Polygalaceae (two), and Sapotaceae (two). With one exception (*Xanthophyllum ellipticum*: two feeding observations), all food species were seen to be consumed only once. The banded leaf monkeys primarily fed on fruits, followed by leaves, then flowers. The monkeys tended to eat more young leaves than mature ones. For twelve species only fruits were eaten, for seven only leaves, for three only flowers, and for one species leaves and flowers (*Bauhinia semibifida*). Fifty-two percent of the 23 food plant species are threatened in Singapore (presumed extinct, critically endangered, endangered, or vulnerable), with the remaining 48% either being common (native) or exotic (Table 1.6). The banded leaf monkeys spent most of their time feeding in the middle (irregular layer of trees which occasionally forms a closed canopy but whose crowns are usually in lateral contact) and upper storeys (consists of trees with broad umbrella-like crowns that form a discontinuous layer) of the forest. Seventy-eight percent of their food sources were trees, 18% were climbers, and the remaining 5% were shrubs.

Table 1.5: Identified plant species consumed by banded leaf monkey in Singapore

Family	Species	Conservation status	Plant Type	Leaves <sup>#</sup>	Flowers	Fruits	N	Months available
Celastraceae	<i>Lophopetalum multinervium</i>	Endangered	Tree			+	1	Apr, May, Jul
Connaraceae	<i>Agelaea macrophylla</i>	Critically endangered	Climber			+	1	Jan, Feb, Jun-Aug
Dilleniaceae	<i>Tetracera indica</i>	Common	Climber			+	1	Year-round
Euphorbiaceae	<i>Hevea brasiliensis</i>	Naturalized (Exotic)	Tree	Y			1	Aug
Fabaceae	<i>Bauhinia semibifida</i>	Vulnerable	Climber	+	+		1	Jan, Feb, Apr-Jun, Aug-Nov*
	<i>Pterocarpus indicus</i>	Casual (Exotic)	Tree	+			1	N/A
Gentianaceae	<i>Fagraea fragrans</i>	Common	Tree	+			1	Dec
Ixonanthaceae	<i>Ixonanthes reticulata</i>	Common	Tree			+	1	Mar-Oct
Lauraceae	<i>Litsea castanea</i>	Endangered	Tree	+			1	Sep
	<i>Litsea elliptica</i>	Common	Tree			+	1	Feb, Sep, Dec
	<i>Litsea firma</i>	Vulnerable	Tree			+	1	Jul, Aug, Oct, Dec
	<i>Nothaphoebe umbelliflora</i>	Common	Tree	Y			1	Jan, Feb, Apr-Jul, Sep-Nov
Menispermaceae	<i>Fibraurea tinctoria</i>	Common	Climber		+		1	Feb-May, Aug, Nov, Dec
Moraceae	<i>Artocarpus elasticus</i>	Common	Tree			+	1	Apr-Aug, Oct, Nov
Myristicaceae	<i>Knema malayana</i>	Endangered	Tree			+	1	Jan-Sep
Myrtaceae	<i>Syzygium grande</i>	Common	Tree	+			1	Oct, Nov
Pentaphylacaceae	<i>Adinandra dumosa</i>	Common	Tree		+		1	Feb-Jun, Sep-Dec
Polygalaceae	<i>Xanthophyllum ellipticum</i>	Critically endangered	Tree			+	2	Mar, Apr, Oct, Nov
	<i>Xanthophyllum eurhynchum</i>	Vulnerable	Shrub	+			1	Jan-Aug, Nov, Dec
Rhizophoraceae	<i>Pellacalyx axillaris</i>	Endangered	Tree			+	1	Oct
Sapindaceae	<i>Nephelium lappaceum</i>	Critically endangered	Tree			+	1	Jan, Apr-Nov
Sapotaceae	<i>Madhuca sp.</i>	Presumed extinct	Tree			+	1	N/A
	<i>Palaquium xanthochymum</i>	Critically endangered	Tree			+	1	Oct

Plant species are native unless otherwise stated.

#: Leaves: There is no differentiation between young and mature leaves in this category unless otherwise stated as Y (young leaves).

n: Number of feeding observations on that item.

\*: Months when either leaves or flowers were available.

Table 1.6: Conservation status of the food plant species (after Chong et al., 2009)

Conservation status	No. of species	% of total species	Total %
<b>Threatened</b>	Presumed Extinct	1	4.35
	Critically Endangered	4	17.39
	Endangered	4	17.39
	Vulnerable	3	13.04
<b>Not Threatened</b>	Common	9	39.13
	Casual/Naturalized (Exotic)	2	8.70

The phenology of food plant species was examined based on herbarium and field samples (Appendix II). Plant specimens dated between 1881 and 2010, with six to 78 individuals of each food plant species being assessed. There were no local herbarium samples for *Fagraea fragrans*, *Hevea brasiliensis* and *Palaquium xanthochyllum*, hence the phenology of these species is based on 1-2 specimens collected during feeding observations. One species of *Madhuca* which was presumed extinct in Singapore until its rediscovery during this study also lacked herbarium specimens. The study reveals that only one species, *Tetracera indica* (Dilleniaceae) provides food (fruit) year-round; all other food plant have seasonal availability. Between April and November, the largest amount of food is available (9-13 species), with two small peaks in April and October (Fig. 1.5). Fewer preferred foods are found between December to March, when seasonal plant parts are relatively uncommon (7-9 species).

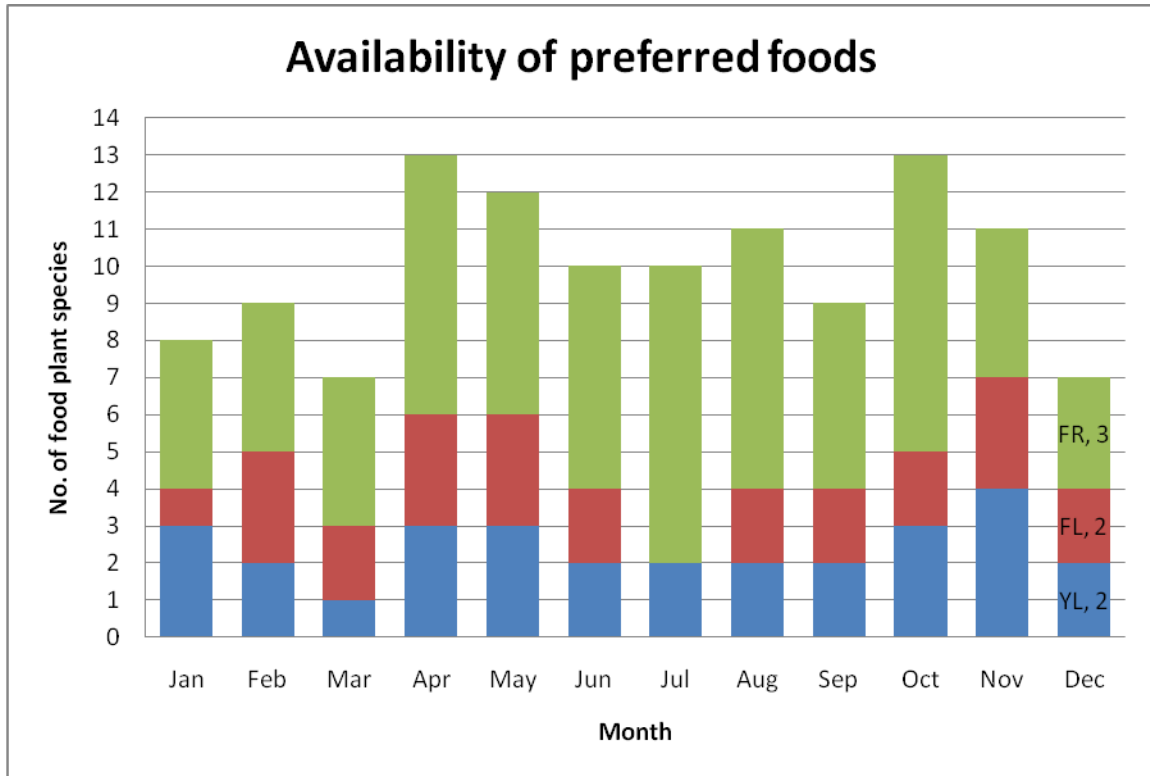


Figure 1.5: Number of food species with the availability of preferred plant parts of banded leaf monkeys. YL: young leaves; FL: flowers; FR: fruit

### 1.3.3 Habitat

Nine vegetation plots were established but only eight plots yielded data. Plot 4 was infested with parasitic mites and hence it was abandoned. A total of 194 species were found including 98 species of trees (35 families) and 96 species of climbers (28 families) (Appendices IIIa and IIIb). In the 1.2 ha study area, 438 trees and 1136 climbers could be identified while 255 individuals could not be identified to species level.

The Myrtaceae contributes the greatest tree biomass based on both basal area and stem density (Figs. 1.6a and 1.6b) and together with the Ixonanthaceae, and Cannabaceae, account for 51.4% of the total basal area and 45.7% of the total stem density. The three dominant tree species by basal area are *Rhodamnia cinerea* (Myrtaceae), *Syzygium*

*grande* (Myrtaceae), and *Ixonanthes reticulata* (Ixonanthaceae) (Table 1.7), of which two species *Syzygium grande* (Myrtaceae) and *Ixonanthes reticulata* (Ixonanthaceae) were consumed by the monkeys (see Table 1.5).

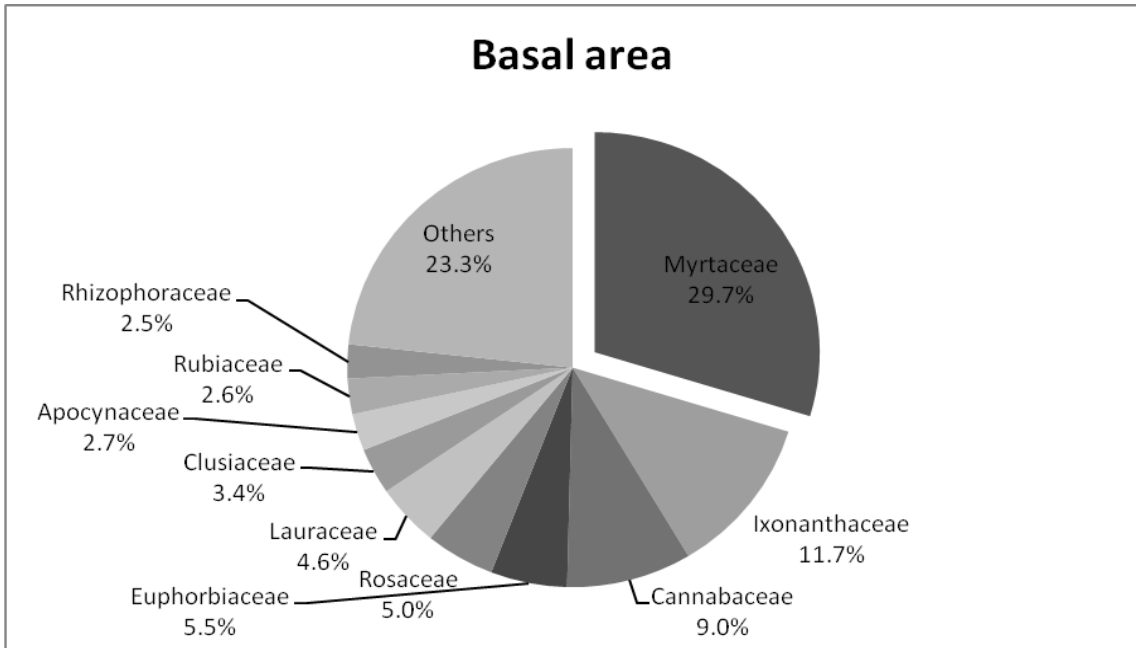


Figure 1.6a: The most common tree families at the CCNR based on basal area

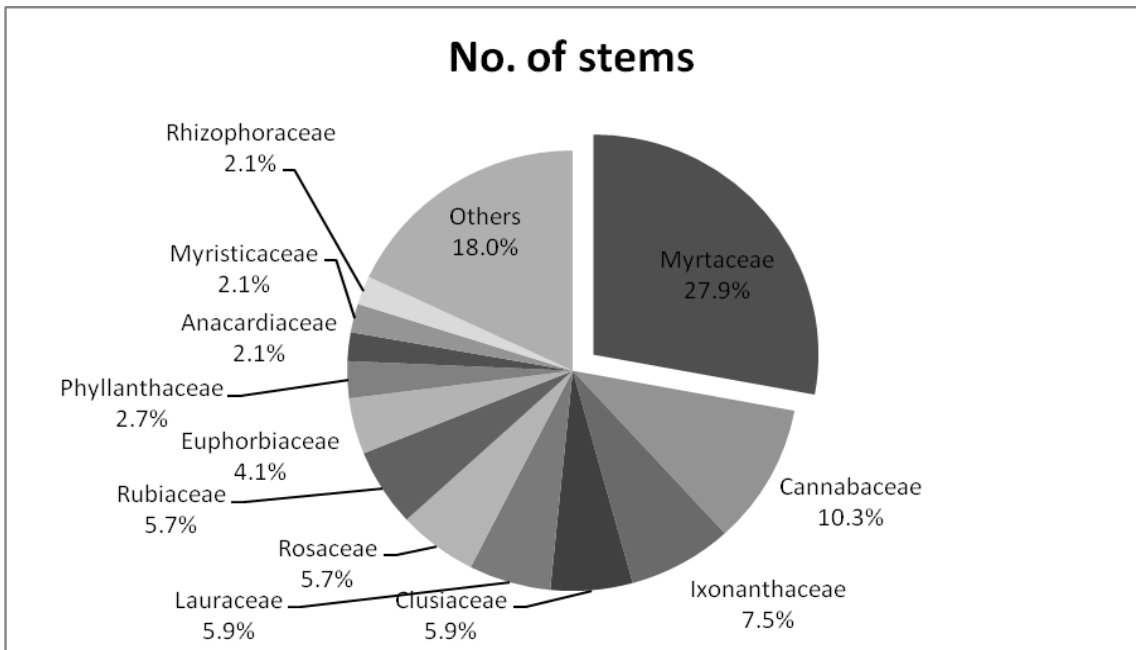


Figure 1.6b: The most common tree families at the CCNR based on number of stems

Table 1.7: The 10 most common tree species based on basal area

Rank	Species	Family	Basal area/ha (cm <sup>2</sup> /ha)	% basal area	Stems
1	<i>Rhodamnia cinerea</i>	Myrtaceae	27888.65437	12.0	72
2	<i>Syzygium grande</i>	Myrtaceae	26686.70297	11.5	27
3	<i>Ixonanthes reticulata</i>	Ixonanthaceae	23338.81243	10.1	24
4	<i>Gironniera nervosa</i>	Cannabaceae	17456.11416	7.5	36
5	<i>Prunus polystachya</i>	Rosaceae	11570.10016	5.0	25
6	<i>Gynotroches axillaris</i>	Rhizophoraceae	5708.092034	2.5	9
7	<i>Shorea platycarpa</i>	Dipterocarpaceae	5309.011014	2.3	8
8	<i>Litsea elliptica</i>	Lauraceae	4944.612509	2.1	10
9	<i>Macaranga gigantean</i>	Euphorbiaceae	4839.503932	2.1	6
10	<i>Timonius wallichianus</i>	Rubiaceae	3938.554325	1.7	15

The three dominant families combined (Menispermaceae, Connaraceae, Apocynaceae) account for 36.7% of the total individual density, with Menispermaceae contributing the largest number of climber individuals (Fig. 1.7). The three dominant climber species are *Fibraurea tinctoria* (Menispermaceae), *Agelaea macrophylla* (Connaraceae), and *Tetracera akara* (Dilleniaceae) (Table 1.8), of which two species *Fibraurea tinctoria* (Menispermaceae) and *Agelaea macrophylla* (Connaraceae) were consumed by the monkeys (see Table 1.5).

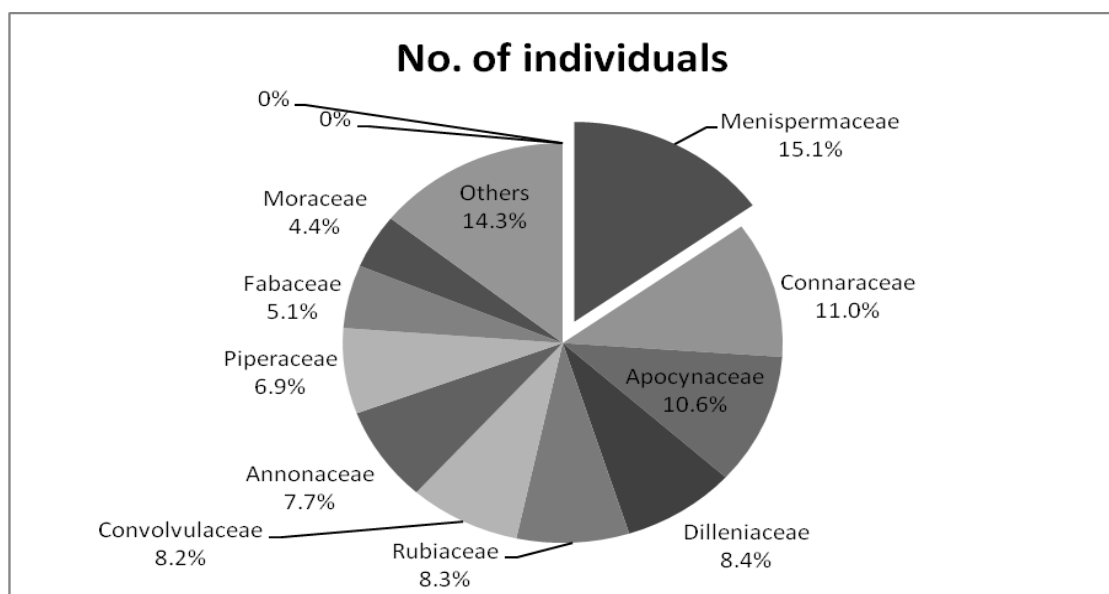


Figure 1.7: The most common climber families at the CCNR based on number of individuals

Table 1.8: The 10 most common climber species based on number of individuals

Rank	Species	Family	No. of individuals	% individuals
1	<i>Fibraurea tinctoria</i>	Menispermaceae	167	14.7
2	<i>Agelaea macrophylla</i>	Connaraceae	69	6.1
3	<i>Tetracera akara</i>	Dilleniaceae	54	4.8
4	<i>Piper porphyrophyllum</i>	Piperaceae	42	3.7
5	<i>Erycibe tomentosa</i>	Convolvulaceae	40	3.5
6	<i>Spatholobus ferrugineus</i>	Fabaceae	40	3.5
7	<i>Smilax setosa</i>	Smilacaceae	38	3.3
8	<i>Gynochthodes sub lanceolata</i>	Rubiaceae	37	3.3
9	<i>Ficus punctata</i>	Moraceae	36	3.2
10	<i>Willughbeia edulis</i>	Apocynaceae	35	3.1

The diversity of climber species exceeds that of tree species in all but one plot (plot 1, Table 1.9). In total, 14 species of climbers presumed to be nationally extinct (Chong et al. 2009) were rediscovered during the survey (Appendix IV).

Table 1.9: Number of tree and climber species in each sampling plot

Plots <sup>1</sup>	No. of tree species	No. of climber species
1	23	4
2	25	31
3	25	31
4	-	-
5	21	25
6	24	35
7	17	30
8	21	43
9	13	15

<sup>1</sup> Area of each plot is 1500m<sup>2</sup>. Total size of 8 plots is 12,000 m<sup>2</sup>. Plot 4 was abandoned.

### 1.3.4 Vocalization

Banded leaf monkeys have one type of loud call (typically termed “machine-gun” call, Hüttche, 1994) which was recorded for more than 60 times during the study. Preliminary analysis was performed on one such recording. This loud call is atonal, lacks specific harmonic structure, can consist of three parts (Fig. 1.8): the first is 1.35 sec long, the second 0.89 sec, and the last 1.07 sec. The dominant frequencies of the three parts are

3669 Hz, 2113 Hz and 2382 Hz, respectively. The first part of the call comprises of six distinguishable notes, second part of six notes, and last part of seven notes.

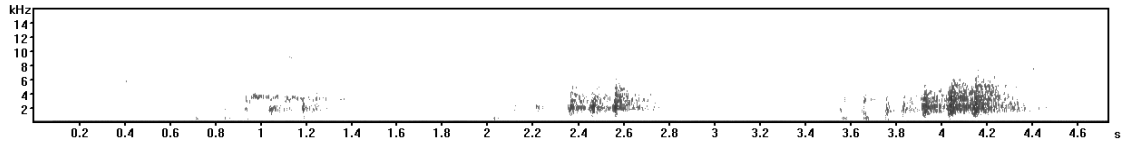


Figure 1.8: Spectrogram of a loud call of banded leaf monkey

Two night surveys to record calling rounds were conducted in order to determine sleeping sites of the banded leaf monkeys. I define a calling round as a period where two or more neighboring troops of banded leaf monkeys call at least once following the first loud call. On 2 February 2010, four calling rounds were recorded (Fig. 1.9a). The first calling round began at 21:35 and ended at 21:37, followed by a second calling round which started at 22:26 and ended at 22:29. The third calling round occurred between 23:22 and 23:24, and the last calling round occurred between 05:58 and 06:00. All calling rounds were initiated by the group in Nee Soon and elicited an immediate response from the troop at Upper Seletar.



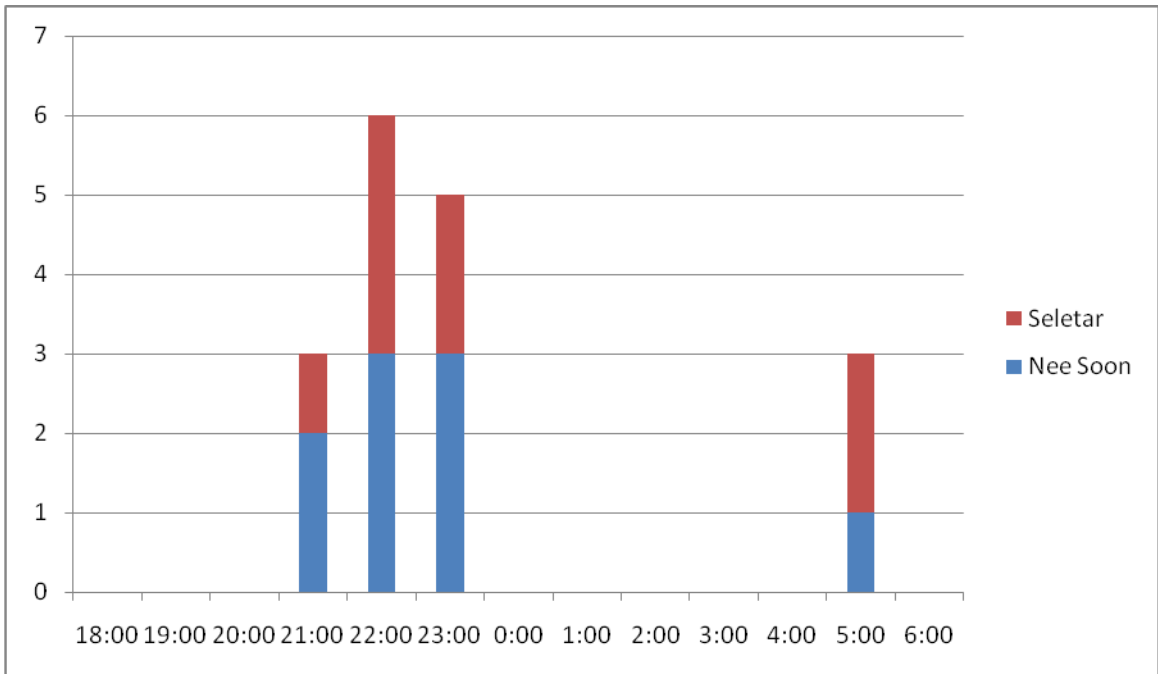


Figure 1.9a: Timing (x-axis) and number of nocturnal loud calls (y-axis) on 2 February 2010. The group at Nee Soon began the calling round first at all times, immediately followed by calls from Seletar group

On 2 March 2010, three calling rounds were heard. The first round began at 01:42 and ended at 01:47. The second round started at 04:52 and ended at 04:56. The last calling occurred between 05:41 and 05:42. Two calling rounds were initiated by the group in Nee Soon (1<sup>st</sup> and 3<sup>rd</sup> rounds) and responded immediately by the troop at Upper Seletar. The second calling round was initiated by the Upper Seletar group and responded by the Nee Soon troop (Fig. 1.9b). Based on triangulation, both the Seletar and Nee Soon troops have one regular sleeping site.

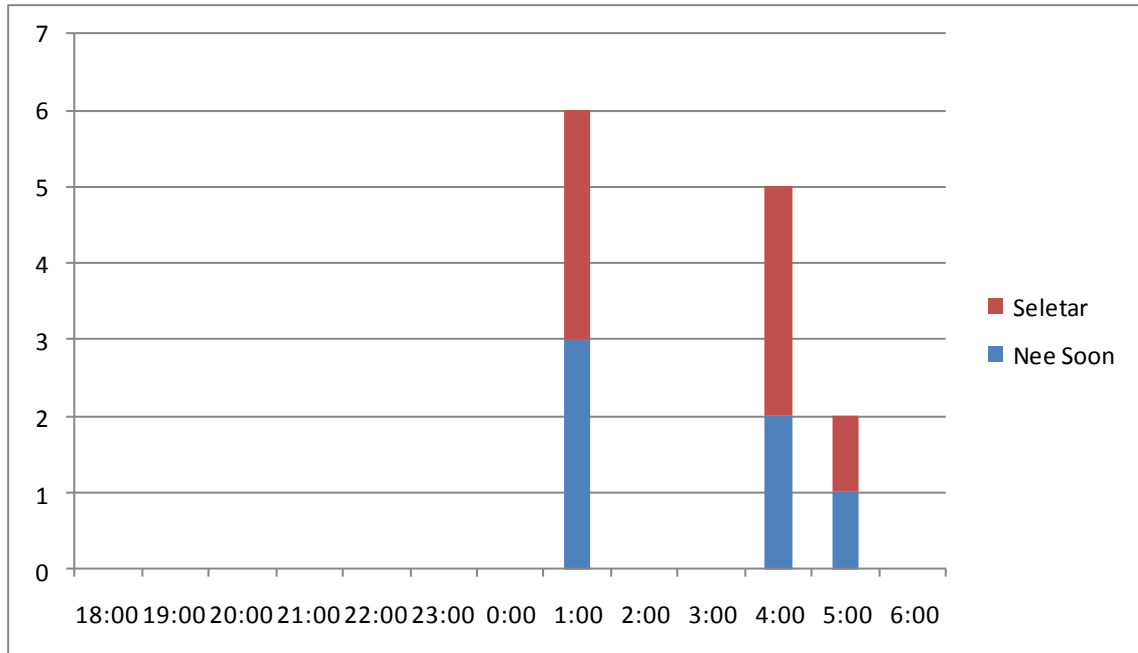


Figure 1.9b: Timing (x-axis) and number of nocturnal loud calls (y-axis) on 2 March 2010. Nee Soon group initiated the calling rounds except the second calling round at 04:52am

## 1.4 DISCUSSION

### 1.4.1 Banded leaf monkey populations in Singapore

Based on two years of field observations, I estimate that there are at least 40 banded leaf monkeys belonging to at least six groups in the Central Catchment Nature Reserve (CCNR). This population estimate is more than two times the previous estimate 15 years ago (Hüttche 1994). It remained unclear whether the higher current population numbers indicate that the banded leaf monkeys are recovering, or that the greater research effort in this study revealed additional populations or greater abundance. Additional monitoring is needed because it could establish whether the troops are successfully reproducing.

Poaching and habitat conversion used to be major reasons for the population decline of banded leaf monkeys in Singapore. With the cessation of direct human threats to the survival of the monkeys, it appears that the species is still not recovering fast, i.e. the species remains rare and no additional populations have become established in the forests adjacent to the CCNR, e.g. MacRitchie forest. Several reasons such as the availability of food resources (see Section 1.4.2 below), and the carrying capacity (see Li et al., 2009) of the current habitat may explain why the population in Singapore is not growing. Determining the carrying capacity of the CCNR habitat will be important in order to assess if the current habitat can support more monkeys in the future or the current habitat limits further population growth.

The small population of banded leaf monkeys in Singapore may experience low genetic diversity owing to increased levels of drift and inbreeding (Lande and Barrowclough, 1987; Nei, 1987). This reduced genetic diversity is often associated with decreased fitness and a higher threat of extinction (Frankel, 1974; Wright, 1977). This situation may be exacerbated by habitat fragmentation: studies of primates in fragments frequently conclude that fragmentation negatively affects some aspect of their biology or ecology (e.g. diet and home range size, Cristóbal-Azkarate and Arroyo-Rodríguez, 2007; population size, Cristóbal-Azkarate et al., 2005; social organization, Zunio et al., 2007) (see Arroyo-Rodríguez and Mandujano, 2009). The forests within and adjacent to the CCNR (namely Nee Soon swamp forest, Upper Seletar, Upper Peirce and Lower Peirce reservoir parks) are divided and governed by different government agencies such as National Parks Board, Public Utilities Board, and Ministry of Defence (Ng and Lim,

1992), resulting in forest fragments being separated by roads and buildings. The degree to which such habitat fragmentation is affecting the gene flow and genetic diversity of the banded leaf monkeys needs to be investigated. Even in the absence of direct evidence for harmful fragmentation, canopy contiguity between forest fragments within the CCNR should be restored in order to promote genetic exchange between the different groups.

#### **1.4.2 Plant food selection and availability**

The banded leaf monkeys have a colobine-typical digestive stomach which allows them to consume relatively large amounts of foliage compared to other primates. Based on feeding observations however, I found that the banded leaf monkeys in Singapore do not depend heavily on leaves; instead they show a strong preference for fruits. I recorded 23 food plant species, of which more than half were sources of fruit only. Fruit-biased diet is relatively uncommon and has only been recorded for some Asian colobines like Western purple-faced langur (*Trachypithecus vetulus nestor*, Dela, 2007) and Tonkin snub-nosed monkeys (*Rhinopithecus avunculus*, Boonratana and Canh, 1998). Within *Presbytis* fruit-preference has not been documented, although two species (*P. melalophos*, *P. rubicunda*) demonstrate approximately equal preference for fruits and leaves (Davies et al., 1988). Though fruits vary widely in biochemistry and quality, they are in general easily digested and contain energy-rich sugars and nonstructural carbohydrates (Kay and Davies, 1994; Waterman and Kool, 1994). On the other hand, as the amount of fiber in the diet increases, the digestibility of the food, the amount of proteins and other nutrients decrease (Janis, 1976). Thus there is a definite tolerance level in the percentage of fiber

(in foliage) allowed in the diet. Therefore, even though the digestive physiology of colobines can support a more folivorous diet, leaf monkeys cannot feed indiscriminately.

Nonetheless, foliage can form a substantial part of their diet (seven food species where only leaves are eaten). Although the preference of banded leaf monkeys for young or mature leaves could not always be determined in the field, it has been documented that leaf monkeys generally consume young leaves significantly more frequently than mature leaves, e.g. Mentawai langur (*Presbytis potenziani*, Fuentes, 1996), Delacour's langur (*Trachypithecus delacouri*, Workman, 2010), and Yunnan snub-nosed monkey (*Rhinopithecus bieti*, Grueter et al., 2009a). Compared to mature foliage, young leaves generally have higher nutritional quality; they have more protein and smaller amounts of fiber and secondary compounds (Oates et al., 1980; McKey et al., 1981; Boonratana, 1993).

Based on these preliminary data, it is difficult to conclude on the feeding preferences of banded leaf monkeys. Although more than half of the 23 plant species were sources of fruit only, each species was observed to be fed on only once. Similarly, for the seven species where only leaves were observed to be eaten, there could be occasions when the monkeys also feed on other plant parts. It is thus important to obtain more feeding observations in order to determine the dietary contribution of different plant parts.

The use of plant food by a primate is also influenced by plant phenology (Bauchop and Martucci, 1968; Freeland and Janzen, 1974; Glander, 1975; Chivers, 1994; Waterman

and Kool, 1994). My phenology study suggests that not all preferred plant parts of the 23 identified food species were available year-round (except for fruit of *Tetracera indica*), although at least seven plant species provided food all months. There was one period with a greater availability of preferred foods (9-13 species): between April and November, with two small peaks in April and October. This period was followed by lower availability of preferred foods between December and March, when seasonal plant parts were relatively uncommon (7-9 species). Between December and early March, Singapore experiences the Northeast Monsoon Season with the first half of the monsoon being characterized by heavy rainfall and the later part of the season by dry and hot weather. As a result of the seasonality in rainfall, plant part production varied, and this will affect food availability for primates. When the banded leaf monkeys experience lower food availability in particular months, they may be exploiting perennial foods like mature foliage, and/or other food resources which are not recorded in this study. It is apparent that more food plant species could be available in the forest.

#### Food preference for uncommon tree species in the habitat

The plant species were not consumed in accordance with their spatial abundance. Instead the banded leaf monkeys predominantly feed on uncommon species. Fifty-one percent of the tree basal areas within their habitat were Myrtaceae, Ixonanthaceae, and Cannabaceae, of which only two species *Syzygium grande* (Myrtaceae) and *Ixonanthes reticulata* (Ixonanthaceae) were of direct dietary importance to the monkeys. Of the 10 most common tree species, banded leaf monkeys exploited only three plant species as food. Based on food selection ratio, Lauraceae contributed the largest proportion (4 of 18

tree species), but only represented 4.6% of the total basal area (6<sup>th</sup> most common tree family). Similar to other leaf monkeys, banded leaf monkeys appear to be selective feeders, seldom eating items in proportion to their abundance in the forest which is likely due to differences in nutritional quality. Bennett (1983) indicated that only 1.1% of feeding observations of mitered leaf monkeys involved the 12 most common tree species, even though those species comprised a large proportion (32%) of the stems in the transects. A high degree of selectivity for rare angiosperm tree species was also observed for Yunnan snub-nosed monkeys (Grueter et al., 2009b).

#### Importance of climbers as a food source

The importance of climbers may be underestimated by my study. Primates are more likely to be seen when they fed on certain food items than on others. For instance, many of the favored fruits of mitered leaf monkeys were taken from large, open emergent trees where the animals were conspicuous, whereas many of the young leaves were taken from lianas and smaller, denser trees in which visibility was poor (Bennett, 1983). Hence feeding observations may be biased towards recording data on tree species. Moreover, it has been shown that many species of *Presbytis* place a high reliance on climbers as their primary food source e.g. *P. potenziani* (35% of feeding observations, Fuentes, 1996) and *P. rubicunda* (32.2%, Davies, 1984). Climbers are very abundant in the CCNR (see Ismail, 2010), and provide a rich and fairly constant source of leafy material (Whitten, 1982a, b). All climber food species of the banded leaf monkeys were among the 10 most common climber families based on individual density: *Fibraurea tinctoria* (Menispermaceae; top most common climber family), *Agelaea macrophylla*

(Connaraceae; 2<sup>nd</sup>), *Tetracera indica* (Dilleniaceae; 4<sup>th</sup>) and *Bauhinia semibifida* (Fabaceae; 9<sup>th</sup>). Therefore it is possible that those less common climber species might have been overlooked although they could be important food sources.

In the eight vegetation plots, 255 individuals (14% of total number of individuals) could not be identified to species due to the lack of plant specimens. These individuals were either trees which were too tall or climbers which were too high in the canopy for observers to confidently identify the leaves; hence no corresponding specimens could be collected from the ground.

#### More than half of food plant species locally threatened

More than half of the 23 identified food plant species of the banded leaf monkeys are threatened in Singapore (Chong et al., 2009). Although it is too premature to conclude that the availability of food species preferred by the banded leaf monkeys is a limiting factor for banded leaf monkey populations, it suggests that this topic should be addressed by future research. In addition to rediscovering one tree species (*Madhuca* sp.) previously thought to be extinct in Singapore, fourteen species of climbers that were also considered extinct were rediscovered. Botanical surveys should thus be carried out more regularly in order to provide an updated status and distribution of the plant species, which is necessary in order to assess the habitat for food availability of the banded leaf monkeys.



### The need for continued monitoring of food selection by banded leaf monkeys

The banded leaf monkeys were not observed at equal frequencies throughout the year; hence feeding observations were biased toward those months with higher visibility. It was especially difficult to find the monkeys during the breeding season (June-July, see Chapter 2), and this is the period where feeding observations would be particularly valuable because the females will have higher nutritional demands. It is therefore important to increase survey efforts during reproductive months as they could be feeding on different food plant species.

The number of food plant species identified is also not exhaustive because it is generally considered impossible to estimate the number of food items ingested in tall, dense rainforests (Bennett, 1983). Here the data in this study are preliminary and only provide baseline information on the feeding ecology of the banded leaf monkeys in Singapore. But the data could be improved through continuous feeding observations. Nonetheless, these observations face two problems: a) the amount of data that can be collected per month is rather small and b) it is time consuming especially when the study subjects cannot be reliably observed and/or when the food items are difficult to discern. Indirect methods of evaluating diets can be employed (van Wyk, 2000). Analyses of plant DNA in feces provides a promising, yet largely unexplored, means of documenting the diets of elusive primates (Bradley et al., 2007). With further optimization, this approach could provide a basic evaluation of minimum primate dietary diversity even when knowledge of local flora is limited. Diet information gleaned from molecular analyses, complemented with continued observational behavioral data will allow the assessment of

consumer-resource relationships in an ecosystem like that in the CCNR. Equipped with such knowledge, we can better understand whether the habitat can sustain banded leaf monkey populations in Singapore.

An important component in a primate's food selection and feeding strategy is optimizing nutrients from quality foods versus the costs of searching (Freeland and Janzen, 1974). As the distribution of fiber and nutrients in different plant parts and species is highly variable, the monkeys have to feed selectively, choosing foods with a relatively high nutrient to fiber ratio. It is well known that protein content and fiber have a strong influence on leaf choice in colobines (Oates et al., 1980; Waterman and Choo, 1981; Davies et al., 1988; Fashing et al., 2007). With a small body size of 6.0 to 6.5 kg, banded leaf monkeys are at the lowest end of the size spectrum for animals capable of fore-stomach fermentation (Hoppe, 1977). As body mass decreases, the energy and protein requirements of an animal increase relative to body weight (Kleiber, 1961; Munro, 1969), as do the energetic costs of traveling (Schmidt-Nielsen, 1972). Moreover, food passage rate increases with body mass (Parra, 1978), so that small colobine monkeys have little opportunity to retain poorly digestible foods for prolonged fermentation. Banded leaf monkeys, therefore, are expected to select high-energy, protein-rich foods. Equipped with feeding data, research can be done to examine the phytochemical components associated with preferred and avoided food items, thereby contributing to a comprehensive understanding of the food selection of the banded leaf monkeys (see Davies et al., 1988).

### 1.4.3 Vocalization

The dominant frequencies of loud calls of banded leaf monkeys in Singapore range from 2113 to 3669 Hz, which are relatively high as compared to other leaf monkeys (e.g. Hanuman langur (270 Hz), Hohmann, 1989; purple-faced langur (292 Hz), Rudran, 1973; Nilgiri langur (310 Hz), Herzog and Hohmann, 1984). A significant negative relationship is found between loud call frequency and home range size of primates, after controlling for the potentially confounding effects of body size and phylogeny (Mitani and Stuht, 1998). Hence it is likely that the high frequencies demonstrated by loud calls of banded leaf monkeys reflect a smaller home range, as loud calls at high frequencies facilitate their transmission over short distances (Mitani and Stuht, 1998). Although only one loud call was sampled for banded leaf monkeys in Singapore, the usually high frequencies demonstrate an interesting aspect of their vocal behavior which requires further examination.

It has been known that leaf monkeys commonly engage in calling rounds (e.g. *Presbytis melalophos*, Curtin, 1980; Bennett, 1983). Around dusk, dawn and at intervals through the night, banded leaf monkeys in Singapore engage in calling rounds. One individual (usually alpha males, see Kitchen, 2004) initiates the calls, soon to be followed by calls from the males of neighboring groups. Within a period of about five minutes, harem males within the area call at least once and up to about three times (see Figs. 1.9a and 1.9b). Through two night surveys, sleeping sites of two groups of banded leaf monkeys were located: Group A of eight individuals from Upper Seletar, and one group (either B

or C) from Nee Soon. Within only two listening stations within the CCNR, it is highly unlikely that nocturnal calls from all groups would be recorded.

The night surveys however, demonstrate that such calling behavior could be used to survey banded leaf monkey populations which are difficult to locate visually. This could help with confirming the number of groups in the vicinity (see Dallmann and Geissmann, 2001). Recently, there are more and more studies employing such auditory survey methods to complement visual-based population surveys, and they were effective in quantifying the number of groups of primate populations (e.g. black howler and spider monkeys, Estrada et al., 2004; Andean titi monkey, Aldrich et al., 2008). More night surveys on the banded leaf monkeys covering more areas within the CCNR should be carried out to provide a more comprehensive understanding of their home range. Moreover, loud calls can be useful for distinguishing vocal patterns of adult males (e.g. Western purple-faced langurs, Eschmann et al., 2008), which can also assist in the identification of groups.

## **1.5 CONCLUSION**

Based on two years of field observations, I find that there are at least 40 banded leaf monkeys in Singapore, an estimate which is two times greater than previously believed. However, it remains uncertain whether the population is recovering. I also identify 23 plant species that are consumed by banded leaf monkeys, of which more than half are locally threatened. The diet consists mainly of fruits, and food species are not consumed in accordance with their abundance; instead, the banded leaf monkeys prefer uncommon species. These preliminary data on food preference of the banded leaf monkeys are interesting and require further research to understand their feeding ecology.

## **CHAPTER 2**

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# **REPRODUCTION AND INFANT PELAGE COLOURATION OF THE BANDED LEAF MONKEY IN SINGAPORE**

## 2.1 INTRODUCTION

The banded leaf monkey (*Presbytis femoralis*) is known from the Malay Peninsula (including Singapore and peninsular Thailand) and eastern Sumatra (Groves, 2001; Meijaard and Groves, 2004) (Fig. 2.1). Several subspecies are recognized; most notably *P. f. robinsoni* in north Malay Peninsula, *P. f. percura* in east-central Sumatra and *P. f. femoralis* in Singapore and Johor, Malaysia. Although *P. f. femoralis* is globally listed as vulnerable (Nijman et al., 2008), it is critically endangered in Singapore due to a small population size (Lim et al., 2008) which was estimated to be only 10 to 15 individuals in the 1990s (Yang et al., 1990).

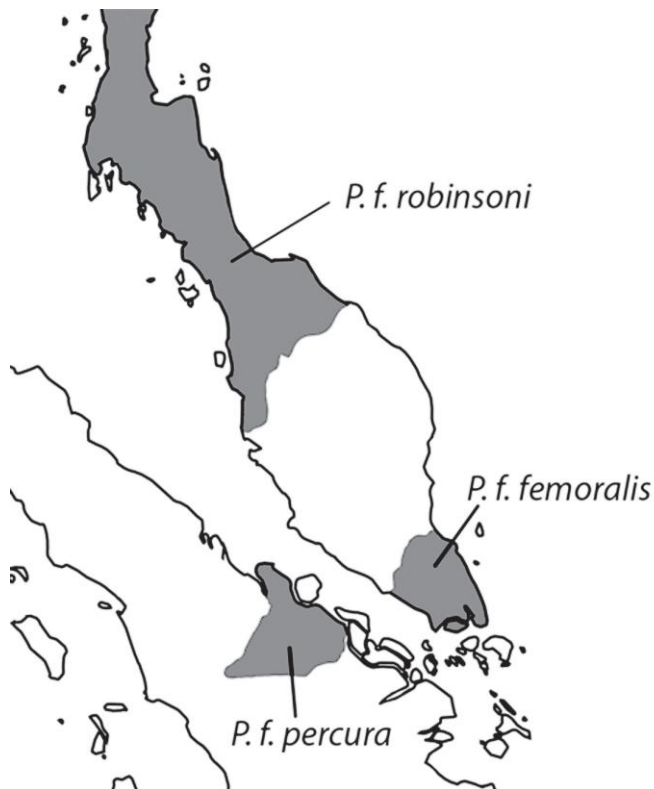


Figure 2.1: Distribution of *Presbytis femoralis*. *Presbytis f. robinsoni* is found in north Malay Peninsula, *P. f. percura* in east-central Sumatra, and *P. f. femoralis* in Johor and Singapore

Unfortunately, it is extremely difficult to obtain data on its reproduction because the species is elusive and shy. Hence, there are no published records of infants in the Singapore population and it remained uncertain whether the population is still reproducing. In order to assess the population status of the banded leaf monkeys in Singapore, it is important to determine if there are new infants and when the species reproduces. Here we report several births in the Singapore population and describe the natal pelage and infant development based on 22 months of field data. We also discuss the implications of our findings for conservation and potential adaptive significance of the pelage coloration.

The taxonomy of this species is still subject of much dispute. Groves (2001) and Brandon-Jones et al. (2004) recognize *P. f. femoralis* (Martin, 1838) from Johor and Singapore. On the other hand, Chasen (1940) suggested that the Singapore population is a unique subspecies from the Johor population based on differences in adult pelage coloration, and Hüttche (1994) described the specimens from Singapore as darker in ventral color than the specimens from Johor. In addition, while the infants of *P. f. percura* in east-central Sumatra are white (Aimi and Bakar, 1996), there were unconfirmed sightings of orange infants in Singapore from occasional nature surveys carried out over the last 20 years, thus potentially supporting the claim that the banded leaf monkeys in Singapore belong to a separate taxonomic unit from the Johor population. Note that flamboyant natal pelage color is common within the Colobinae (Newton and Dunbar, 1994). Across primates the natal pelage can either resemble the adult pelage, be flamboyant (striking, highly visible), or dark (different from adult pelage



but dark and not highly visible). Hrdy (1976) argues that flamboyant pelage may have evolved to facilitate infant-handling by attracting other females, conferring a benefit to infants from being handled.

## **2.2 MATERIALS AND METHODS**

Field surveys were carried out from September 2008 to June 2010 in the Central Catchment Nature Reserve (CCNR). The precise locations of study sites were retained in order to minimize disturbance to the banded leaf monkeys from the public. Teams of two observers walked on existing trails from 0630 hours to 1130 hours and from 1600 hours to 1900 hours whenever the weather permitted. Once the banded leaf monkeys were found, they were followed for as long as possible. We recorded all instances of observing infants, birth seasons, number of infants, and their physical characteristics throughout development. Observations were made using binoculars (Nikon Monarch 10x42 DCF) and video camera (Sony Handycam HDD HDRSR12E), and data was recorded using *ad libitum* sampling (Altmann, 1974). Video recordings of the infants were analyzed using iMovie '09 (ver. 8.0.2 Apple Inc.).

## **2.3 RESULTS**

The total time in the field was 52,762 minutes (879 hours). A total of at least six births were recorded from 2008 to 2010. Two infants were first observed in a group of 14 banded leaf monkeys (Group A) on 1 Aug.2009. This group comprised of at least six

adults of which at least two were females. A sex ratio is not provided because the sex of banded leaf monkeys is difficult to determine in the field. Both infants were white with a broad black line from head, along the spine, to tail, and intersected by a black line passing along the shoulders to the outer surfaces of both forearms, forming a distinctive cruciform (cross-like) black pattern on the dorsum. A faint black line ran from the dark spinal stripe along the hind legs to the feet. From the frontal view, the crown and facial fur were completely white. The forehead was slightly dark as compared to the pale color of the face. These two infants were identified as newborns based on the same characters described for newly-born young of *P. femoralis* from the Malay Peninsula (Pocock, 1928). This neonatal pelage coloration of the banded leaf monkeys is distinct from the adult pelage, which is a uniform black on the dorsum with white bands traversing the underside of the hind limbs. Although there is no published description of the infant of the banded leaf monkeys in Johor, one white infant with natal pelage pattern identical to that of the infants of the Singapore population was recently observed in Panti Forest Reserve, Johor (Fig. 2.2; Yong Ding Li, pers. comm.).



Figure 2.2: Natal pelage pattern of an infant banded leaf monkey from Pantí Forest Reserve (Johor). Photo: D. L. Yong

We sighted white infants from Group A again on 23 August 2009, 24 August 2009 and 29 August 2009. There were slight changes in the pelage coloration. The infants had more grayish black fur spreading from the black lines (Fig. 2.3). One infant was able to move unassisted by an adult for a few seconds before the carrying adult, who was close, pulled it back (Figs. 2.4a and 2.4b). It appeared that the infants were carried less frequently by the adults even though they might not yet be fully weaned.



Figure 2.3: Infant banded leaf monkey (red arrow) from Singapore

On 13 October 2009, we saw one white infant in another group of 11 monkeys (Group B). This group comprised of at least five adults of which at least two were females. The natal pelage pattern of this infant did not deviate much from what is shown in Figure 2.2. It was able to move and jump beside the carrying adult. On 22 October 2009, the white infant in Group B was actively locomoting while the rest of the group was feeding. Loud crashes were heard when some banded leaf monkeys in the group were travelling from tree to tree. This loud noise triggered the infant to leap towards the carrying adult. On 26 October 2009, the same white infant was seen playing with members of its group. In the afternoon when the group was not moving, either resting or sleeping, the infant was still very active.

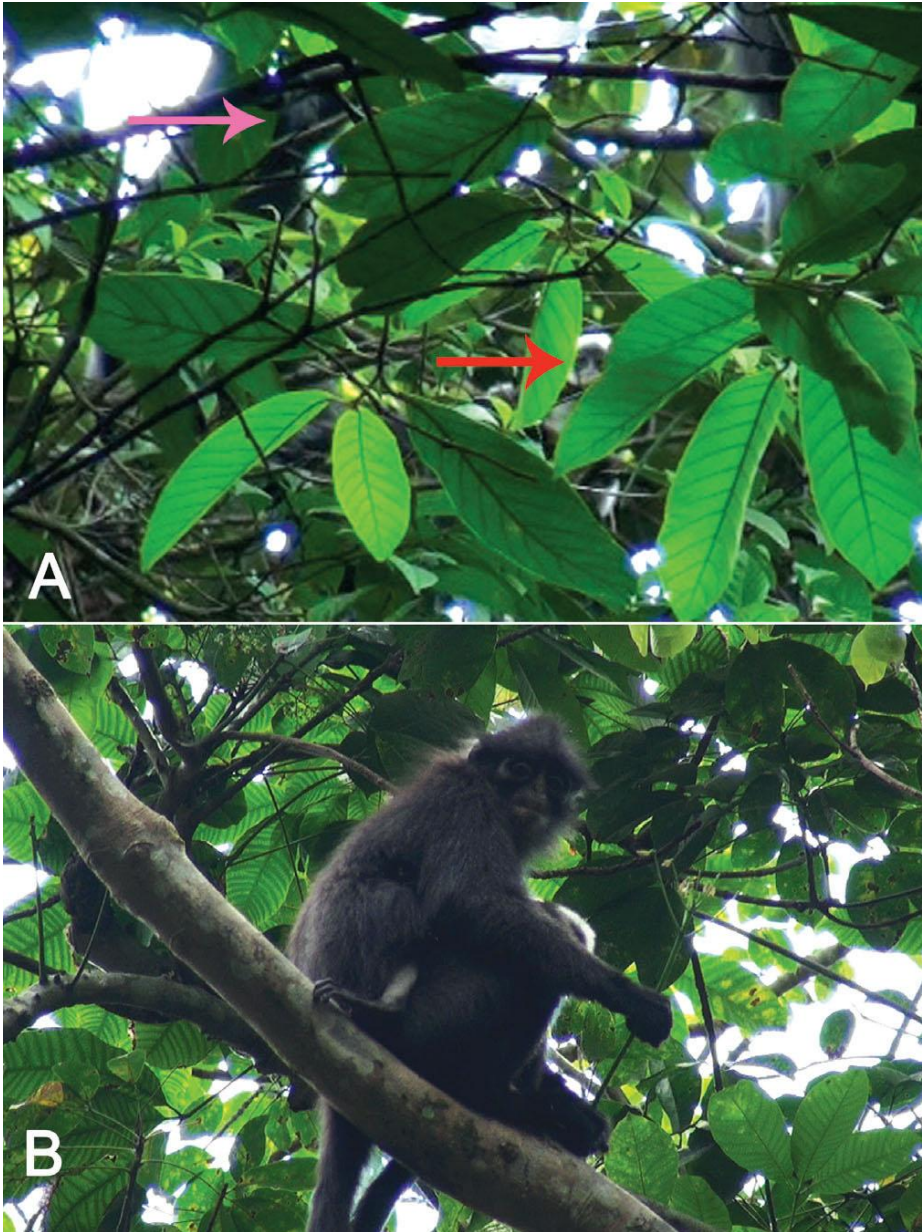


Figure 2.4: (A) Infant banded leaf monkey from Singapore (red arrow) separated from carrying adult (pink arrow); (B) Banded leaf monkey infant being carried by an adult

On 10 November 2009 and 12 November 2009, we saw the same white infant, but also one older, almost completely black infant in Group B. We considered this black infant older because its pelage color was almost identical to that of an adult, even though some white fur was still visible along its hind legs. This black infant was feeding on its own

(Fig. 2.5), but it was still being carried during travel. On 20 January 2010 and 21 January 2010, we again observed two black infants travelling independently of their carrying adults in Group B. They were believed to be approximately seven months old. In November 2008, we also observed one black infant with some white fur being carried by an adult in another group of five monkeys (Group C). This group comprised of at least two adults of which one was female. Based on pelage coloration, this infant was likely to have been born in July 2008. This black infant had successfully developed into a two-year old juvenile when it was sighted again on 14 June 2010, where there was also a new birth of a white infant in the same group.



Figure 2.5: Black infant banded leaf monkey feeding on vegetation

## 2.4 DISCUSSION

The banded leaf monkeys are critically endangered in Singapore due the small population size and it was unclear whether this species is still reproducing. Based on 22 months of field data, we confirmed that the banded leaf monkeys in Singapore are regularly reproducing, with at least six births from 2008 to 2010. Moreover infants have survived beyond seven months, indicating low infant mortality. Two births were recorded for a group of 14 monkeys (including infants) and two births in a group of 11 monkeys in 2009. In 2008 and 2010, one birth in a group of five monkeys was observed each year. According to the age categories and distinguishing criteria described by Bennett (1983) for *P. melalophos* (*P. femoralis* was previously recognized as a subspecies of *P. melalophos* (Chasen, 1940; Oates et al., 1994), it takes approximately six months from birth for the pattern on the head to change to adult color. Therefore, we estimate that the infants from Group A were born around July 2009. Even though we did not observe the infants' birth for Group B, we estimate based on size and pelage coloration that they were also born in July 2009. Similar inferences lead to the conclusion that one infant in Group C was also born in July 2008 and we observed a new infant in June 2010. Thus we can conclude that the banded leaf monkeys had at least one birth period in 2008, one birth period in 2009, and at least one birth period in 2010, all during the months of June/July for three consecutive years. For 2009 we can confirm that this was the only birth period, but since our field work was only carried out from September 2008 to June 2010, it remains unclear whether there were additional birth periods in 2008, and whether there will be a second birth period in the second half of 2010.

Singapore's weather is traditionally classified into four periods according to average prevailing wind directions (National Environmental Agency, 2009): *Northeast Monsoon Season* from December to early March, *Inter-monsoon Period* from late March to May, *Southwest Monsoon Season* from June to September, and *Inter-monsoon Period* from October to November. It appears that the birth season for the banded leaf monkeys in Singapore coincides with the Southwest Monsoon Season in June/July, which is a relatively dry month. *Presbytis melalophos* in Kuala Lompat, Malaysia gives birth not only in June, but also in the drier months of January and February (Bennett, 1983). Continuous monitoring from July 2010 to December 2010 is thus necessary to investigate if the banded leaf monkeys in Singapore also reproduce during the second half of the year, or if they only have one birth season each year.

The infants of the banded leaf monkeys in Singapore are white with distinctive cruciform black pattern on the dorsum, and no orange pelage color was observed throughout each infant's development. This finding is consistent with the natal pelage pattern observed for *P. f. percura* in east-central Sumatra and Johor. Thus, we can confirm that the infant coloration does not support a differentiation between the banded leaf monkeys in Singapore and in Johor.

The young of many primate species have distinctively colored, marked, or patterned natal pelage which is different from the adult pelage. Such age-specific physical traits could be used to distinguish dependent young from older, relatively independent individuals,



which may elicit care-giving and protection from older group members (Alley, 1980), hence increasing the survival chances of the young. The neonatal pelage coloration of the banded leaf monkeys is conspicuous and distinct from the adult pelage. As with other leaf monkeys (Newton and Dunbar, 1994), infant transfer and allocare were observed in the monkeys in Singapore. It is expected that the benefits of such social behavior provided by the reactions of conspecifics to the natal coat would outweigh any increased risk of attracting predators (Alley, 1980). The seemingly conspicuous infant coloration of the banded leaf monkey infants may also be a good camouflage such that it is difficult to catch sight of the infants when they are being carried. The infants are being carried ventrally, between the hind legs of the adult. As such, the white fur on the sides of the body, the arms and legs of infants are concealed by the black forearms of the adult, and the white crown of infants blends in well with the white chest pelage of the adult. Only the central black portion of the dorsum and the head of the infant, which is the same as adult pelage, are visible. This may explain the sighting of the black infant in Group B only in November.

## **2.5 CONCLUSION**

Overall, we provide evidence that the banded leaf monkey population in Singapore is reproducing. However, the population viability remains in doubt given the social constraints on reproduction and group formation (with most surviving individuals suspected to be genetically related since the population estimates are low, and mate choice very restricted), fertility, and genetic constraints. These data on infant coloration

and development of the banded leaf monkeys in Singapore also highlight the importance of long-term study and comparative work in order to understand the reproductive behavior of these endangered primates. In addition to pelage coloration and descriptive behavioral data, future genetic analysis is important for resolving the taxonomy of *Presbytis femoralis* and in order to develop a management plan for the species.

## **CHAPTER 3**

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# **MITOCHONDRIAL SEQUENCES AND THE TAXONOMY OF SINGAPORE'S BANDED LEAF MONKEY**

## 3.1 INTRODUCTION

### 3.1.1 Asian colobines and genus *Presbytis*

The classification and phylogeny of the family Cercopithecidae (Old World monkeys), especially the Asian colobines (subfamily Colobinae) have been debated for decades (Kirkpatrick, 2007; see Karanth et al., 2008). Asian colobines have been tentatively divided into two groups, the odd-nosed group consisting of the genera *Nasalis*, *Pygathrix*, *Rhinopithecus*, and *Simias*, and the langur/leaf monkey group consisting of genera *Presbytis*, *Semnopithecus*, and *Trachypithecus* (Groves, 1970; Jablonski, 1998). Although monophyly has recently been supported for the odd-nosed group, the sister group remains contentious (Sternler et al., 2006). The relationships among the langur and leaf monkey group have proved even more difficult to resolve. At one point langurs and leaf monkeys were all grouped in one genus (*Presbytis*) and only five species (*P. aygula*, *P. melalophos*, *P. frontata*, *P. rubicunda* and *P. potenziani*) were recognized (Napier and Napier, 1967; Tilson, 1976; Weitzel, 1983). However, today 32 species are recognized that fall into three genera, although the monophyly of the group is still being challenged (Ting et al., 2008). This species list keeps growing as species concepts change and more studies yield more information on the diversity of leaf monkeys (Redmond, 2008).

Studying the taxonomy of species has traditionally involved on morphological work based on museum specimens. However these data are now complemented with molecular data because of the paucity of specimens in collections. This applies in particular to rare and endangered species. As recently promoted, integrating morphology and DNA sequence

information has many advantages and provides a stronger basis for taxonomic conclusions (Dayrat, 2005). Thus, most work today relies on field observations and DNA-based analysis which complement morphology (Wiens, 2004).

Resolving the relationships of langurs and leaf monkeys has long been impeded by the lack of molecular data because obtaining DNA samples from these elusive species is difficult (Jablonski and Peng, 1993). Only a few DNA sequences are available for mostly two genera of this group, namely *Trachypithecus* and *Semnopithecus* (Messier and Stewart, 1997; see Osterholz et al., 2008). The lack of molecular data is especially pronounced in the genus *Presbytis*, one of the most diverse genera within Colobinae with complex distribution patterns. *Presbytis* is either not included, or only represented by one species in studies that investigate phylogenetic relationships of Asian colobines (Zhang and Ryder, 1998; Sterner et al., 2006; Whittaker et al., 2006; Osterholz et al., 2008; Ting et al., 2008). Hence, the number of species and subspecies of *Presbytis*, and their relationships are still disputed.

### **3.1.2 Taxonomy of banded leaf monkey**

The banded leaf monkey (*Presbytis femoralis*) is a species of Asian colobine which is found in the Malay Peninsula and Sumatra (Groves, 2001; Meijaard and Groves, 2004). At least three subspecies are currently recognized and the taxonomy is still unresolved: *P. f. femoralis* occurs in Singapore and Johor, Malaysia, *P. f. robinsoni* in the northwest Malay Peninsula, extending north throughout peninsular Thailand and Burma, and *P. f. percura* in eastern Sumatra (Fig. 2.1).

Although *P. f. femoralis* and *P. f. robinsoni* are currently classified as “banded leaf monkeys” (Brandon-Jones et al., 2004), recent molecular analysis using mitochondrial DNA markers (ND3, ND4L and ND4) and associated tRNAs genes clustered *P. f. robinsoni* as part of an unresolved polytomy with *P. f. femoralis* (Fig. 3.1, Md-Zain et al., 2008).

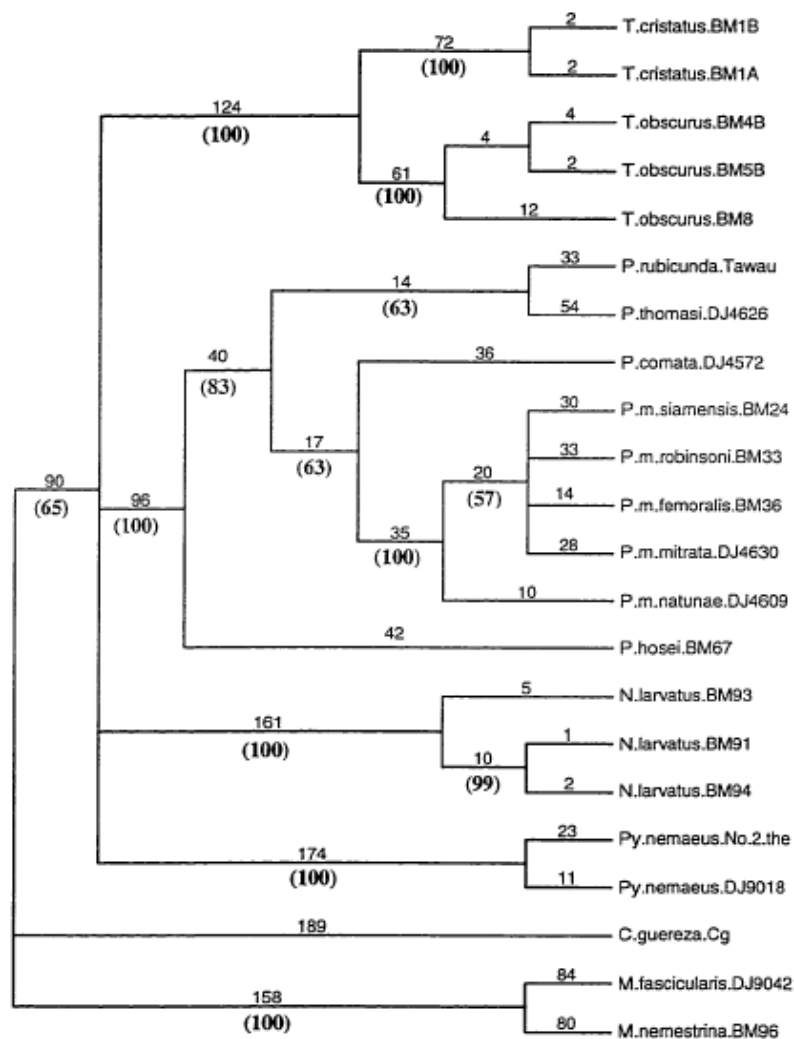


Figure 3.1: Maximum parsimony heuristic bootstrap mtDNA tree for *Presbytis* and *Trachypithecus* species (Figure 4 in Md-Zain et al., 2008). The bootstrap support values are shown below the branches of the tree

*Presbytis femoralis* was first described by Martin in 1838 based on one specimen from Singapore, making Singapore the type locality (see Miller, 1934). The taxonomy of *P. f. femoralis* in Singapore and Johor is in dispute based on alleged difference in adult pelage coloration. Chasen (1940) described that the banded leaf monkeys from South Johor have more white fur on the underparts, although in some cases specimens from Singapore and Johor populations were difficult to separate. More recently Hüttche (1994) examined potential subspecies characteristics such as pelage coloration, vocalization and cranial measurements in order to infer taxonomic affinities between banded leaf monkeys. Based on 12 specimens, six from Singapore and six from Johor, he concluded that both populations shared similarities in most pelage features, except for a minor difference in the coloration of the ventral pelage, with the Singapore specimens being generally darker than those from Johor. Hence he deduced that this may represent individual-level variability that does not warrant the recognition of a separate subspecies. Similarly, analyses of their vocalizations and cranial measurements revealed no obvious differentiation between the two populations. However, there continued to be recurrent speculations that the Singapore population belongs to a different subspecies (Chasen, 1940; Lucas et al., 1988; Ng and Lim, 1992; Pitra et al., 1995).

Prior to my study, there was no genetic data of banded leaf monkey from Singapore. However, molecular information for banded leaf monkey from Singapore would be valuable for clarifying subgeneric relationships within *Presbytis*, and for testing the subspecies boundary based on morphology.

### **3.1.3 Conservation of banded leaf monkey in Singapore**

The banded leaf monkey (*P. f. femoralis*) is critically endangered in Singapore (Lim et al., 2008) and threatened by widespread deforestation in southern Malaysia. As a species first described from Singapore, this species represents part of our national and natural heritage and deserves a high conservation priority. Several conservation strategies have been proposed to ensure the long-term survival of the species in Singapore; one strategy may include the release of banded leaf monkeys from Johor. Such reintroduction or restocking provides an immediate and direct means of reviving declining populations by adding new reproductive individuals. Before the actual translocation of individuals could take place, one has to determine if the different populations belong to the same species and subspecies. However, because the subspecies status between these two populations in Singapore and Johor remained in doubt, introductions should not even be considered until genetic data from these two populations become available and confirm that the populations belong to the same subspecies.

### **3.1.4 Genetic markers**

Mitochondrial rather than nuclear sequence data are usually used in population-level assays of genetic variation among species. Mitochondrial DNA (mtDNA) evolves more rapidly than most nuclear DNA (Wilson et al., 1985; Mindell and Thacker, 1996). Thus mtDNA has become the molecular marker of choice in many phylogenetic and phylogeographic studies (see Avise, 1986; Moritz et al., 1987) and a large comparative database of mitochondrial sequence data is available. Specifically, 12S rRNA and cytochrome *b* (*cyt-b*) of the mtDNA are the markers of choice in most primate genetic



studies (Zhang and Ryder, 1998; Heckman et al., 2006; Karanth et al., 2008; Osterholz et al., 2008). However, use of mitochondrial DNA to infer phylogenetic relationships also carries several disadvantages that could produce incorrect or biased inferences, including the possible sequencing of nuclear transferred mitochondrial fragments (numts) as has been demonstrated by Collura and Stewart (1995). However, by applying methods that mitigate the risk of amplifying numts (see Raaum et al., 2005; Thalmann et al., 2004), it is possible to avoid this problem.

While mitochondrial genes such as 12S rRNA and *cyt-b* are often appropriate for phylogenetic analysis of temporally deep branches, a more quickly evolving locus is necessary to identify intra-generic species relationships of groups that may have radiated over a relatively shorter time span (Whittaker et al., 2007). The hypervariable region I of the displacement loop, or *D-loop*, is the most rapidly evolving part of the mitochondrial genome (Walberg and Clayton, 1981; Chang and Clayton, 1985). This region is useful for examining intra-specific relationships between closely related species (Avice, 2000) and can complement other mtDNA genes in phylogenetic analyses. Thus for this study, three gene loci namely, 12S rRNA, *cyt-b* and *D-loop* were used for comparisons between banded leaf monkeys in Singapore and Johor, and other colobines.

## **3.2 MATERIALS AND METHODS**

### **3.2.1 Fecal collection and DNA extraction**

Given that it is extremely risky and difficult to acquire fresh blood from unhabituated and rare arboreal primates, an alternative source of DNA from feces was used. Fecal samples were collected from the field opportunistically. Whenever a group of banded leaf monkeys were found, they were followed for as long as possible. If defecation was observed, the “drop-off” point was immediately located and searched for excrement. Feces were collected into zip-lock bags and stored at -70°C in lab. Genomic DNA was extracted from 50mg of feces using QIAamp® DNA Stool Mini Kit (QIAGEN, Singapore). The DNA was recovered in 40µl of elution buffer, aliquotted and stored at -20°C. Amplification of genomic DNA was carried out for fecal samples that worked the best in order to preserve sufficient template DNA for further genetic work.

### **3.2.2 DNA amplification and sequencing**

Mitochondrial DNA was sequenced using known and designed primers (Table 3.1). A direct polymerase chain reaction (PCR) amplification was carried out in a total volume of 25µl consisting of 1.0µl BSA, 2.0µl dNTP, 0.8µl Thai Taq, 2.5µl Taq buffer, 1.2µl each primer, and between 2µl and 5µl template DNA. Nuclease-free water was added accordingly. Amplification conditions on a thermal cycler (Eppendorf®, BRAUN Singapore) were: initial denaturation at 95°C for 5 minutes, 40 cycles of 1 minute at 94°C, 1 minute at 45°C to 61°C (see annealing temperatures, Table 3.1), 1 minute at 72°C, and a final extension of 5 minutes at 72°C. The resulting amplification products

were electrophoresed on 1% agarose gels, and visualized by ethidium staining. A negative PCR control was processed along with each set of PCR reactions. For each fecal extraction multiple PCRs (six or more) were attempted at three gene regions (12S rRNA, *cyt-b* and *d-loop*). A successful PCR reaction was defined as one producing specific product in the expected size range. Amplified products were purified using Bioline SureClean (Randolph, MA). Terminator sequencing reactions were then performed in both forward and reverse directions in 10µl volumes using BigDye ver. 3.1 (Applied Biosystems, Foster, CA). A final purification was performed with Agencourt® CleanSEQ® kit (Agencourt Bioscience Corporation, Beverly, MA) followed by direct sequencing in an ABI PRISM® 3100 Genetic Analyzer (Perkin Elmer Applied Biosystems, Norwalk, CT) following the manufacturer's instructions.

Table 3.1: Primer sequences utilized in analysis of DNA from banded leaf monkey feces

Gene region	Primer name	Primer sequences (5'-3')	Gene size range (bp)	Annealing temp. (°C)	Reference
12S rRNA	L14724	F: CTGGGATTAGATACCCCACTAT	300 – 400	56, 58	pers comm. Md-Zain
	H15149	R: GAGGGTGACGGGCGGTGTGT			
Cyt- <i>b</i>	L14724*	F: CGAGATCTGAAAAACCATCGTTG	1140	54	Karanth et al. 2008
	H15915	R: AACTGCAGTCATCTCCGGTTTACAAGA			
	L15369*	R: TTCCTACACGAAACAGGATCAAAYAAAYCC	55, 57, 60	Karanth et al. 2008	
	690415	F: CTCCTCATTGAAACATGAAATAT	620	45	Geissmann et al. 2004
	690416	R: CTTTGTTGTTTGGATTTGTG			
	6720	F: CTMTTCTACACGAAACAGG	600 – 700	56	pers comm. Meyer
	6721	R: AATACAGAAAGTAGTTTAAATAG			
6435	F: ACAAGAGTGCTACTCTCCTC	650 – 700	56	pers comm. Meyer	
2068	R: ATTGATTTACGGAGGATGGT				
6405	F: AATGATATGAAAARYCATCGTTG	650 – 800	56	pers comm. Meyer	
6719	R: TTRTCTGGGTCGCTYAAAAG				
D-loop	hf_dloop_F 6234_HVI_r	F: GCCCTTATGTAATTCGTGCATTAC R: TGATAGACCCGTGATCCATC	480	58	This study; pers comm. Meyer

\*Also semi-nested PCR.

### 3.2.3 Primer design

Due to the lack of *Presbytis*-specific primers in published literature, published primers of *cyt-b* gene of *Trachypithecus* and *Semnopithecus* from Karanth et al. (2008) and of leaf monkeys from Geissmann et al. (2004) were initially used in this study. Specific 12S rRNA, *cyt-b* and *d-loop* primers for *Presbytis* were later obtained from Md-Zain (pers. comm.) and Meyer (pers. comm.). However, the *d-loop* region was so variable that the *Presbytis*-group specific *d-loop* primers were still too general for *P. femoralis femoralis*, especially the forward primer. In order to design a forward primer that is sufficiently specific to the *d-loop* region of banded leaf monkeys, I compared published *d-loop* sequences for colobine monkeys in GenBank (National Center for Biotechnology Information, Bethesda, MD) using Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI) and searched for highly conserved regions. The region which was the most conserved was selected as a primer binding site. The designed oligonucleotide sequence (hf\_dloop\_F; see Table 3.1) was tested for functionality and checked against the formation of hairpin, self-dimer or hetero-dimer using OligoAnalyzer 3.1 (Integrated DNA Technologies Inc. 2010).

### 3.2.4 Sequence alignment and phylogenetic analysis

Sequences were edited, checked for accuracy (e.g. translatable to amino acid sequence), concatenated and aligned in Sequencher 4.6. 12S rRNA and *cyt-b* sequences obtained from the banded leaf monkey in Singapore were compared with four populations in Johor, Malaysia: Kluang, Kota Tinggi, Mersing and Pontian (pers. comm., Md-Zain). Cytochrome *b* sequences from Singapore and Johor were also aligned with sequences

from Colobinae obtained from Genbank. Parsimony analysis to infer the gene-tree for *cyt-b* of the colobines was performed in TNT ver. 1.1 (Goloboff et al., 2008). Strict consensus tree was obtained and support was determined with bootstrap resampling for 250 replicates with 10 random addition sequences per replicate. One African colobine, the Angolan black-and-white colobus monkey (*Colobus angolensis*) was designated as outgroup.

### 3.3 RESULTS

#### 3.3.1 Fecal samples

A total of five fecal samples were collected in the field (Table 3.2). One defecation event of a banded leaf monkey was caught on video and can be viewed from <http://www.youtube.com/watch?v=fSJ33zc0UTM>. All collections were made within one to 10 hours of defecations. If initial PCRs failed, additional optimization of thermal profiles and reaction volumes, and re-extraction of difficult samples were performed. Of the five fecal samples, four were used in this study while one remained untouched. Only one sample yielded positive results (sample collection on 27 Jan 2010). I also amplified the genomic DNA from this sample for future analysis.

Table 3.2: Fecal sample collections

Date	Location	Time defecated	Time collected	Remarks
05 Apr 2009	Nee Soon	0845	1200 (1)	Landed on swamps
29 Aug 2009	Old Thomson Road	1105	1145 (1); 1315 (2)	Landed on grass along road
27 Jan 2010	Upper Seletar	0725	0825 (2)	Run over by truck
11 Mar 2010	Upper Seletar	Same day	1650 (1)	Landed on road
30 Mar 2010	Old Thomson Road	Morning	1035 (2)	Run over by bicycle

Number in brackets indicates the number of samples collected; may or may not be from the same individual.

### 3.3.2 Molecular data

I obtained three mitochondrial sequences from one fecal sample from one individual: 12S rRNA of 346 bases long, *cyt-b* of 453 bases and *d-loop* of 432 bases. They were obtained using primer pairs L14724 and H15149, 6720 and 6721, hf\_dloop\_F and 6234\_HVI\_r respectively. All other *cyt-b* primers which were not *Presbytis*-specific, namely from Geissmann et al. (2004) and Karanth et al. (2008), did not generate amplifications. 12S rRNA and *cyt-b* sequences were also obtained from 12 specimens of banded leaf monkeys from four populations in Johor (pers. comm., Md-Zain). The 12S rRNA sequences from banded leaf monkeys in Singapore and all four populations in Johor are identical, and for *cyt-b* only small differences ranging from 0.258% (1bp) to 1.804% (7bp) are observed (Table 3.3). *D-loop* sequences were not available from specimens in Johor for comparison with Singapore population.

I retrieved 93 sequences representing 25 species of colobine monkeys from Genbank, of which only four specimens representing three species were from *Presbytis* (*P. femoralis robinsoni*, *P. melalophos* and *P. comata comata*; Table 3.3). For *cyt-b*, genetic differences otherwise typical for species were found between *P. f. femoralis*, *P. melalophos* and *P. c. comata* (9.278% to 13.918%). There is also a 9.536% pairwise distance between the supposedly closely-related subspecies *P. f. femoralis* and *P. f. robinsoni*.

Table 3.3: Pairwise distances between Singapore population and other *Presbytis* species for cytochrome *b* sequences

Species	Localities/Specimens	Pairwise distances (%)
<i>Presbytis femoralis femoralis</i> (Johor)	PmfBM39 Mersing	0.258 (1bp)
	PmfBM41 Mersing	0.258
	PmfBM47 Pontian	0.258
	PmfBM37 Kluang	0.515 (2bp)
	PmfBM38 Kluang	0.515
	PmfBM40 Mersing	0.515
	PmfBM42 Mersing	0.515
	PmfBM45 Kota Tinggi	0.515
	PmfBM46 Kota Tinggi	0.515
	PmfBM35 Kluang	0.773 (4bp)
	PmfBM36 Kluang	0.775
	PmfBM43 Kota Tinggi	1.804 (7bp)
<i>P. melalophos</i>	gi 157361360	9.278
<i>P. f. robinsoni</i>	gi 46410401	9.536
<i>P. comata comata</i>	gi 46410399	11.340
<i>P. melalophos</i>	gi 88174260	13.918

The parsimony analysis of *cyt-b* data of 25 species of colobines found four trees. Bootstrap consensus tree was obtained (Fig. 3.2). African (*Colobus* and *Ptilocolobus*) and Asian colobines formed distinct clades. However, within Asian colobines, *cyt-b* was not sufficient to resolve the phylogenetic relationships of all species; only *Nasalis*, *Presbytis*, *Pygathrix*, and *Rhinopithecus* formed distinct clades. All banded leaf monkey specimens from Johor clustered with the Singapore population. Several *Trachypithecus* species (*T. geei*, *T. johnii*, *T. pileatus* and *T. vetulus*) cluster with *Semnopithecus*.



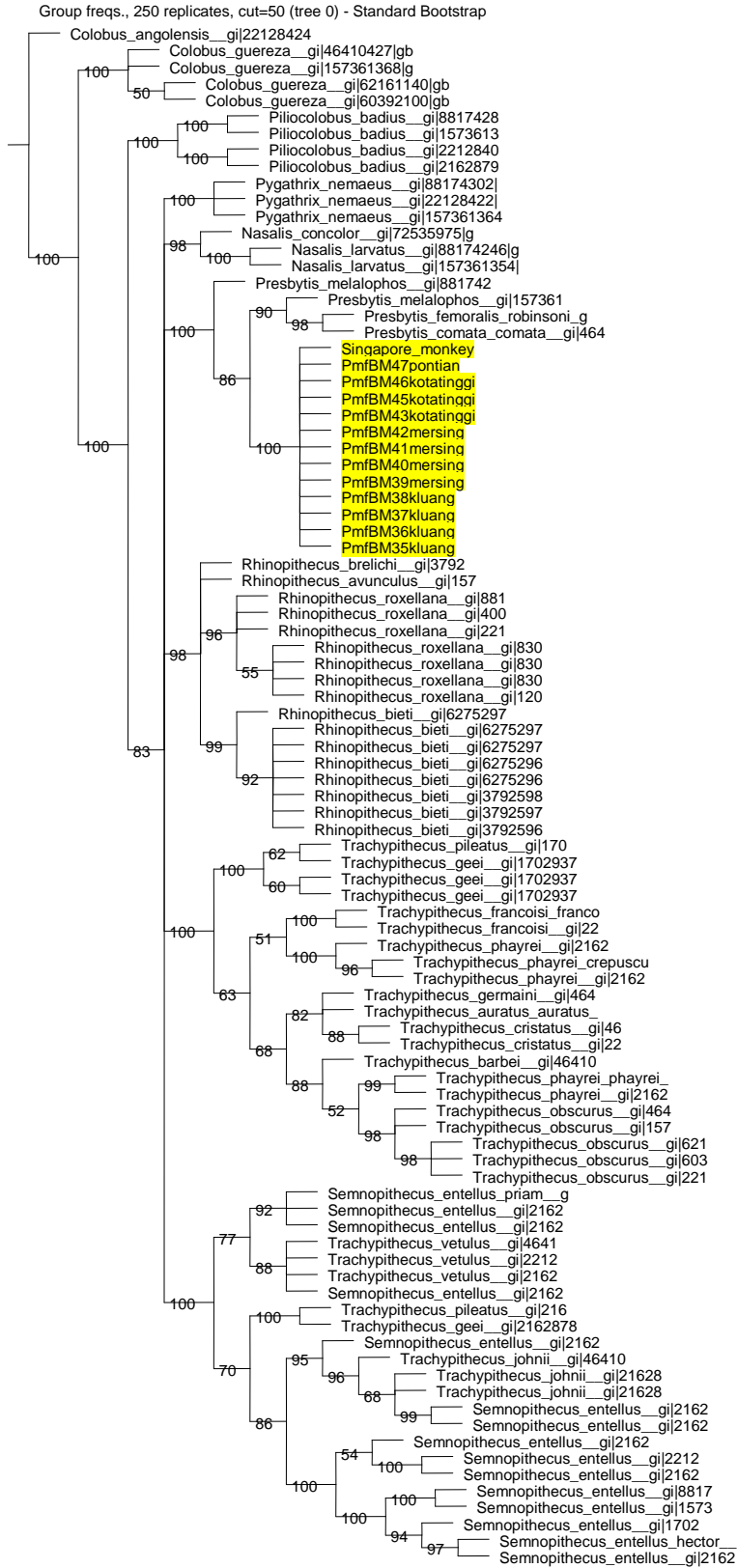


Figure 3.2: Consensus tree of 25 taxa (93 sequences) of colobines using maximum parsimony. Bootstrap support values are shown on the branches

## 3.4 DISCUSSION

### 3.4.1 Conservation of banded leaf monkey in Singapore

#### Genetic relationship of populations in Singapore and Johor, Malaysia

The banded leaf monkeys of Singapore and Johor share identical 12S rRNA sequence, and there is only 0.25% to 0.51% genetic differentiation in cytochrome *b* (*cyt-b*). Since most primate species differ by more than 3% for *cyt-b* (Zhang and Ryder, 1998; Li et al., 2001) and intraspecific variation in the more conservative barcode region of *COI* rarely exceeds 2% (Avice 2000), it appears likely that the Johor and Singapore populations belong to the same species and the genetic data also do not indicate the presence of two subspecies. Only one specimen (PmfBM43) from Kota Tinggi is rather different from all other specimens (Fig. 3.3), which indicates that either there is some population-level differentiation within the Johor population of *Presbytis femoralis*, or that a nuclear pseudogene may have been sequenced. Under certain circumstances, nuclear pseudogenes can be amplified more efficiently than the intended mtDNA target (Collura et al., 1996), which will then confound results. Hence it is important to check this specimen from Kota Tinggi for the presence of pseudogenes and morphological differences before further comparison should be made.

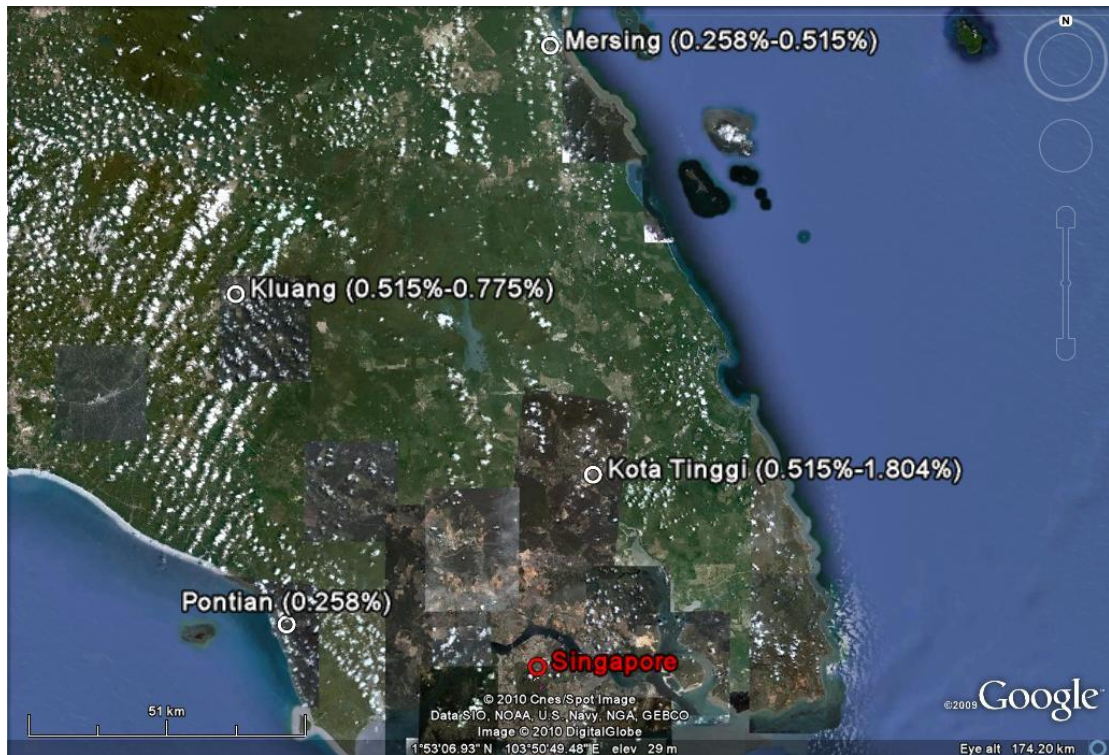


Figure 3.3: Map of Singapore with reference to four localities in Johor where genetic data of banded leaf monkeys are available. Percentages in parentheses indicate *cyt-b* pairwise distances as compared to Singapore population

### Reintroduction and restocking of banded leaf monkeys in Singapore

The banded leaf monkey is critically endangered in Singapore due to its small population and its limited range (an isolated 455ha forest reserve in the Central Catchment Nature Reserve, CCNR, National Parks Board, 2007). In Johor, the banded leaf monkeys face threats from habitat destruction and widespread deforestation. Loss of habitat can accelerate the pace of extinction of banded leaf monkeys (Pitra et al., 1995). In order to address the species' decline in Singapore, a reintroduction or restocking initiative could be considered (Hüttche, 1994; Pitra et al., 1995). Since I have provided evidence that the Singapore and Johor populations of banded leaf monkeys likely belong to the same species and subspecies, captive individuals from Johor could be translocated into

Singapore to increase population size. Such a reintroduction could involve transferring individuals into a formerly occupied habitat like the Bukit Timah Nature Reserve (BTNR), while restocking would involve adding individuals into the present range which is the CCNR. Banded leaf monkeys have historically been found in the BTNR, but they went extinct in the 1980s. Therefore, even though the BTNR is still the prime choice for reintroduction because of its intact primary and secondary forests, the habitat has to be assessed for its carrying capacity and food resource availability to support populations before translocation of the monkeys could be supported. Continuous monitoring of the existing populations in the CCNR is important to collect additional data on diet (see Chapter 1), reproduction (see Chapter 2), movement patterns (Molony et al., 2006), and range use (Ostro et al., 1999), complemented with habitat parameters of the BTNR, to predict the population viability of banded leaf monkeys in the reserve.

Smaller populations occurring in separate ranges (e.g. CCNR and BTNR) may be more viable than a single larger one, provided that the environmental variation influencing each different areas is at least partially independent (see Soulé, 1987). However, the existing small population of banded leaf monkeys in the CCNR is likely to experience genetic bottlenecks as demonstrated in many small populations of primates (e.g. Delacour's langur, Cat Ba langur, Mittermeier et al., 2005; Bornean orangutan, Gossens et al., 2006). Populations that are small will lose genetic diversity more rapidly through genetic drift and inbreeding (Wright, 1931; Lande and Barrowclough, 1987; Nei, 1987), resulting in loss of fitness and decreased population viability (Frankel, 1974; Wright, 1977). Hence, a more pressing issue in the conservation of banded leaf monkeys in Singapore may be to

increase the genetic diversity through restocking. In this case, the carrying capacity of the CCNR and intraspecific interactions will be important components in determining the long-term feasibility of restocking. If the habitat in the CCNR can support more banded leaf monkeys at present, and additional individuals from Johor can integrate with the local population to elevate genetic diversity, restocking may be a viable solution to conserve the species.

The National Parks Board is developing an “Eco-Link” by 2013 in order to reestablish arboreal connectivity between the CCNR and the BTNR (National Parks Board, 2009). Such linkage may promote biodiversity exchange between the two reserves. It is thus important to monitor if it is being actively used by the banded leaf monkeys in the CCNR to recolonize the habitat at the BTNR. If they are utilizing the green corridor, habitat availability (and probably the food resource) is effectively increased. This may potentially encourage population growth of banded leaf monkeys, and plans for reintroduction and restocking will have to be reassessed through further monitoring efforts.

Overall, while ex-situ translocation of banded leaf monkeys is one approach to support dwindling wild populations, it is necessary to first examine population and demographic changes, and genetic diversity of the banded leaf monkeys in Singapore. If the population in Singapore grows well and there is sufficiently high genetic variability among individuals, the population may potentially recover without intervention. After all, only 44% of translocations of threatened species have been successful (Griffith et al., 1989);

translocation of primate species is a long-term and high-risk process involving captive studies and management, and post-release monitoring which may be too stressful for animals to follow through with.

#### Inter-group genetic relatedness of banded leaf monkeys in Singapore

One important component in the conservation of banded leaf monkeys is to analyze population structure and dispersal patterns of the species, assess their parentage and individual identities, estimate degree of relatedness between populations or pairs of individuals (Di Fiore, 2003), and reproductive strategies (Bradley et al., 2000). Microsatellite loci allow for elucidating the genetic structure of individual populations and the extent of inbreeding. Microsatellites are regions of the genome comprising variable numbers of tandem repeats of a 1-6 base-pair nucleotide motif. Using amplified genomic DNA, microsatellite sequences can be elucidated. Most importantly for field applications, microsatellite marker genotype requires only small amounts of template DNA since it is based on PCR (Mullis and Faloona, 1987). Therefore in addition to mitochondrial sequences, microsatellites should be explored as an additional tool for investigating the genetic diversity of banded leaf monkeys in Singapore.

#### **3.4.2 Asian colobines**

Johns and Avise (1998) reported that the mean genetic distances for congeneric mammal and reptile species generally exceed 3% in the mitochondrial *cyt-b* gene. Hence based on *cyt-b*, the banded leaf monkeys in Johor and Singapore are likely distinct species from the populations in north Malay Peninsula, given their 9.536% distance for this gene. This

would elevate *P. f. femoralis* to species status and restrict its distribution to the Southern Malay Peninsula only, highlighting a higher conservation priority for the native primate of Singapore. A reclassification of its IUCN species status will be necessary after evaluating the genetic relatedness between *P. f. femoralis* and *P. f. percura* in Sumatra.

The evolutionary history of Asian colobines is not well understood. Although monophyly of the odd-nosed monkeys (*Nasalis*, *Pygathrix*, and *Rhinopithecus*) was recently confirmed (Sterner et al., 2006), the genetic relationships among langurs (*Presbytis*, *Semnopithecus*, and *Trachypithecus*) remained unclear. The main problem in *Presbytis* is the paucity of data. Currently, sequences are only available for few species like *P. comata*, *P. femoralis*, *P. hosei*, *P. melalophos*, *P. rubicunda*, and *P. thomasi* (Md-Zain et al., 2008), making the task of elucidating evolutionary relationships within *Presbytis* and with other langur genera difficult. In addition, the phylogenetic relationships among the three langur genera may need to be resolved based on nuclear data. For example, Osterholz et al. (2008) have suggested a sister grouping of *Semnopithecus* and *Trachypithecus* using mitochondrial *cyt-b* and a portion of sex-determining region, Y-chromosome (SRY) gene combined with presence/absence analysis of retroposon insertions. This approach analyzes paternal-, maternal- and biparental-inherited markers which allow the detection of hybridization or introgression events between different lineages (Tosi et al., 2002; Arnold and Meyer, 2006). Moreover, retroposon insertions have been proven useful to resolve relationships in various primate lineages e.g. Strepsirrhine (Roos et al., 2004) and Old World monkeys (Xing et al., 2005).

In order to increase the resolution of the phylogenetic relationships within *Presbytis* and among Asian colobines, retroposon insertion analyses should be employed in combination with various other genetic markers, for example a faster evolving *d-loop* gene. Although it is difficult to obtain *d-loop* gene especially via feces, I have successfully retrieved *d-loop* information for the banded leaf monkeys in Singapore. The data can be used in future comparative analyses to provide a better understanding of the molecular relationships among *Presbytis*.

### **3.4.3 Shortcomings of this study and suggested improvements**

The genomic DNA of the banded leaf monkeys was successfully extracted from their feces and all target genes (i.e. 12S rRNA, *cyt-b* and *d-loop*) were amplified, which is difficult to achieve. However, I did encounter two problems that usually arise when noninvasively-collected samples like feces are used as sources of DNA. Firstly, fecal samples yield extremely low quantities of DNA and because of initial low template copy number, PCR amplification frequently fails. Secondly, DNA extracted from fecal samples is commingled with DNA from plant items in the diet and from intestinal-tract microbes, which may provide a competing template for PCR and produce spurious alleles as amplification artifacts (Bradley and Vigilant, 2002). Moreover, some plant secondary compounds can inhibit PCR. As such, only 25% of fecal samples yield positive results. In order to avoid the latter problem, only highly-specific primers were selected to be used in the genetic analyses. Several molecular analyses of colobines relied on universal primers recommended by Kocher et al. (1989), Li et al. (2004), and Whittaker et al. (2006), but these primers were not useful in amplifying target genes of the banded leaf monkeys.



The third and most fundamental reason behind failure in DNA extraction from fecal samples is degraded DNA if feces are not sufficiently dessicated in the field before freezing at  $-70^{\circ}\text{C}$  in lab. Banded leaf monkey feces were collected in zip-lock bags and brought back to the lab for storage immediately after collection. Improvements can be made to the collection of fecal samples in the field. The fecal sample that yielded data was collected within an hour of defecation and stored in  $-70^{\circ}\text{C}$  within another 30 minutes. The use of silica gel beads (e.g. Sigma® Type II 1/8" silica gel beads) to dessicate fecal samples stops hydrolytic degradation of DNA and has been shown to help with many primate genetic analyses (e.g. Bradley et al., 2000). Samples stored using this method are stable at room temperature for many months.

Full-length genes can be amplified using high quality DNA samples like blood or tissue samples (see Karanth et al., 2008). However if only low quality DNA samples from fecal samples are available, it is difficult to obtain sequence for an entire gene locus. Amplification products which are shorter have a higher rate of amplification efficiency and accuracy (Bradley et al., 2000). Therefore in the case of the banded leaf monkeys where only fecal samples are currently available, shorter-length gene regions will yield better success rates.

### 3.5 CONCLUSION

The banded leaf monkeys in Singapore and Johor, Malaysia are likely to belong to the same species and subspecies, *Presbytis femoralis femoralis*, based on mitochondrial DNA sequences. Conservation plans to translocate captive individuals from Johor into Singapore to encourage population growth of banded leaf monkeys could then be explored. However, whether the nature reserves in Singapore could support additional populations, and whether it is necessary to intervene recovery of the Singapore population require further investigations. In addition, contrary to current subgeneric classification of *P. f. femoralis* and *P. f. robinsoni*, cytochrome *b* gene reveals that they are likely different species. An examination of the genetic distance between *P. f. femoralis* and the other subspecies, *P. f. percura*, will be necessary to reevaluate the taxonomy of the species and the IUCN conservation status of banded leaf monkeys.

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## APPENDICES

Appendix I: The number of banded leaf monkeys recorded range from 1 to 14, with the highest number last recorded in 1998. Excluding data from my study, from 2001 to present, the highest number of banded leaf monkeys spotted was no more than 8; the most common group size seen was 2 to 3.

No.	Date	Time	Quantity	Location	Observations	Observer(s)
1	19-Jul-86		2	Seletar Reservoir	Adult with orangey youngster	SY, SRJ
2	07-Aug-86		1	Seletar Reservoir	Adult sitting	SRJ
3	14-Aug-86		1	Seletar Reservoir	Adult moving	SY, SRJ
4	15-Mar-87		4	Nee Soon	2 adults, 2 immatures	Several
5	10-Apr-87		10	Nee Soon	4 adults, 3 subadults, 3 juveniles	SS, SRJ
	24-Apr-87		≥2	Nee Soon	1 adult, 1 black infant	CJH
6	04-Oct-87		1	Bukit Timah	Female found dying, RMBR	HKL, CMY
7	02-Nov-87		1	Nee Soon	Male sitting on tree	SRJ
8	03-Dec-87		2	Nee Soon	1 adult, 1 immature	PB
9	03-Dec-87		4	Nee Soon	Moving	SY
10	01-Apr-88		2	Bukit Timah		DHM
11	25-Mar-89		3	Nee Soon	2 adults, 1 juvenile foraging	YSH
12	13-May-89	18:00	2	Mandai	Call was heard	YSH
13	28-May-89		1	Nee Soon	On trees	HWH
14	17-Sep-89	12:00		Mandai	Call was heard	TML, YSH
15	31-Dec-89	18:30	3	Nee Soon		KVL, PNG, TSL
16	10-Mar-90	16:15	8	Nee Soon		TML, YSH
17	01-Apr-90		10	Nee Soon	Include 2 subadults, 1 infant	TML, YSH
18	22-Apr-90		13	Seletar Reservoir		TML, YSH
19	05-May-90		6	Seletar Reservoir		TML, YSH
20	15-Jul-90		2	Nee Soon		TML, YSH
21	26-Aug-90		6	Seletar Reservoir		Several
22	25-Dec-90			Seletar Reservoir	Call was heard	YSH
23	01-May-91		10	Old Upp Thomson Rd	At least 10	RFO, EL, UR
24	04-Aug-91			Nee Soon	Call was heard	Several
25	31-Aug-91		14	Nee Soon		TML, YSH



26	01-Dec-91		5	Nee Soon		TML, YSH
27	19-Jan-92		5	Nee Soon		TML, YSH
28	03-Feb-92		7	Nee Soon		YSH
29	15-May-92		4	Nee Soon	At least 4	KVL, HKL, CMY
30	18-May-92		14	Nee Soon	2 separate troops	TML, YSH
31	22-May-92		4	Nee Soon	At least 4	KVL, VCO
32	02-Jun-92		5	Nee Soon		Several
33	19-Mar-93		3	Nee Soon	Moving fast	CH, ST
34	23-Mar-93	19:10	5	Nee Soon	Feeding on leaves	TML, YSH
35	25-Mar-93	17:40	6	Nee Soon	3 adults, 3 juveniles	CH
36	29-Mar-93	18:10	1-2	Nee Soon	Foraging, long rests	CH
37	06-Apr-93	18:15	6-8	Nee Soon	Machine gun call due to observer; moved away	CH, DBJ
38	07-Apr-93	07:20	2-3	Nee Soon	Moving to north	CH
39	25-Apr-93	09:11	1	Nee Soon	1 adult moving to north	CH
40	01-May-93	09:15	1	Nee Soon	Moving away fast	CH
		17:46	1	Nee Soon		CH
41	03-May-93	17:43	8	Nee Soon	Machine gun call due to observer; moved away	CH, LK
42	04-May-93	08:05	2	Nee Soon	Moving to south	CH
43	05-May-93	08:24	6	Nee Soon	Several adults, 1 juvenile; Moving to north	CH, LK
44	06-Jun-93		3	Nee Soon	Include 1 infant	TML, YSH
45	12-Jun-93	08:49	2-3	Nee Soon	Moving away	CH
46	22-June-93	19:45		Nee Soon	Two groups alternating calls	CH
47	23-Jun-93	06:10		Nee Soon	Two groups alternating calls	CH
		20:05		Nee Soon	Two groups alternating calls	CH
48	05-Jul-93	04:15		Seletar Reservoir	Soft calls	CH
49	10-Jul-93		10	Nee Soon	Include 3 infants	TML, YSH
50	19-Jul-93	19:40		Nee Soon	1 call	CH
51	03-Oct-93		5	Nee Soon		TML, YSH
52	09-Feb-94	12:00	8	Nee Soon	Feeding	YSH
53	27-Feb-94		7	Nee Soon	6 adults, 1 juvenile	SRJ
54	21-May-94		8	Nee Soon		TML, YSH
55	25-Sep-00	Morning		Nee Soon		Ding Li
56	19-Aug-01	18:00	8	Nee Soon	Followed by drongos	Ding Li

57	04-Dec-01	Morning	2	Nee Soon		Ding Li
58	12-Oct-04		3	Seletar Reservoir	Moving	Ben Lee
59	17-Oct-04	Afternoon	7	Old Upp Thomson Rd	Moving	Kwokwai
60	30-Jan-05	12:00	6	Upper Peirce Reservoir	4 adults, 2 juveniles	Several
61	18-Feb-05	15:30	2	Upper Peirce Reservoir	Feeding	Several
62	10-Apr-05	08:15	1	Nee Soon		Kelvin Lim
		Afternoon	3	Nee Soon	Feeding	Kwokwai
63	11-Sep-05	Morning	2	Nee Soon		Kwokwai
64	03-Dec-05	Afternoon	2	Seletar Reservoir		Kwokwai
65	09-Dec-05	Morning	3	Upp Thom Rd, lamppost 299	Sitting on raintrees	Kwokwai
66	18-Dec-05		2	Lower Peirce Boardwalk	Moving	Kwokwai
67	23-Dec-05	15:30	5	Nee Soon	4 adults, 1 juvenile	YSH
68	30-Mar-06	Afternoon	7	Nee Soon		YSH
69	22-Apr-06	Morning	5	Upper Peirce Reservoir		LKC
70	23-Apr-06		5	Upper Peirce Reservoir		Ding Li
71	24-Apr-06	15:30	7	Nee Soon	Feeding	YSH
72	01-Oct-06	18:20	3	Nee Soon		YSH
73	05-Oct-06		1	Upper Peirce Reservoir		Genevie
74	20-Oct-06		2	Upper Peirce Reservoir	Feeding on tembusu	Derek Liew
75	21-Oct-06	17:45	5	Seletar Reservoir	2 black & white infants	Several
76	27-Oct-06		3	Nee Soon	Feeding on fruits	Ding Li
77	28-Oct-06		4	Nee Soon		Ding Li
78	19-Apr-07	17:40	2	Seletar Reservoir	Moving	John Sha
79	08-Jul-07	07:20	2	Seletar Reservoir	Foraging	John Sha
80	13-Aug-07	16:30	2	Seletar Reservoir	Moving	John Sha
81	31-Aug-07	17:30	2	Lower Peirce Reservoir	Moving, macaques around	John Sha
82	02-Sep-07	Morning	6	Upper Peirce Reservoir	Feeding and resting	Ding Li
83	13-Sep-07	18:15	2	Seletar Reservoir	Foraging	John Sha
84	19-Mar-08	07:30	5	Nee Soon		Ding Li
85	06-Jul-08		6	Old Upp Thomson Rd		Ding Li
86	13-Sep-08	09:00	7	Nee Soon	Moving	Ding Li
87	18-Nov-08	18:30	5	Seletar Reservoir	Feeding, moving	Shun Deng
88	20-Nov-08	18:00	5	Seletar Reservoir	Moving	Shun Deng

89	27-Mar-09	07:10	3	Nee Soon	In between two ranges	YSH
90	05-Apr-09	18:00	2	Nee Soon	Call heard at 8pm	Kwokwai
91	30-Apr-09		9	Nee Soon	In between 2 ranges	YSH
92	01-Jun-10	14:40	3	Seletar	Feeding	Uncle S
93	02-Jun-10	09:45	7 to 8	Seletar		Uncle S
94	12-Jul-10	11:00		Seletar		Alvin
95	18-Jul-10	09:00	8	Nee Soon	2 groups; Cllas	Shun Deng, Ding Li

Appendix II: Availability of preferred plant parts in different months

Months	Flowers	Fruits	Young leaves		No. of specimens with seasonal plant parts (period)
Jan	X	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		78 (1884-2007)
	X	<i>Agelaea macrophylla</i>	X		11 (1949-2009)
	X	X	<i>Artocarpus elasticus</i>		26 (1888-2010)
	<i>Bauhinia semibifida</i>	X	<i>Bauhinia semibifida</i>		20 (1889-2009)
	X	X	X	<i>Fagraea fragrans</i>	1 (2009)
	X	<i>Fibraurea tinctoria</i>	X		24 (1884-2009)
	X	X	X	<i>Hevea brasiliensis</i>	2 (2009-2010)
	X	X	X	<i>Ixonanthes reticulata</i>	37 (1889-2010)
	X	<i>Knema malayana</i>	X		35 (1883-2010)
	X	<i>Litsea castanea</i>	X		26 (1883-2010)
	X	X	X	<i>Litsea elliptica</i>	26 (1883-2009)
	X	X	<i>Litsea firma</i>		25 (1885-2010)
	X	X	X	<i>Lophopetalum multinervium</i>	6 (1924-2010)
	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>		25 (1886-2010)
	<i>Nothaphoebe umbelliflora</i>	<i>Nothaphoebe umbelliflora</i>	<i>Nothaphoebe umbelliflora</i>		25 (1886-2010)
	X	X	X	<i>Palaquium xanthochymum</i>	1 (2009)
	<i>Pellacalyx axillaris</i>	X	<i>Pellacalyx axillaris</i>		14 (1881-2009)
	<i>Pterocarpus indicus</i>	<i>Pterocarpus indicus</i>	X		17 (1888-2008)
	<i>Syzygium grande</i>	<i>Syzygium grande</i>	X		40 (1885-2009)
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		57 (1884-2009)
	X	X	X	<i>Xanthophyllum ellipticum</i>	6 (1938-2009)
	X	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		71 (1883-2010)
Feb	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	X		
	<i>Agelaea macrophylla</i>	<i>Agelaea macrophylla</i>	<i>Agelaea macrophylla</i>		
	X	X	<i>Artocarpus elasticus</i>		
	<i>Bauhinia semibifida</i>	X	X		

	X	X	X	<i>Fagraea fragrans</i>	
	<i>Fibraurea tinctoria</i>	<i>Fibraurea tinctoria</i>	X		
	X	X	X	<i>Hevea brasiliensis</i>	
	X	X	X	<i>Ixonanthes reticulata</i>	
	<i>Knema malayana</i>	<i>Knema malayana</i>	X		
	X	<i>Litsea castanea</i>	X		
	<i>Litsea elliptica</i>	<i>Litsea elliptica</i>	<i>Litsea elliptica</i>		
	X	X	X	<i>Litsea firma</i>	
	X	X	X	<i>Lophopetalum multinervium</i>	
	X	X	X	<i>Nephelium lappaceum</i>	
	<i>Nothaphoebe umbelliflora</i>	X	<i>Nothaphoebe umbelliflora</i>		
	X	X	X	<i>Palaquium xanthochyllum</i>	
	<i>Pellacalyx axillaris</i>	X	X		
	X	X	X	<i>Pterocarpus indicus</i>	
	<i>Syzygium grande</i>	<i>Syzygium grande</i>	X		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	X	X	<i>Xanthophyllum ellipticum</i>	
	X	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		
Mar	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		
	<i>Agelaea macrophylla</i>	X	X		
	X	X	X	<i>Artocarpus elasticus</i>	
	X	X	X	<i>Bauhinia semibifida</i>	
	X	X	X	<i>Fagraea fragrans</i>	
	<i>Fibraurea tinctoria</i>	<i>Fibraurea tinctoria</i>	X		
	X	X	X	<i>Hevea brasiliensis</i>	
	X	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>		
	<i>Knema malayana</i>	<i>Knema malayana</i>	<i>Knema malayana</i>		
	X	<i>Litsea castanea</i>	X		
	<i>Litsea elliptica</i>	X	X		

	<i>Litsea firma</i>	X	<i>Litsea firma</i>		
	X	X	X	<i>Lophopetalum multinervium</i>	
	<i>Nephelium lappaceum</i>	X	X		
	<i>Nothaphoebe umbelliflora</i>	X	X		
	X	X	X	<i>Palaquium xanthochymum</i>	
	X	X	X	<i>Pellacalyx axillaris</i>	
	<i>Pterocarpus indicus</i>	<i>Pterocarpus indicus</i>	X		
	<i>Syzygium grande</i>	<i>Syzygium grande</i>	X		
	X	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	<i>Xanthophyllum ellipticum</i>	<i>Xanthophyllum ellipticum</i>		
	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		
Apr	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		
	X	X	X	<i>Agelaea macrophylla</i>	
	X	<i>Artocarpus elasticus</i>	<i>Artocarpus elasticus</i>		
	<i>Bauhinia semibifida</i>	<i>Bauhinia semibifida</i>	<i>Bauhinia semibifida</i>		
	X	X	X	<i>Fagraea fragrans</i>	
	<i>Fibraurea tinctoria</i>	<i>Fibraurea tinctoria</i>	<i>Fibraurea tinctoria</i>		
	X	X	X	<i>Hevea</i>	
	X	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>		
	<i>Knema malayana</i>	<i>Knema malayana</i>	<i>Knema malayana</i>		
	<i>Litsea castanea</i>	<i>Litsea castanea</i>	X		
	X	X	X	<i>Litsea elliptica</i>	
	X	X	X	<i>Litsea firma</i>	
	<i>Lophopetalum multinervium</i>	<i>Lophopetalum multinervium</i>	<i>Lophopetalum multinervium</i>		
	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>	X		
	<i>Nothaphoebe umbelliflora</i>	<i>Nothaphoebe umbelliflora</i>	<i>Nothaphoebe umbelliflora</i>		
	X	X	X		
	<i>Pellacalyx axillaris</i>	X	<i>Pellacalyx axillaris</i>		
	<i>Pterocarpus indicus</i>	X	X		

	<i>Syzygium grande</i>	<i>Syzygium grande</i>	X		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	<i>Xanthophyllum ellipticum</i>	X		
	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		
May	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		
	X	X	<i>Agelaea macrophylla</i>		
	X	<i>Artocarpus elasticus</i>	<i>Artocarpus elasticus</i>		
	<i>Bauhinia semibifida</i>	X	<i>Bauhinia semibifida</i>		
	X	X	X	<i>Fagraea fragrans</i>	
	<i>Fibraurea tinctoria</i>	<i>Fibraurea tinctoria</i>	X		
	X	X	X	<i>Hevea brasiliensis</i>	
	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>		
	X	<i>Knema malayana</i>	X		
	<i>Litsea castanea</i>	<i>Litsea castanea</i>	X		
	<i>Litsea elliptica</i>	X	X		
	<i>Litsea firma</i>	X	X		
	X	<i>Lophopetalum multinervium</i>	X		
	X	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>		
	<i>Nothaphoebe umbelliflora</i>	X	<i>Nothaphoebe umbelliflora</i>		
	X	X	X		
	<i>Pellacalyx axillaris</i>	X	<i>Pellacalyx axillaris</i>		
	X	<i>Pterocarpus indicus</i>	X		
	<i>Syzygium grande</i>	<i>Syzygium grande</i>	X		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	X	<i>Xanthophyllum ellipticum</i>		
	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		
June	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		
	X	<i>Agelaea macrophylla</i>	X		

	X	<i>Artocarpus elasticus</i>	<i>Artocarpus elasticus</i>		
	<i>Bauhinia semibifida</i>	X	X		
	X	X	X	<i>Fagraea fragrans</i>	
	X	<i>Fibraurea tinctoria</i>	<i>Fibraurea tinctoria</i>		
	X	X	X	<i>Hevea brasiliensis</i>	
	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>		
	<i>Knema malayana</i>	<i>Knema malayana</i>	<i>Knema malayana</i>		
	<i>Litsea castanea</i>	<i>Litsea castanea</i>	X		
	<i>Litsea elliptica</i>	X	X		
	X	X	X	<i>Litsea firma</i>	
	X	X	X	<i>Lophopetalum multinervium</i>	
	X	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>		
	X	X	<i>Nothaphoebe umbelliflora</i>		
	X	X	X	<i>Palaquium xanthochymum</i>	
	X	X	<i>Pellacalyx axillaris</i>		
	X	<i>Pterocarpus indicus</i>	X		
	<i>Syzygium grande</i>	<i>Syzygium grande</i>	X		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	X	X	<i>Xanthophyllum ellipticum</i>	
	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		
July	X	<i>Adinandra dumosa</i>	X		
	<i>Agelaea macrophylla</i>	<i>Agelaea macrophylla</i>	<i>Agelaea macrophylla</i>		
	X	<i>Artocarpus elasticus</i>	X		
	X	X	X	<i>Bauhinia semibifida</i>	
	X	X	X	<i>Fagraea fragrans</i>	
	X	<i>Fibraurea tinctoria</i>	X		
	X	<i>Hevea brasiliensis</i>	X		
	X	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>		
	X	<i>Knema malayana</i>	X		



	<i>Litsea castanea</i>	X	X		
	X	X	X	<i>Litsea elliptica</i>	
	X	<i>Litsea firma</i>	<i>Litsea firma</i>		
	<i>Lophopetalum multinervium</i>	<i>Lophopetalum multinervium</i>	<i>Lophopetalum multinervium</i>		
	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>		
	X	<i>Nothaphoebe umbelliflora</i>	<i>Nothaphoebe umbelliflora</i>		
	X	X	X	<i>Palaquium xanthochymum</i>	
	<i>Pellacalyx axillaris</i>	X	<i>Pellacalyx axillaris</i>		
	<i>Pterocarpus indicus</i>	<i>Pterocarpus indicus</i>	X		
	<i>Syzygium grande</i>	X	X		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	X	X	<i>Xanthophyllum ellipticum</i>	
	X	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		
Aug	X	X	X	<i>Adinandra dumosa</i>	
	X	<i>Agelaea macrophylla</i>	<i>Agelaea macrophylla</i>		
	X	<i>Artocarpus elasticus</i>	X		
	<i>Bauhinia semibifida</i>	X	X		
	X	X	X	<i>Fagraea fragrans</i>	
	<i>Fibraurea tinctoria</i>	X	X		
	X	X	<i>Hevea brasiliensis</i>		
	X	<i>Ixonanthes reticulata</i>	X		
	<i>Knema malayana</i>	<i>Knema malayana</i>	<i>Knema malayana</i>		
	<i>Litsea castanea</i>	<i>Litsea castanea</i>	X		
	<i>Litsea elliptica</i>	X	X		
	<i>Litsea firma</i>	<i>Litsea firma</i>	<i>Litsea firma</i>		
	X	X	X	<i>Lophopetalum multinervium</i>	
	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>		
	X	<i>Nothaphoebe umbelliflora</i>	X		
	X	X	X	<i>Palaquium xanthochymum</i>	

	X	X	<i>Pellacalyx axillaris</i>		
	X	X	X	<i>Pterocarpus indicus</i>	
	<i>Syzygium grande</i>	X	X		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	X	X	<i>Xanthophyllum ellipticum</i>	
	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		
Sep	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		
	X	X	X	<i>Agelaea macrophylla</i>	
	X	X	X	<i>Artocarpus elasticus</i>	
	<i>Bauhinia semibifida</i>	<i>Bauhinia semibifida</i>	X		
	X	X	X	<i>Fagraea fragrans</i>	
	X	<i>Fibraurea tinctoria</i>	<i>Fibraurea tinctoria</i>		
	X	X	X	<i>Hevea brasiliensis</i>	
	X	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>		
	X	<i>Knema malayana</i>	X		
	<i>Litsea castanea</i>	<i>Litsea castanea</i>	<i>Litsea castanea</i>		
	<i>Litsea elliptica</i>	<i>Litsea elliptica</i>	X		
	<i>Litsea firma</i>	X	X		
	<i>Lophopetalum multinervium</i>	X	<i>Lophopetalum multinervium</i>		
	X	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>		
	<i>Nothaphoebe umbelliflora</i>	X	<i>Nothaphoebe umbelliflora</i>		
	X	X	X	<i>Palaquium xanthochyllum</i>	
	X	X	X	<i>Pellacalyx axillaris</i>	
	X	<i>Pterocarpus indicus</i>	X		
	<i>Syzygium grande</i>	<i>Syzygium grande</i>	X		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	X	X	<i>Xanthophyllum ellipticum</i>	
	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>	X		

Oct	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		
	X	X	X	<i>Agelaea macrophylla</i>	
	X	<i>Artocarpus elasticus</i>	X		
	<i>Bauhinia semibifida</i>	<i>Bauhinia semibifida</i>	<i>Bauhinia semibifida</i>		
	X	X	X	<i>Fagraea fragrans</i>	
	X	X	X	<i>Fibraurea tinctoria</i>	
	X	X	X	<i>Hevea brasiliensis</i>	
	X	<i>Ixonanthes reticulata</i>	<i>Ixonanthes reticulata</i>		
	X	X	X	<i>Knema malayana</i>	
	<i>Litsea castanea</i>	X	X		
	X	X	<i>Litsea elliptica</i>		
	<i>Litsea firma</i>	<i>Litsea firma</i>	<i>Litsea firma</i>		
	X	X	<i>Lophopetalum multinervium</i>		
	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>	<i>Nephelium lappaceum</i>		
	X	X	<i>Nothaphoebe umbelliflora</i>		
	X	<i>Palaquium xanthochymum</i>	X		
	X	<i>Pellacalyx axillaris</i>	X		
	X	X	X	<i>Pterocarpus indicus</i>	
	<i>Syzygium grande</i>	<i>Syzygium grande</i>	<i>Syzygium grande</i>		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	<i>Xanthophyllum ellipticum</i>	X		
	X	X	X	<i>Xanthophyllum eurhynchum</i>	
Nov	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		
	X	X	X	<i>Agelaea macrophylla</i>	
	<i>Artocarpus elasticus</i>	<i>Artocarpus elasticus</i>	<i>Artocarpus elasticus</i>		
	<i>Bauhinia semibifida</i>	<i>Bauhinia semibifida</i>	<i>Bauhinia semibifida</i>		
	X	X	X	<i>Fagraea fragrans</i>	
	<i>Fibraurea tinctoria</i>	X	X		
	X	X	X	<i>Hevea brasiliensis</i>	

	X	X	X	<i>Ixonanthes reticulata</i>	
	X	X	X	<i>Knema malayana</i>	
	X	<i>Litsea castanea</i>	X		
	<i>Litsea elliptica</i>	X	X		
	X	X	X	<i>Litsea firma</i>	
	X	X	X	<i>Lophopetalum multinervium</i>	
	X	<i>Nephelium lappaceum</i>	X		
	X	X	<i>Nothaphoebe umbelliflora</i>		
	X	X	X	<i>Palaquium xanthochymum</i>	
	X	X	X	<i>Pellacalyx axillaris</i>	
	<i>Pterocarpus indicus</i>	X	X		
	<i>Syzygium grande</i>	X	<i>Syzygium grande</i>		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	<i>Xanthophyllum ellipticum</i>	X		
	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		
Dec	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>	<i>Adinandra dumosa</i>		
	X	X	X	<i>Agelaea macrophylla</i>	
	X	X	<i>Artocarpus elasticus</i>		
	X	X	X	<i>Bauhinia semibifida</i>	
	X	X	<i>Fagraea fragrans</i>		
	<i>Fibraurea tinctoria</i>	X	<i>Fibraurea tinctoria</i>		
	X	X	X	<i>Hevea brasiliensis</i>	
	X	X	X	<i>Ixonanthes reticulata</i>	
	X	X	X	<i>Knema malayana</i>	
	X	<i>Litsea castanea</i>	X		
	<i>Litsea elliptica</i>	<i>Litsea elliptica</i>	<i>Litsea elliptica</i>		
	<i>Litsea firma</i>	<i>Litsea firma</i>	<i>Litsea firma</i>		
	X	X	X	<i>Lophopetalum multinervium</i>	
	X	X	X	<i>Nephelium lappaceum</i>	

	X	X	X	<i>Nothaphoebe umbelliflora</i>	
	X	X	X	<i>Palaquium xanthochymum</i>	
	X	X	X	<i>Pellacalyx axillaris</i>	
	X	X	X	<i>Pterocarpus indicus</i>	
	<i>Syzygium grande</i>	X	X		
	<i>Tetracera indica</i>	<i>Tetracera indica</i>	<i>Tetracera indica</i>		
	X	X	X	<i>Xanthophyllum ellipticum</i>	
	X	<i>Xanthophyllum eurhynchum</i>	<i>Xanthophyllum eurhynchum</i>		

Species highlighted in gray indicate availability of preferred plant parts during particular months.

Appendix IIIa: Tree species and their proportions in the botanical transects at the CCNR

Family	Species	National status	No. of stems	Species basal area	Family basal area
Anacardiaceae	<i>Buchanania sessifolia</i>	VU	1	346.6394661	5218.849738
	<i>Camposperma auriculata</i>	Common	2	1480.936745	
	<i>Camposperma squamatum</i>	Common	6	3391.273527	
Annonaceae	<i>Cyathocalyx ramuliflorus</i>	Common	5	2591.997403	3326.815775
	<i>Cyathocalyx ridleyi</i>	VU	2	377.5951025	
	<i>Xylopia malayana</i>	Common	1	357.2232698	
Apocynaceae	<i>Alstonia angustifolia</i>	Common	3	2036.705807	7431.580913
	<i>Alstonia angustiloba</i>	Common	1	1052.412061	
	<i>Dyera costulata</i>	Common	2	4342.463045	
Aquifoliaceae	<i>Ilex cymosa</i>	Common	1	215.1774831	215.1774831
Burseraceae	<i>Canarium littorale</i>	Common	4	2514.807256	5764.194152
	<i>Santiria laevigata</i>	VU	3	3249.386896	
Cannabaceae	<i>Girouneria nervosa</i>	Common	36	20947.33699	24935.60071
	<i>Girouneria subaequalis</i>	EN	9	3988.263719	
Celastraceae	<i>Bhesa paniculata</i>	Common	2	287.5134047	1530.911398
	<i>Lophopetalum wightianum</i>	VU	1	1243.397993	
Clusiaceae	<i>Calophyllum ferrugineum</i>	Common	3	1680.914931	9579.37687
	<i>Calophyllum pulcherrimum</i>	Common	6	2835.186156	
	<i>Calophyllum tetrapterum</i>	VU	2	551.3127229	
	<i>Calophyllum teysmannii</i>	VU	1	191.0655092	
	<i>Calophyllum wallichianum var incrassatum</i>	VU	3	1259.711375	
	<i>Garcinia eugeniifolia</i>	VU	1	346.6394661	
	<i>Garcinia nigrolineata</i>	CR	1	127.3239545	
	<i>Garcinia parvifolia</i>	Common	9	2587.222755	
Dipterocarpaceae	<i>Shorea platycarpa</i>	CR	8	6370.813217	6370.813217
Elaeocarpaceae	<i>Elaeocarpus mastersii</i>	Common	1	630.3331521	1066.099386
	<i>Elaeocarpus nitidus var salicifolius</i>	VU	1	435.7662342	
Euphorbiaceae	<i>Hevea brasiliensis</i>	Naturalized	3	3043.838287	15155.05199
	<i>Macaranga conifer</i>	Common	6	4060.201753	
	<i>Macaranga gigantea</i>	Common	6	5807.404718	
	<i>Mallotus paniculatus</i>	Common	1	175.7866346	
	<i>Pimelodendron griffithianum</i>	VU	1	1223.583202	
	<i>Trigonopleura malayana</i>	CR	1	844.2373956	
Fabaceae	<i>Archidendron clypearia</i>	Common	2	825.8549997	

	<i>Archidendron jiringa</i>	VU	1	336.2148173	1162.069817
Fagaceae	<i>Lithocarpus bennettii</i>	CR	1	1263.371938	1263.371938
Gentianaceae	<i>Fagraea fragrans</i>	Common	1	175.7866346	175.7866346
Hypericaceae	<i>Cratoxylum maingayi</i>	CR	1	183.3464944	183.3464944
Ixonanthaceae	<i>Ixonanthes icosandra</i>	VU	9	4403.419388	
	<i>Ixonanthes reticulata</i>	Common	24	28006.57491	32409.9943
Lauraceae	<i>Beilschmiedia madang</i>	EN	1	140.3746598	
	<i>Cryptocarya ferrea</i>	CR	1	240.7218514	
	<i>Lindera lucida</i>	VU	2	469.825392	
	<i>Litsea costalis</i>	CR	2	838.5078177	
	<i>Litsea elliptica</i>	Common	10	5933.535011	
	<i>Litsea firma</i>	VU	2	1009.758536	
	<i>Litsea grandis</i>	EN	1	718.1866807	
	<i>Nothaphoebe umbelliflora</i>	Common	7	3355.94113	12706.85108
Malvaceae	<i>Pentace triptera</i>	EN	2	1513.961396	1513.961396
Melastomataceae	<i>Pternandra coeruleascens</i>	VU	2	2839.722072	2839.722072
Meliaceae	<i>Aglaia exstipulata</i>	CR	2	288.4683344	288.4683344
Moraceae	<i>Artocarpus dadah</i>	EN	1	3025.933356	
	<i>Artocarpus integer</i>	Casual	2	667.4958313	
	<i>Artocarpus kemando</i>	EN	1	412.5296125	
	<i>Artocarpus rigidus</i>	VU	1	2219.336104	
	<i>Ficus binnendykii</i>	CR	1	198.9436789	6524.238583
Myristicaceae	<i>Horsfieldia polyspherula</i>	VU	3	1403.746598	
	<i>Knema malayana</i>	EN	6	3111.001673	4514.748271
Myrtaceae	<i>Rhodamnia cinerea</i>	Common	72	33466.38524	
	<i>Syzygium borneense</i>	Common	4	3362.705215	
	<i>Syzygium filiforme</i>	EN	7	1458.177589	
	<i>Syzygium grande</i>	Common	27	32024.04356	
	<i>Syzygium incarnatum</i>	EN	3	4142.246126	
	<i>Syzygium lineatum</i>	Common	2	370.6718625	
	<i>Syzygium maingayi</i>	CR	4	3157.315761	
	<i>Syzygium nemestrinum</i>	EN	1	1559.718442	
	<i>Syzygium palembanicum</i>	VU	1	133.7697297	
	<i>Syzygium ridleyi</i>	EN	1	2694.174877	82369.2084
Pentaphylacaceae	<i>Adinandra dumosa</i>	Common	6	1688.793101	
	<i>Eurya acuminata</i>	Common	1	315.8429846	
	<i>Ternstroemia corneri</i>	CR	1	140.3746598	2145.010745

Phyllanthaceae	<i>Aporosa falcifera</i>	CR	2	574.9472319	
	<i>Aporosa globifera</i>	NA	1	305.8958006	
	<i>Aporosa lucida</i>	CR	2	786.3049963	
	<i>Aporosa symplocoides</i>	Common	2	453.2732779	
	<i>Baccaurea griffithii</i>	CR	1	296.1077716	
	<i>Baccaurea maingayi</i>	CR	2	599.1387833	
	<i>Baccaurea minor</i>	CR	1	258.5472051	
	<i>Baccaurea parviflora</i>	Common	1	2782.744602	6056.959669
Polygalaceae	<i>Xanthophyllum affine</i>	EN	1	140.3746598	
	<i>Xanthophyllum flavescens</i>	NA	3	2968.000956	3108.375616
Rhizophoraceae	<i>Gynotroches axillaris</i>	Common	9	6849.710441	6849.710441
Rosaceae	<i>Prunus polystachya</i>	Common	25	13884.12019	13884.12019
Rubiaceae	<i>Diplospora malaccensis</i>	CR	3	978.4845901	
	<i>Psydrax sp10 of Wong</i>	NA	7	1578.498726	
	<i>Timonius wallichianus</i>	Common	15	4726.26519	7283.248506
Rutaceae	<i>Citrus deliciosa</i>	NA	1	127.3239545	
	<i>Melicope lunu-ankenda</i>	CR	1	522.1077908	649.4317453
Sapindaceae	<i>Dimocarpus longan</i>	Casual	2	2744.865726	
	<i>Guioa pubescens</i>	VU	1	191.0655092	2935.931235
Sapotaceae	<i>Palaquium obovatum</i>	VU	1	535.0789187	
	<i>Palaquium xanthochymum</i>	CR	1	389.9296106	
	<i>Pouteria malaccensis</i>	VU	1	459.6394756	1384.648005
Staphyleaceae	<i>Turpinia sphaerocarpa</i>	CR	1	357.2232698	357.2232698
Symplocaceae	<i>Symplocos fasciculata</i>	VU	1	367.9662284	367.9662284
Thymelaeaceae	<i>Aquilaria malaccensis</i>	VU	2	4153.625705	
	<i>Enkleia malaccensis</i>	CR	1	424.0683459	
	<i>Gonystylus confuses</i>	EN	1	1650.11845	6227.812501
<b>Total basal area per 1.2ha</b>					<b>277796.6771</b>
<b>Total no. of individuals identified</b>			<b>438</b>		
<b>Total no. of individuals unidentified</b>			<b>23</b>		
<b>Total no. of species</b>			<b>98</b>		

The national status of each species is referred based on Chong et al., 2009. Only natives are categorized as either 'NE' (extinct), 'CR' (critically endangered), 'EN' (endangered), 'VU' (vulnerable), or 'Common' (not threatened). Exotics are listed as either 'Casual' or 'Naturalized'.



Appendix IIIb: Tree species in the 1.2ha plots within the study area in the CCNR

Family	Basal area (cm <sup>2</sup> ) per 1.2ha	Basal area (cm <sup>2</sup> ) per ha	% Basal area	Stems	% Stems
Myrtaceae	82369.2084	68641.00768641	29.65089786	122	27.85388128
Ixonanthaceae	32409.9943	27008.3285827008	11.66680413	33	7.534246575
Cannabaceae	24935.60071	20779.6672620780	8.976205512	45	10.2739726
Euphorbiaceae	15155.05199	12629.2099912629	5.455447525	18	4.109589041
Rosaceae	13884.12019	11570.10016	4.997943207	25	5.707762557
Lauraceae	12706.85108	10589.0425710589	4.574155163	26	5.936073059
Clusiaceae	9579.37687	7982.8140587983	3.448341047	26	5.936073059
Apocynaceae	7431.580913	6192.9840946193	2.675187108	6	1.369863014
Rubiaceae	7283.248506	6069.3737556069	2.621791074	25	5.707762557
Rhizophoraceae	6849.710441	5708.0920345708	2.46572799	9	2.054794521
Moraceae	6524.238583	5436.8654865437	2.348566093	6	1.369863014
Dipterocarpaceae	6370.813217	5309.011014	2.293336718	8	1.826484018
Thymelaeaceae	6227.812501	5189.8437515190	2.241859963	4	0.913242009
Phyllanthaceae	6056.959669	5047.4663915047	2.180357129	12	2.739726027
Bursereaceae	5764.194152	4803.4951274803	2.074968713	7	1.598173516
Anacardiaceae	5218.849738	4349.041448	1.878658081	9	2.054794521
Myristicaceae	4514.748271	3762.290226	1.625198798	9	2.054794521
Annonaceae	3326.815775	2772.346479	1.197572196	8	1.826484018
Polygalaceae	3108.375616	2590.313013	1.118939088	4	0.913242009
Sapindaceae	2935.931235	2446.609363	1.056863335	3	0.684931507
Melastomataceae	2839.722072	2366.43506	1.022230393	2	0.456621005
Pentaphragaceae	2145.010745	1787.508955	0.772151331	8	1.826484018
Celastraceae	1530.911398	1275.759498	0.551090607	3	0.684931507
Malvaceae	1513.961396	1261.634497	0.544989021	2	0.456621005
Sapotaceae	1384.648005	1153.873337	0.498439368	3	0.684931507
Fagaceae	1263.371938	1052.809948	0.454782954	1	0.228310502
Fabaceae	1162.069817	968.3915142	0.418316672	3	0.684931507
Elaeocarpaceae	1066.099386	888.4161553	0.383769667	2	0.456621005
Rutaceae	649.4317453	541.1931211	0.233779522	2	0.456621005
Symplocaceae	367.9662284	306.6385237	0.13245883	1	0.228310502
Staphyleaceae	357.2232698	297.6860582	0.128591628	1	0.228310502
Meliaceae	288.4683344	240.3902787	0.103841535	2	0.456621005
Aquifoliaceae	215.1774831	179.3145692	0.077458624	1	0.228310502
Hypericaceae	183.3464944	152.7887453	0.066000247	1	0.228310502
Gentianaceae	175.7866346	146.4888622	0.063278883	1	0.228310502

Appendix IV: Climber species and their proportions in the botanical transects at CCNR

Family	Species	Local status	No. of individuals	%	Family %
Annonaceae	<i>Artabotrys costatus</i>	VU	2	0.176056338	
	<i>Artabotrys crassifolius</i>	CR	2	0.176056338	
	<i>Artabotrys suaveolens</i>	EN	9	0.792253521	
	<i>Cyathostemma viridiflorum</i>	VU	4	0.352112676	
	<i>Fissistigma fulgens</i>	VU	1	0.088028169	
	<i>Fissistigma lanuginosum</i>	EN	1	0.088028169	
	<i>Fissistigma latifolium var ovoideum</i>	VU	26	2.288732394	
	<i>Fissistigma manubriatum</i>	VU	6	0.528169014	
	<i>Friesodielsia borneensis</i>	VU	31	2.728873239	
	<i>Friesodielsia glauca</i>	NE	4	0.352112676	
	<i>Melodrum aberrans</i>	NA	1	0.088028169	
	<i>Mitrella kentia</i>	Common	20	1.76056338	
	<i>Pyramidanthe prismatica</i>	EN	5	0.440140845	
	<i>Uvaria curtisii</i>	NE	1	0.088028169	
	<i>Uvaria leptopoda</i>	CR	2	0.176056338	
<i>Uvaria pauci-ovulata</i>	CR	1	0.088028169	7.658450704	
Apocynaceae	<i>Anodendron candolleum</i>	CR	14	1.232394366	
	<i>Leuconotis griffithii</i>	VU	2	0.176056338	
	<i>Urceola brachysepala</i>	EN	4	0.352112676	
	<i>Urceola elastic</i>	CR	1	0.088028169	
	<i>Urceola torulosa</i>	EN	7	0.616197183	
	<i>Willughbeia angustifolia</i>	NE	2	0.176056338	
	<i>Willughbeia edulis = W. coriacea</i>	NE	35	3.080985915	
	<i>Willughbeia flavescens</i>	CR	26	2.288732394	10.56338028
Araceae	<i>Scindapsus hederaceus</i>	Common	2	0.176056338	0.176056338
Celastraceae	<i>Salacia viminea</i>	CR	5	0.440140845	0.440140845
Combretaceae	<i>Combretum sundaicum</i>	VU	3	0.264084507	0.264084507
Connaraceae	<i>Agelaea borneensis</i>	VU	18	1.584507042	
	<i>Agelaea macrophylla</i>	CR	69	6.073943662	
	<i>Connarus semidecandrus</i>	CR	2	0.176056338	
	<i>Rourea acutipetala</i>	NE	1	0.088028169	
	<i>Rourea asplenifolia</i>	CR	19	1.672535211	
	<i>Rourea mimosoides</i>	EN	16	1.408450704	11.00352113
Convolvulaceae	<i>Argyreia ridleyi</i>	CR	1	0.088028169	
	<i>Erycibe leucoxyloides</i>	WUO	30	2.64084507	

	<i>Erycibe malaccensis</i>	CR	22	1.936619718	
	<i>Erycibe tomentosa</i>	Common	40	3.521126761	8.186619718
Dilleniaceae	<i>Tetracera akara</i>	VU	54	4.753521127	
	<i>Tetracera fagifolia</i>	VU	26	2.288732394	
	<i>Tetracera macrophylla</i>	VU	11	0.968309859	
	<i>Tetracera maingayi</i>	NA	4	0.352112676	8.362676056
Dioscoreaceae	<i>Dioscorea laurifolia</i>	Common	9	0.792253521	
	<i>Dioscorea orbiculata</i> var. <i>tenuifolia</i> = <i>D. tenuifolia</i>	NE	2	0.176056338	
	<i>Dioscorea prainiana</i>	CR	5	0.440140845	1.408450704
Fabaceae	<i>Bauhinia semibifida</i>	VU	4	0.352112676	
	<i>Dalbergia parviflora</i>	NE	1	0.088028169	
	<i>Entada spiralis</i>	Common	13	1.144366197	
	<i>Spatholobus ferrugineus</i>	Common	40	3.521126761	5.105633803
Gentianaceae	<i>Fagraea acuminatissima</i>	NE	2	0.176056338	0.176056338
Gleichinaceae	<i>Dicranopteris linearis</i>	Common	5	0.440140845	0.440140845
Gnetaceae	<i>Gnetum latifolium</i> var. <i>furniculare</i>	CR	3	0.264084507	
	<i>Gnetum macrostachyum</i>	CR	15	1.320422535	
	<i>Gnetum microcarpum</i>	CR	2	0.176056338	1.76056338
Icacinaceae	<i>Iodes cirrhosa</i>	CR	1	0.088028169	
	<i>Iodes ovalis</i>	EN	7	0.616197183	
	<i>Phytocrene bracteata</i>	VU	13	1.144366197	1.848591549
Loganiaceae	<i>Strychnos axillaris</i>	NE	1	0.088028169	
	<i>Strychnos ignatii</i>	VU	1	0.088028169	0.176056338
Malvaceae	<i>Grewia laevigata</i>	VU	3	0.264084507	0.264084507
Melastomataceae	<i>Dissochaeta gracilis</i>	VU	2	0.176056338	0.176056338
Menispermaceae	<i>Fibraurea tinctoria</i>	Common	167	14.70070423	
	<i>Limacia scandens</i>	VU	3	0.264084507	
	<i>Tinomisium petiolare</i>	EN	1	0.088028169	15.0528169
Moraceae	<i>Ficus apiocarpa</i>	EN	1	0.088028169	
	<i>Ficus globosa</i>	EN	3	0.264084507	
	<i>Ficus heteropleura</i>	Common	2	0.176056338	
	<i>Ficus microsyce</i>	CR	1	0.088028169	
	<i>Ficus punctata</i>	Exotic	36	3.169014085	
	<i>Ficus sagittata</i>	CR	5	0.440140845	
	<i>Ficus sundaica</i>	CR	1	0.088028169	
	<i>Ficus trichocarpa</i>	CR	1	0.088028169	4.401408451

Myrsinaceae	<i>Grenacheria amentacea</i>	NE	7	0.616197183	0.616197183
Olacaceae	<i>Erythralum scandens</i>	VU	2	0.176056338	0.176056338
Orchidaceae	<i>Vanilla griffithii</i>	VU	1	0.088028169	0.088028169
Piperaceae	<i>Piper caninum</i>	Common	33	2.904929577	
	<i>Piper pedicellosum</i>	CR	3	0.264084507	
	<i>Piper porphyrophyllum</i>	EN	42	3.697183099	6.866197183
Rhamnaceae	<i>Ventilago maingayi</i>	NA	18	1.584507042	
	<i>Ventilago malaccensis</i>	EN	3	0.264084507	1.848591549
Rubiaceae	<i>Coptosapelta tomentosa</i> = <i>C. flavescens</i>	NE	6	0.528169014	
	<i>Gynochthodes sublanceolata</i>	Common	37	3.257042254	
	<i>Morinda rigida</i>	NE	21	1.848591549	
	<i>Morinda umbellate</i>	Common	2	0.176056338	
	<i>Oxyceros fragrantissimus</i>	EN	4	0.352112676	
	<i>Oxyceros scandens</i>	NE	2	0.176056338	
	<i>Psychotria penangensis</i>	VU	11	0.968309859	
	<i>Psychotria sarmentosa</i>	CR	2	0.176056338	
	<i>Schradera membranacea</i>	CR	5	0.440140845	
	<i>Uncaria lanosa</i> var <i>glabrata</i>	CR	1	0.088028169	
	<i>Uncaria longiflora</i> var <i>pteropoda</i>	CR	3	0.264084507	8.274647887
Rutaceae	<i>Luvunga crassifolia</i>	CR	6	0.528169014	
	<i>Paramignya scandens</i>	CR	1	0.088028169	0.616197183
Smilacaceae	<i>Smilax setosa</i>	Common	38	3.345070423	3.345070423
Vitaceae	<i>Cissus nodosa</i>	CR	1	0.088028169	
	<i>Nothocissus spicifera</i>	CR	4	0.352112676	
	<i>Tetrastigma leucostaphylum</i>	NE	3	0.264084507	0.704225352
<b>Total no. of individuals identified</b>			<b>1136</b>		
<b>Total no. of individuals unidentified</b>			<b>232</b>		
<b>Total no. of species</b>			<b>96</b>		

The national status of each species is referred based on Chong et al., 2009. Only natives are categorized as either 'NE' (extinct), 'CR' (critically endangered), 'EN' (endangered), 'VU' (vulnerable), or 'Common' (not threatened). Exotics are listed as either 'Casual' or 'Naturalized'. Species without biogeographical or historical evidence of being non-native, but are restricted to only human-disturbed habitats are classified as 'WUO' (weed of uncertain origin).

## Appendix V: Special notes on behavior

- 1) In larger groups like group A which consisted of 13 individuals, group traveling usually involved an adult remaining at the main crossing point, with group members traveling one-by-one in front of the adult. This behavior of the adult may serve to indicate traveling paths to other group members, and has been observed in Guizhou snub-nosed monkeys with troop sizes of up to 300 individuals (pers. comm., Niu).
- 2) Most interactions with macaques were cordial, and they did not seem to actively avoid each other. Several occasions when banded leaf monkeys were observed, macaques were similarly seen in proximity. Actually quantifying of degree of competition of these two species of sympatric primates would have to involve the in-depth examination of spatial and temporal utilization of habitat and food resources.