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A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Molecular Phylogenetic Status and Ecological Characteristics of Phayre's Leaf Monkey (*Trachypithecus phayrei*) in Myanmar

미얀마에 서식하는 Phayre's Leaf Monkey (*Trachypithecus phayrei*)의 분자계통학적 위치와 생태학적 특성

BY

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DEPARTMENT OF FOREST SCIENCES

GRADUATE SCHOOL

SEOUL NATIONAL UNIVERSITY

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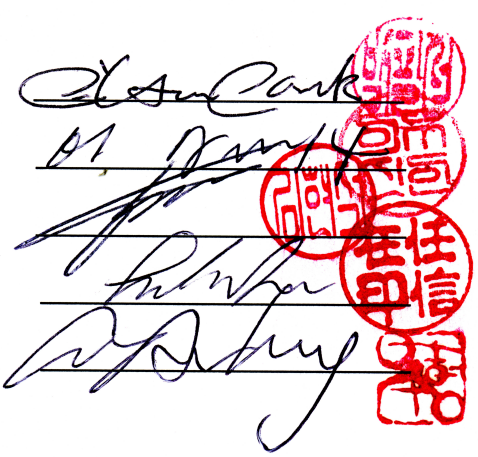

Abstract

Phayre's leaf monkey (*Trachypithecus phayrei*) is a medium sized primate and endemic to tropical forests in Asia. It is categorized as 'Endangered' by IUCN and listed as CITES Appendix II species. Although three subspecies (*T. p. phayrei*, *T. p. crepusculus*, and *T. p. shanicus*) have been identified, little is known about the taxonomic status and ecology of Phayre's leaf monkey in the region. This study aimed at confirming the taxonomic status of Phayre's leaf monkey in Myanmar and also understanding its behaviors and habitat uses in order to provide species conservation and habitat management.

This study was the first molecular work using non-invasive samples (hair and feces) and non-destructive sample (bones from dead animal) to investigate the phylogenetic status of Phayre's leaf monkey in Myanmar particularly Popa Mountain Park (PMP) and Alaungdaw Kathapa National Park (AKNP). Two DNA markers, nuclear protamine P1 (*Prm1*) and mitochondrial cytochrome *b* (*Cyt-b*) genes were used. The population from PMP is more closely related to the subspecies *T. p. shanicus* from the southwest China, while that from AKNP cluster together with subspecies *T. p. phayrei* from the northeast India.

Three groups of Phayre's leaf monkey (mean 41 ± 0.67, 41 ± 0.58 and 21 ± 0.67 individuals for group-1, group-2, and group-3, respectively) were recorded in PMP. The monkey highly occupied semi-evergreen forest with little or no disturbances (occupancy value: ψ = 0.7679, SE = 0.182) in PMP. The occurrence of monkey was positively related with the forest stand characteristics

(Spearman's rank correlation: tree density (tree/plot): Υ_s = 0.305, P = 0.039; basal area (m^2/plot): Υ_s = 0.469, P < 0.0001; mean tree height (m): Υ_s = 0.583, P < 0.0001).

The availability of food resources was significantly different between the forest stands occupied by Phayre's leaf monkey and those were not used by the monkey. In this study, food plants for the monkey consisted of fourteen different families including Moraceae and Myrtaceae. Moreover, Phayre's leaf monkey also eats climbers (*Cissus vitiginea*, *Piper attenuatum* and *Smilax perfoliata*) which have not been recorded elsewhere in its range.

Phayre's leaf monkeys spent most of their time for feeding (54%) followed by resting (32%), travelling (7%) and other activites (7%). The monkeys travelled randomly on ground and tree strata (Kruskal-Wallis H test: H = 3.527, df = 4, P = 0.474) but they displayed non-randomly for feeding, resting and other behviors (i.e grooming, playing) (Kruskal-Wallis H test: feeding: H = 23.214, df = 4, P < 0.0001; resting: H = 23.291, df = 4, P < 0.0001; others: H = 11.107, df = 4, P = 0.025).

Phayre's leaf monkey spent slightly more time for resting in winter (31.65%) than that in summer (28.73%) and rainy (27.63%). Two feeding peaks were recognized in the early morning (0600 – 0800 h) and another in the late evening (1600 -1800 h). However, in terms of the pooled data set for all time intervals, there were no significant differences except for other behaviors (i.e. grooming or playing) (Kruskal-Wallis H test: H = 13.28, df = 5, P = 0.021). The study suggests that seasonal and time differences will not affect the

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Habitat suitability for Phayre's leaf monkey in PMP was created using the landscape habitat suitability index (HSI) model. The highly suitable habitat (5.28%) was lying to the northern part of PMP, followed by suitable (12.63%), moderately suitable (23.54%), least suitable (26.31%) and unsuitable (32.24%) habitats. The outputs of habitat suitability map were easily understandable and it can be used for park management plans particularly conserving the suitable habitats for Phayre's leaf monkey.

In conclusion, taxonomic information provided by this study can be used as basis to conserve genetic resources of threatened species particularly Phayre's leaf monkey from PMP as this population revealed genetic characters different from other subspecies of *T. phayrei*. Moreover, information on the food resources and the suitable habitats of Phayre's leaf monkey can be considered for upgrading the park management plan by conserving main food resources and protecting the suitable habitats.

Keywords: diurnal behavior, habitat suitability, habitat uses, non-invasive sample, Phayre's leaf monkey (*Trachypithecus phayrei*), phylogenetic

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Chapter I. General Introduction and Literature Review

1.1 Background

Tropical forests are the most ancient, more diverse, and the most ecologically complex of land communities (Myers *et al.* 2000). Though occupying only 7% of the earth's land surface, tropical forests probably sustain over half of the life forms (Wilson 1988, Myers 1992). However, these forests are being cleared, burned, logged, and converted to agricultural and industrial lands without historical precedent (Laurance and Bierregaard 1997). Approximately 5.8 million hectares of the remaining tropical forests were cleared or logged annually (Achard *et al.* 2002).

About 90% of primates live in tropical forests and they perhaps are the best flagship species for these forests (Rowe and Myers 2013). However, over 40% of the total 634 primate species are threatened to extinction due to combination of habitat degradation and anthropogenic activities (PCI 2013). This is especially problematic in Southeast Asia where most of the forests are being logged or converted to agricultural and settlements leaving very little habitats for primates. For instance, the habitats of the orangutans, endemic primates to the islands of Indonesia, have been reduced due to the massive palm oil plantations (Orangutan Conservancy 2013). In addition, the unsustainable commercial and illegal bushmeat trade is also threatening some primate such as apes, chimpanzees, and gorillas. Over 1 million metric tons of bushmeat are taken each year from African forests alone (Save the Primates

2013). Not only are the primates killed for food and body parts, orphaned primates are being sold on the exotic pet market. Hundreds of infant orangutans are taken from the wild for the pet trade every year (Orangutan Conservancy 2013). In order to highlight the dangers facing primate species, the world conservationists and primatologists announced the list of the world's 25 most endangered primates (Russell *et al*. 2012). Among the listed species, Asia possess 9 species (36%) followed by Madagascar representing 6 species (24%) in Africa and Neotropics represent 5 species (20%). In Asia, 6 out of total 9 most endangered primates belong to the Colobinae subfamily (Russell *et al*. 2012). Most colobine monkeys are arboreal species and mainly feed on leaf-biased diets (Strasser and Delson 1987, Davies and Oates 1994). Undoubtedly, habitat loss and degradation cause serious threats to such arboreal primates which mainly depend on vegetative parts for their foods (PCI 2013). Management action for assessing the suitable habitats is badly needed to save such threatened species.

As one of the endangered primates in Asia, Phayre's leaf monkeys (*Trachypithecus phayrei*) are being threatened and their population is continuously declining (Molur *et al*. 2003). They predominantly distribute to the southeast Asia (Gupta 2001, Bleisch *et al*. 2008) but their distribution also extends to Bangladesh, India, and China (Karanth *et al*. 2008, Bleisch *et al.* 2008). In Bangladesh and India, this species is at risk of habitat disturbances especially due to the establishment of tea gardens, paper mills, timber plantations, livestock ranching, shifting agriculture, firewood collection, charcoal production, and human settlements (Molur *et al*. 2003). Further to

the east of the range (China, Vietnam, Thailand, Myanmar, and Lao PDR), the major threat is hunting for traditional medicine and bushmeat (Bleisch *et al*. 2008). Indeed, in northern Thailand, they have been nearly entirely hunted out, and now only survive in the protected areas (Bleisch *et al*. 2008). The range of the species in Vietnam, Lao PDR and Thailand coincides with ethnic minorities that favour shifting cultivation, with a continual resulting loss of forest (Bleisch *et al*. 2008).

The authentic population status of the Phayre's leaf monkey, in its entire distribution range, is available from Tripura, northeast of India and it was approximated 1,000 individuals (Gupta 1994, 2001). In Bangladesh, about 1,300 individuals were estimated in past two decades (Gittins and Akonda 1982). Data is deficient for other range countries: Myanmar, China, Vietnam, Thailand and Laos. With isolated populations scattered throughout their range, the viability of these populations in the region is currently unknown.

Phayre's leaf monkey is categorized as Endangered by IUCN Red List and listed in CITES Appendix II (Bleisch *et al*. 2008). In Myanmar, this monkey is found in more than five sites particularly at the western Rakhine Range, eastern Shan Plateau and the central Bago Range (Pocock 1939, Bleisch *et al.* 2008). Although Phayre's leaf monkey has been listed as a completely protected animal by the national wildlife law (MoF 1994), its current status and habitat use throughout the country remains unknown. Only one population is confined to Popa Mountain Park (PMP) located in the

central plain. About 150 ~ 200 individuals of Phayre's leaf monkey was recorded in PMP (Min 2006).

On the other hand, due to the lack of detailed studies at molecular level, in Myanmar, the identification of animals including Phayre's leaf monkey, continues to be based on morphological features and geographical distribution (Pocock 1939). Moreover, after an intensive field survey in PMP (Oo 1995), there was an erroneous identification for Phayre's leaf monkey as Dusky leaf monkey (*T. obscurus*) which distributes in southern part of Myanmar (Pocock 1939, Geissmann *et al.* 2004). Both species have similar morphological characteristics, especially pale eye-patches (Pocock 1939, Geissmann *et al.* 2004). No comprehensive molecular studies have been conducted yet to confirm the identity of Phayre's leaf monkey in Myanmar, both at species and subspecies level. As a result, the taxonomic status of *T. phayrei* from Myanmar is still controversial. Wildlife management aimed at species conservation generally needs the adequate information of species identities and their phylogenetic positions among species, subspecies as well as closely related taxa (Issac *et al.* 2007, Kuntner *et al*. 2010, Medina-Romero *et al.* 2012). Molecular works to clarify the taxonomic status of Phayre's leaf monkey in Myanmar is badly needed to conserve the remaining populations.

Today habitat suitability models have been used to quantify the range of habitat conditions that will provide most of the requirements for a particular species (Turner *et al*. 1995, Larson *et al.* 2003, Nazeri *et al.* 2010). The output of habitat suitability models are usually simple, easily understandable and can be used for the assessment of habitat requirements or

prioritization of conservation efforts to save threatened species (Kushwaha *et al.* 2004, Zarri *et al*. 2008). Despite the fact that many studies have been conducted for habitat suitability models for each particular species, little is known for primates particularly Phayre's leaf monkey. Although the historical distribution of Phayre's leaf monkey had been recorded throughout Myanmar (Pocock 1939), information is still lacking for current distribution and its potential remaining habitats.

1.2 Study objectives

This study aimed at confirming the taxonomic status of Phayre's leaf monkey in Myanmar and also understanding its behavior and habitat use. Understanding the taxonomic status of a species can provide genetic conservation especially for highly threatened species. Every single species need specific habitat requirements for their life requisites of food, cover and reproduction. Thus, understanding of habitats and species adaption to habitat helps to develop the conservation implications for threatened species.

To address the above mentioned aims, the study had three specific objectives:

1) To clarify the taxonomic status of Phayre's leaf monkey in Myanmar particularly populations from Popa Mountain Park and Alaungdaw Kathapa National Park

2) To describe the population size, habitat use and behavior of Phayre's leaf monkey in Popa Mountain Park in order to realize that the habitats' influences on the species survival and its behaviors

3) To examine suitable habitat for Phayre's leaf monkey in Popa Mountain Park in order to support conservation implications

1.3 Literature Review

1.3.1 Phayre's leaf monkey

Phayre's leaf monkey (*Trachypithecus phayrei*) is rare endemics species to Asia and it is predominantly distributed in Southeast Asia (Karanth *et al.* 2008). Three subspecies (*T. p. phayrei, T. p. crepusculus,* and *T. p. shanicus*) have been identified (Pocock 1939, Yin 1993, Bleisch *et al*. 2008). *Trachypithecus phayrei phayrei* distributes in Bangladesh, northeastern India, and western Myanmar (Bleisch *et al.* 2008). *Trachypithecus phayrei crepusculus* can be found in Southwestern China, Lao PDR, Myanmar (south of the range of *T. p. phayrei*), Thailand, and northern Vietnam (*Geissmann et al*. 2004, Bleisch *et al*. 2008, He *et al*. 2012). *Trachypithecus phayrei shanicus* distributes in Southwestern China, northern and eastern Myanmar (Gupta 2001, Bleisch *et al.* 2008). The distribution of langurs and leaf monkeys including Phayre's leaf monkey and subspecies distribution of Phayre's leaf monkey were shown in **Figure 1.1a** and **Figure 1.1b**, respectively.

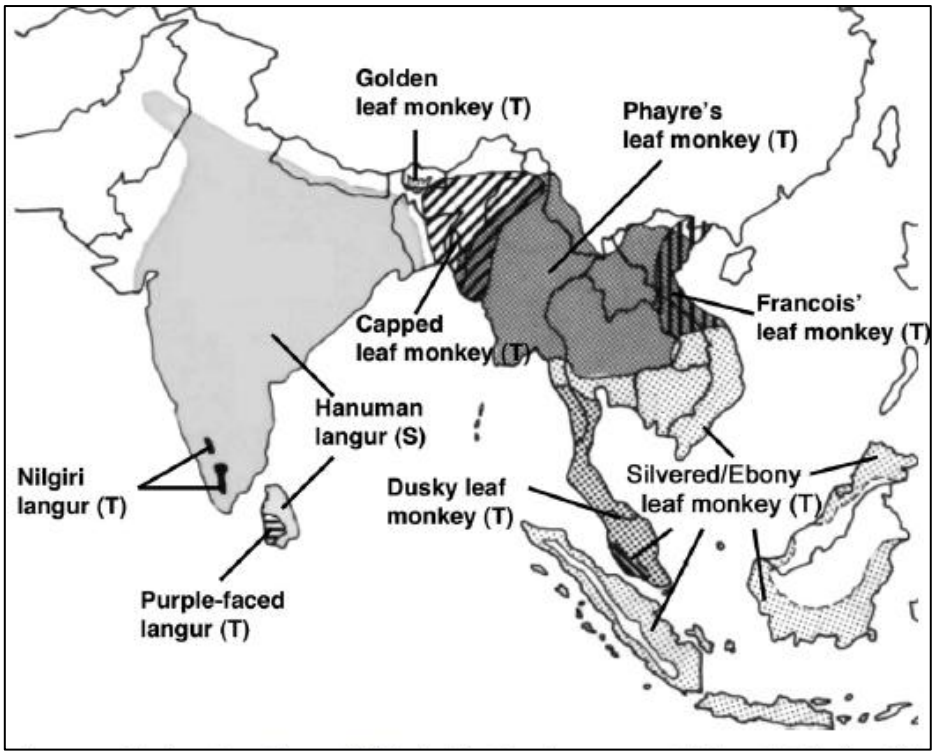


Figure 1.1a Map showing the distribution of Asian langurs and leaf monkeys including Phayre's leaf monkey (*Trachypithecus phayrei*). Map was adopted from Karanth *et al.* (2008). Hanuman langur (*Semnopithecus entellus*), Nilgiri langur (*Trachypithecus johnii*), Purple-faced langur (*T. vetulus*), Capped leaf monkey (*T. pileatus*), Golden leaf monkey (*T. geei*), Francois' leaf monkey (*T. francois*), Dusky leaf monkey (*T. obscurus*), Silvered/Ebony leaf monkey (*T. cristatus*)

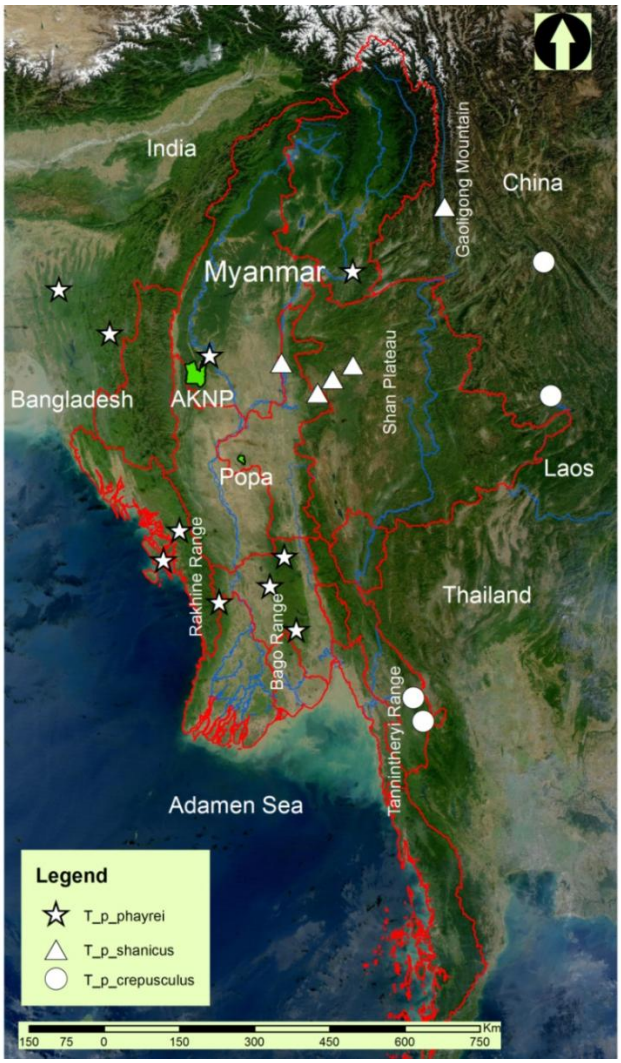
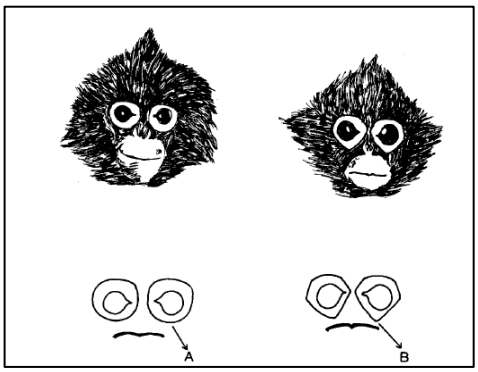


Figure 1.1b Subspecies distribution of Phayre's leaf monkey in Myanmar and neighboring countries (India and China). Map was created according to existing information for localities (Pocock 1939, Gupta 2001, He *et al*. 2012, this study 2012). AKNP: Alaungdaw Kathapa National Park, Popa: Popa Mountain Park

The striking morphological character of Phayre's leaf monkey is the white ocular rings around the eyes and sex can be identified by observing differences between the ocular markings (Bhattacharya and Chakraborty 1990) (**Figure 1.2**). Male is slightly larger than female, average 7.4 kg and 6.2 kg for male and female, respectively (Bhattacharya and Chakraborty 1990). The typical color of Phayre's leaf monkey represents dark grey-blue in color with some brown on the dorsal side and has mostly brownish-white pelage on the ventral side. The upper arms, legs and tail are silvery-grey in color, and, in general, the head and tail are darker than the rest of the chest (Cantwell 2011). The young monkey exhibits yellowish colored fur until about 3 months of age when the pelage begins to change (Cantwell 2011). Females give birth to one offspring at a time, which nurse for almost a year. Breeding of Phayre's leaf monkeys can be intermittent, and birth generally occurs between November and March (Gupta, 2001).



(a) Bhattacharya and Chakraborty (1990)

(b) Photo courtesy: Wikipedia

(c) Photo: Thaung Win

(d) Photo: Thaung Win

Figure 1.2 (a) Identification of male and female by ocular marking: in male (A), the white ocular rings around the eyes are parallel to the side of the nose, resulting in a back strip uniform in width. In female (B), the white ocular rings around the eyes bend inwards toward the nose causing more of black triangular shape; (b) Female with her infant exhibiting yellowish color fur; (c) Adult male taking a rest on big tree's branch; (d) Female with her one year old baby changed the fur color to dark grey already

Phayre's leaf monkey has either a unimale or multimale-multifemale social system and there are two males in the latter. The number of females in a group will range from 3 to 6. Solitary individuals have been reported. Since this species is a territorial one, they used to defend its territory against conspecific groups (Cantwell 2011). Little information is known regarding the lifespan of the Phayre's leaf monkeys. Other species within the family Cercopithecidae have life spans of 20 to 30 years in the wild (Johnson 2008).

Phayre's leaf monkey selects a wide variety of food items. For instance, the monkey selected about 81 different plant species in India (Gupta 2001) and 29 species in Bangladesh (Aziz and Feeroz 2009), but data are deficient for other countries. Phayre's leaf monkey prefers families Moraceae and Leguminosae and sometimes eats bamboo shoots (Gupta 1998, Aziz and Feeroz 2009). Since Phayre's leaf monkey is a folivore (Gupta and Kumar 1994), immature leaves to more mature ones were preferred by this monkey (Bose and Bhattacharjee 2002). It is also arboreal and diurnal species, although the monkey will move around the ground occasionally to move or to feed (Gupta 2001). As a consumer of fruits, Phayre's leaf monkey may play a role in seed dispersal. The considerable consumption of foliages by this monkey may also influence plant composition in local ecosystems (Cantwell 2011).

1.3.2 Taxonomic status of the Phayre's leaf monkey

The colobine monkeys are unique among primates on that they are predominantly leaf eaters and ex-habit foregut fermentation (Chivers and Hladik 1980). It is generally agreed that the colobines consist of two groups or clades; the African and the Asian (Oates *et al.*1994, Morales and Melnick 1998, Disotell 2000). Zhang and Ryder (1998) reported that Asian colobines appear to have migrated from Africa in the late Miocene. Asian colobines are more diverse than their African relatives (Osterholz *et al.* 2008). The Asian colobines are further split into odd-nosed monkey (*Pygathrix, Rhinopithecus, Nasalis, Simias*) and langur (*Presbytis, Trachypithecus, Semnopithecus*) groups, which are both belived to be monophyletic.

Phayre's leaf monkey is listed in Asian colobine monkeys (Karanth 2010) and it has been scientifically classified as follow:

Kingdom: Animalia
Phylum: Chordata
Class: Mammalia
Order: Primate
Family: Cercopithecidae
Subfamily: Colobinae
Genus*: Trachypithecus*
Species*: T. phayrei*
Subspecies: *T. p. phayrei, T. p. crepusculus, T. p. shanicus*

In comes after Karanth (2010), the langurs and leaf monkeys of South Asia were grouped into three clades: India, Southeast Asia, and northeast India including Bhutan and Bangladesh. Phayre's leaf monkey had been clustered to Southeast Asia clade along with other leaf monkeys: Silvered (*T. cristatus*), Francois' (*T. francoisi*), and Dusky (*T. obscurus*) (**Table 1**). Within the clade, the affiliations of Phayre's and Dusky leaf monkeys are disputed, and hence, the phylogenetic position of these monkeys is still in controversy (Geissmann *et al.* 2004, Karanth *et al.* 2008). Due to limited molecular work on Phayre's leaf monkey in Myanmar, the species identification is still continuing based on the geographical distribution and morphological characteristics.

Table 1 Classification of the langurs and leaf monkeys in Asia

	Groves (2001)	**Brandon-Jones *et al.* (2004)**	**Karanth (2010)**
Langurs of South Asia (Indian clade)			
Hanuman langur	*S. entellus*	*S. entellus*	*S. entellus*
Nilgiri langur	*T. johnii*	*T. johnii*	*T. johnii*
Purple-faced langur	*T. vetulus*	*T. vetulus*	*T. vetulus*
Langurs of northeast (northeast Indian, Bhutan, Bangladesh)			
Capped langur	*T. pileatus*	*T. pileatus*	*?. pileatus* *
Golden langur	*T. geei*	*T. geei*	*?. geei* *
Leaf monkeys of Southeast Asia (Southeast Asia clade)			
Phayre's leaf monkey	*T. phayrei*	*T. phayrei*	*T. phayrei*
Silvered leaf monkey	*T. cristatus*	*T. cristatus*	*T. cristatus*
Francois' leaf monkey	*T. francoisi*	*T. francoisi*	*T. francoisi*
Dusky leaf monkey	*T. obscurus*	*T. obscurus*	*T. obscurus*

Note: *S* = *Semnopithecus*, *T* = *Trachypithecus*. Adopted from Karanth *et al.* (2008, 2010). *These species could not be placed in either *Semnopithecus* or *Trachypithecus* groups due to incongruence between the mitochondrial and nuclear genes. Karanth (2010) reported that mtDNA tree strongly suggests that Capped and Golden langur belong to the *Semnopithecus* group, whereas the nuclear-coded *Lzm* gene supports that they may belong to the *Trachypithecus* group.

1.3.3 Phylogenetic analysis for species conservation

Conservation biology aims to preserve many species from throughout the phylogeny of a species group, rather than large number from one branch (Karanth 2010). By doing so, one preserves a wider array of species, but it means that one has to know something about the relationship of the organisms involved. In order to conserve such a species, systematic molecular works is one of best tools and it can provide important insights into movement of individuals between groups, how successful individuals are at breeding and how individuals are related to one another (Vigilant *et al.* 2012).

Knowledge on genetic status of a species enhances the genetic diversity conservation which is an explicit goal of Convention on Biological Diversity (CBD) (Laikre 2010). From genetic data, different kinds of information can be reconstructed, including the evolutionary relatedness of subspecies and populations, times estimates for evolutionary divergences, past population dynamics, extent of gene flows over geographical landscapes, and group social structure (Ruvolo 1997). Species conservationists can at least begin to incorporate this information into their plans for species conservation.

Among these genetic data, phylogenetic information mainly supports the evolutionary relationships among organisms (Nei 1996). Phylogenetic analysis is conducted to clarifying the evolutionary pattern of multigene families (Atchley *et al.* 1994) and to understanding the adaptive evolution at molecular level (Chandrasekharan *et al.* 1996). This technique also provides much deeper insight into the mechanism of maintenance of polymorphic

alleles in populations (Figuero *et al.* 1988). From the results of phylogeny analysis, several different insights of evolutionary history can be figured out. The patterns of relatedness among species, subspecies, and populations can be inferred—who is related to whom? From the same data set, it can also be estimated when species, subspecies, and populations diverged from each other—when did two groups last share a common ancestor?

Knowledge of phylogeny supports the species conservation in many aspects. For example, using the taxonomic weighting, dispersion and distinctness among eight Mexican marsupials (koala and possums group), potential conservation zones in Mexico were established for species that were listed under conservation categories (Medina-Romero *et al*. 2012). In Africa, the 55 afrotherian species (elephants, mammoths, dugong, manatees group) including five extinct species have been analysed for species-level phylogeny analysis and these were ranked top evolutionary unique species for conservation priority (Kuntner *et al.* 2010). Similarly, Issac *et al.* (2007) calculated evolutionary distinctiveness (ED) scores for mammals and then these scores were combined with the values for species' extinction risk at IUCN Red List. They ranked top 100 mammalian species that were both evolutionarily distinct and globally endangered (EDGE) species in order to provide a set of priorities for mammalian conservation. On the other hand, based on the genetic features and habitat isolation process, two types of conservation units were discussed among conservationists. Firstly, the evolutionarily significant unit (ESU) concerning with historical population structure, mtDNA phylogeny and long-term conservation needs; and,

secondly, the management unit (MU), addressing current population structure, allele frequencies and short-term management issues (Moritz 1994). To use genetic information effectively for species conservation purpose, distinguish between these two types of units is very important and phylogeny analysis is the best tool to infer the genetic status of a species.

1.3.4 Behavior and habitat use of the monkey

Habitat stands at front and center in wildlife management (Morris *et al.* 2008). Since all wildlife species need specific habitat requirements, understanding species adaptation to the habitat sources is essential for the effectiveness of species conservation plan (Morris *et al.* 2008). For instance, Blouin-Demers and Weatherhead (2008) reported that black ratsnakes (*Elaphe obsolete*) in the deserts of Israel spent a disproportionate amount of time in habitats that provide optimal thermal conditions for avoiding lethal temperatures, as well as for capturing prey and evading predators. Fortin *et al.* (2008) revealed that lifetime reproductive success of female red deer (*Cervus elaphus*) living on the Isle of Rum at west coast of Scotland was related to their specialization on specific plant communities located near the coast and inland. In the study of Tana River Red Colobus (*Procolobus rufomitratus*) in eastern Kenya, Mbora and Meikle (2004) pointed out that the abundance of monkeys had a positive relation with the diversity, density and abundance of food trees. Similarly, in order to avoid owl predation risk, the gerbil (*Gerbilliscus spp.*) shifted foraging activity to the bush microhabitat (Kotler *et al.* 1991).

There are great variations in ecological requirements and habitat uses among primates (Crandlemire-Sacoo 1988). In the study of Neotropical primates in Southeast Peru, Warner (2002) reported that among the nocturnal primates, *Aotus spp*. preferred dense bamboo stands for daytime resting while other species like *Saguinus sciureus* and *Cebus moloch* used mostly upper understory of tropical lowland rainforests. In the comparative locomotor ecology of gibbon (*Hylobates spp*.) and macaques (*Macaca spp.*) in Southeast Asia tropical rainforests, the gibbons strongly preferred the emergent canopy layer and traveled higher than the macaques in large trees but the latter preferred to cross narrower gaps than the first (Cannon and Leighton 1994). In China, a group of Sichuan Snub-nosed monkey (*Rhinopethicus roxellana*) highly preferred some specific trees not only for their vegetative parts but also the lichens that grew on the trees (Yiming 2006). One of the colobine monkeys, Francois' leaf monkey (*T. francoisi*) in Lao PDR was believed to be strongly associated with karst limestone reflected the thermal benefits of sleeping within caves in colder areas but the monkeys inhabit in warmer areas would not need these benefits, and so might be less tied to karst areas (Duckworth *et al.* 2010)

Similarly, habitat uses of Phayre's leaf monkey vary within its ranges. In Lao, no records for Phayre's leaf monkey came from deep within extensive closed-canopy forest. Instead, forests with broken canopy and extensive tall bamboo that features were possibly due to the results of human land-use in ancient or recent and mostly concentrated near water sources (Timmins *et al.* 2011). The use of degraded areas is also well known for Phayre's leaf monkey

in India. In Assam and Mizoram, northeast of India, Phayre's leaf monkey was found to live in depleted evergreen forests containing bamboo as well as habitats dominated by the shrub *Macaranga senticulata* and the herb *Alpinia allughas* (Choudhury 1994, 1996). In Tripura, small state located in northeast India, Phayre's leaf monkeys inhabit a wide range of habitats, for example, dense natural patches of evergreen forests, semi-evergreen forests, deciduous secondary forests after shifting cultivation, logging, clear felling, fire, and mixed forestry plantations of both timber and non-timber species (Gupta 2001). In this state, of the 56 groups identified during the survey in 1989, most (46.6%) were recorded from secondary forests followed by plantations (32.2%) and primary forests (21.4%) (Gupta 2001). In Thailand and Bangladesh, Phayre's leaf monkey lives in semi-evergreen forests and semi-deciduous forests (Roonwal and Mohnot 1977, Gittins and Akonda 1982). Phayre's leaf monkeys used to live in dense high forests, bamboo forests along hillsides and along the banks of streams in Myanmar (Roonwal and Mohnot 1977) and they are mostly forest dwellers and rarely visited cultivated grounds (Yin 1993). However, the current distribution and habitat uses of Phayre's leaf monkey in Myanmar are still unknown. Though Min (2006) had recently studied about feeding ecology of Phayre's leaf monkey inhabiting Popa Mountain Park in Myanmar, there is still lack of information for relationship between habitat uses and behavior of this monkey.

1.3.5 Habitat suitability modelling of the monkey

To develop a conservation management plan for a species, knowledge of its ecological characteristics and availability of preferred habitat is essential (Gibson *et al.* 2004). Nowadays, biodiversity extinction is a global crisis and this is, undoubtedly, due to human disturbances on natural habitats (Nazeri *et al.* 2010). With increase of human population and demand on food supply, natural habitats are converting to rural and agricultural areas and many species are going to extinct (Pimm *et al.* 1995). Habitat degradation in the tropical rain forests is the greatest threats to biodiversity as they harbour high species richness and complexity (Gaston 2000). Tropical forests represent less than 7% of land surface, but contain more than half of the species of plants and animals (Myers 1992, Roy *et al.* 2002).

Most of globally threatened mammals such as rhino, tiger, leopard, sun bear, tapir including Phayre's leaf monkey inhabit the tropical forests. It is well recognized that the disappearance of these invaluable tropical forest ecosystem will create a number of serious environmental problems that have negative impacts on biodiversity (Roy *et al.* 2002). In order to save the remaining biodiversity, habitat assessment is considered important for wildlife conservation and developing geospatial maps of habitat suitability has become imperative for habitat conservation implications (Nazeri *et al.* 2010). Conservationists considered that habitat quality plays a vital role for the survival of species particularly herbivores since their behavior, performance and dynamics are operating through vegetation distribution (Pettorelli *et al.* 2005a).

Working on wildlife conservation and management always faces with difficulties due to the lack of data on species and habitat suitability of different species (Kushwaha *et al.* 2004). Traditional methods of wildlife habitat evaluation have many limitations due to inaccessibility to the whole study area, especially in dense tropical forests. Predictive habitat modelling which is part of habitat suitability modelling can be used to fulfill this need. Geospatial technology including Remote Sensing (RS), Geographic Information System (GIS) and Global Positioning System (GPS) provide an efficient method for determining habitat suitability and quality which has great implication in biodiversity conservation. Many studies have been reported for successful implication of geospatial technology in the habitats suitability of tiger (Imam *et al.* 2009), wild boar (Park and Lee 2003), sambar deer (Podchong *et al.* 2009), and wild turkey (Glennon and Porter 1999).

Similarly, geospatial technology has also been applied for primate conservation works with several approaches. For instance, Stickler and Southworth (2008) examined the relative performance of two high-resolution satellite sensors (2.5 m resolution Quickbird and 30 m resolution Landsat ETM+) in capturing pattern of habitat selection by the medium-sized primate, Redtail monkey (*Cercopithecus ascanius*) in Uganda. Peck *et al.* (2011) generated a species-specific landscape map to know the remaining forest with potential to hold the brown-headed spider monkeys (*Ateles fusciceps*) in Ecuador. Torres *et al.* (2008) developed the habitat suitability model by using the different satellite images for three decades to examine habitat degradation in order to pinpoint the remaining potential habitats for conserving the most

endangered primate, chimpanzee (*Pan troglodytes*) inhabiting in Western Africa. Similarly, Shanee *et al*. (2013) evaluated the effectiveness of the protected area network by the integration analysis of habitat suitability model and the human risk assessment throughout the distribution of Andean titi monkey (*Callicebus oenanthe*) in northeastern Peru.

Although the conservation efforts have been given to the threatened primates inhabiting the Southeast Asia tropical forests, the application of habitat suitability model using the geospatial technology is very limited. Similarly, until today, there is little literature on the study of habitat suitable model for the Phayre's leaf monkey in its range countries.

1.4 Methodology

1.4.1 Study areas

Popa Mountain Park

Popa Mountain Park (PMP) is located in central dry zone of Myanmar and it covers an area of about 150 km^2 including core and buffer zones. The park was declared as a protected area in 1989 and it lies between 20˚47' to 20˚56' N and 95˚11' to 95˚19' E in Mandalay Division (**Figure 1.3a**). It is an extinct volcanic mountain with elevation ranges from 300 m to 1,500 m a.s.l. (Htun *et al.* 2011). In Myanmar, the dry zone is geographically a distinct rain shadow area because of surrounding mountain ranges representing climatic barriers for South-west monsoon in summer and North-east monsoon in winter. Mt. Popa is more striking in the dry zone because other mountain only raise

slightly above the remainder of the range, while Mt. Popa stands completely alone with its sleep sides and craggy top. Because of its height, the park receives heavy rainfall and therefore has a climate and vegetation that are quite distinct from those of the surrounding region. The mean annual rainfall during 1994 - 2010 was about 1,000 mm, and mean maximum and minimum monthly temperature for the same period were 28˚C and 14˚C, respectively (Popa Agricultural Office 2010).

PMP stands as main water catchment for the Kyetmauk-Taung dam in the southern part and it was completed in 1967 for irrigation purposes (FD 1981). Most of streams in and around PMP are the purely rain-fed streams which flow only for a few hours even after heavy rain. It is presumably due to the relatively low annual rainfall in the area and the porous nature of the volcanic soils on the upper slopes of Mt. Popa. The only streams known to be perennial are the Kyaukpon stream flowing into the Kyetmauk-Taung dam, and the Chaung-Ma stream which drains in north. The only permanent water resources in PMP are natural springs and about 12 of significant large size were recorded (FD 1981).

The main rock types of Mt. Popa itself are lava and pyroclastic rocks (i.e. rocks made up of different sizes, including volcanic ash) resulting very fertile soil and supporting vegetation cover which is relatively luxuriant compared with the surrounding plains (FD 1981). Mount Popa's name comes from the Sanskrit for flowers (*Pushhpa*) as its slopes are covered with flowering trees (FD 1981, Yin 1958) and the mountain is also famous for its

herbal plants. Vegetation types of PMP vary along the elevational ranges from dry than-dahat forest occurred at low elevation (300-600 m) and mainly dominated by *Terminalia oliveri*, *Jatus hamiltonianus*, *Acacia catechu*, scrub-indaing forest also found at low elevation (300-600 m) and commonly predominated by *Dipterocarpus tuberculatus*, *Shorea obtusa*, *Pentacme siamensis* dry mixed-deciduous forest occupied at elevation between 600-900 m and dominated species are *Jatus hamiltonianus*, *Ulmus lancifolia*, *Shorea obtusa*, dry-hill forests or semi-evergreen forest found at higher elevation (> 900 m) and dominated by *Pittospotum napaulense*, *Carallia brachiata*, *Trevia nudiflora*, *Quercus serrata*, and small grassland area is found above the elevation of 1,200 m. The mountain was opened up to the northern side during the eruption and the crater became a valley. The crater is about 3 km long (north-south), 2 km wide (east-west) and about 1,500 m deep in some part (Zin, 1998). The vegetation types in the crater are very luxuriant and included trees which attain to a height 23 to 27 meters (Min 2006, Htun 2008, Htun *et al.* 2011). The underneath of the forest is very moist and covers with dense herbs as in evergreen forests. The inside of the crater is dominated by the evergreen species such as Kadut (*Ficus hispida*) and Pyinma (*Largestromia speciosa*). The dry mixed deciduous forests can be found at the edge of the crater comprised with Teak (*Tectona grandis*), Pyinkado (*Xylia xylocarpa*), Didu (*Bombax insigne*), Nabe (*Lannea coromandelica*), Panga (*Terminalia chebula*) and Pet-wun (*Berrya mollis*).

PMP was believed to be largely covered with high forests in the past, so did to have continued an abundance of wildlife. Past records mentioned the

occurrence of elephant, rhinoceros, sambar and tigers (FD 1981). But by the time of the faunal survey carried out by Shortbridge in 1913, all these large species had already disappeared (Yin 1958, FD 1981). Apart from a variety of bats, rodents and other small mammal, the specimens of the Rhesus macaque (*Macaca mulatta*), Phayre's leaf monkey (*Trachypithecus phayrei*), jungle cat (*Felis chaus*), jackal (*Canis aureus indicus*) and the wild dog (*Cuon alpinus*) were collected in this survey. Other interesting species were two flying squirrels (*Ratufa bicolor* and *Hylopetes phayrei*) and the Burmese ferret badger (*Melogale personata*).

The area surrounding the PMP is somewhat densely populated and there are 45 villages (population about 55,000 in 2010) around the foot of the mountain (Htun *et al.* 2011). It is assumed that the park's vegetation is secondary or tertiary generation due to severe impact of local people in the early 20th century (FD 1981). During 30 years (1955–1984), the Forest Department of Myanmar, United Nations Development Program and Food and Agricultural Organization (UNDP-FAO) undertook reforestation programmes which included both gap planting and forest enriching to stimulate natural regeneration. Since then, the forest cover has gradually recovered, and once-depleted natural springs have been restored (Htun *et al.* 2011). Despite its protection status, encroachment of villagers from the surrounding villages for firewood, farming, cattle grazing and collecting non-wood forest products has not been stopped (Rao *et al.* 2002).

Alaungdaw Kathapa National Park

The Alaungdaw Kathapa National Park (AKNP) is located about 200 km far north of PMP. It lies between 22° 10' to 22° 42' N and 94° 15' to 94° 40' E Sagaing Division (**Figure 1.3b**). In 1893, Patolon and Taungdwin reserved forests were firstly established (FD 1984). About a century afterward, the combined reserved forests were designated as Alaungdaw Kathapa Wildlife Sanctuary in 1983 under the project for the Nature Conservation and National Parks (NCNP) jointly implemented by Forest Department, Myanmar and the UNDP-FAO organization (FD 1984). In 1989, it was gazetted as the first national park in Myanmar and covers about 1,400 km^2 in area. AKNP had been announced as ASEAN heritage park in 2003 (ACB 2003).

The AKNP has a monsoonal climate but it is sheltered to some extent from the full effects of the southwest monsoon by the western Chill Hills (FD 1984). Rainfall occurs mainly between May and October and it is heaviest in August and September. The mean annual rainfall was recorded about 1,500 mm (Oo *et al.* 2006). The mean annual temperature ranges from a mean minimum of about 10˚C in January to a mean maximum of about 42˚C in April (Oo *et al.* 2006).

Topographically, AKNP is marked by a series of well-defined north-south ridges, with altitudes ranging from about 400 m in the lowest part of the Patolon valley to just over 1,500 m at the highest point, the summit of Hlaingma Taung. The area is well watered since the main rivers,

Patolon and Taungdwin, are flowing northward as tributaries of the Chindwin River. Both rivers are perennial, flowing through generally steep sided valley, often with much exposed rock and large boulders along their beds. There are also a few perennial springs in the park (FD 1984).

In AKNP, the whole area is well forested and the major forest types are mixed deciduous forest dominated by teak (*Tectona grandis*) mainly associated with pyinkado (*Xylia xylocarpa*), taukkyan (*Terminalia crenulata*), dry upper mixed deciduous forest mainly dominated by pyinkado followed by teak, taukkyan, hnaw (*Adina cordifolia*), semi-indaing forest typically includes in (*Dipterocarpus tuberculatus*), ingyin (*Pentacme siamensis*) and an admixture of *Quercus* and *Castanopsis spp*. occurs on the higher ridges, pine forest occurred at several places along the crest of the higher ridges over 800 m, and evergreen forest occurred to a very limited extent near the crest of eastern ridge, the Mahutaung and along the certain rocky streams. Bamboo species occurred in AKNP are tinwa (*Cephalostachyum pergracile*), wabo (*Dendrocalamus hamiltonii*), myin (*Dendrocalamus strictus*), and thaik (*Bumbusa tulda*).

The forested scenery of AKNP is attractive and there is an abundance of wildlife including Asian elephant (*Elephas maximus*), leopard (*Panthera pardus*), Himalayan bear (*Ursus arctos*), gaur (*Bos gaurus*), banteng (*Bos banteng*), barking deer (*Muntiacus muntjak*) and various bird species. Primates include at least one macaque (*Macaca assemensis*) and so called capped langur (*Trachypithecus pileatus*) (FD

1984). Although tiger (*Panthera tigris*) was reported (FD 1984), there was no field sign for tiger presence during the recent nationwide tiger survey in 1997-2001 (Lynam *et al.* 2006).

About 30 villages are located around AKNP and most local people primarily are engaged to agricultural practices (Oo *et al*. 2006). The villagers collect a variety of forest products from the park, including timber for houses, building poles, fuelwood, bamboos, canes, agarwood (*Aquilaria agallocha*), shaw (*Sterculia spp.),* thanatka *(Limonia acidissima*), resin, gums and honey. In AKNP, there is one of the famous religious shrines attracting large numbers of pilgrims who travel mostly on food by various routes passing through the park (FD 1984). Consequently, high pressures from anthropogenic activities were found in AKNP such as hunting, extraction of non-timber forest products, grazing by domestic cattle and also high number of pilgrims to the shrine (Rao *et al*. 2002).

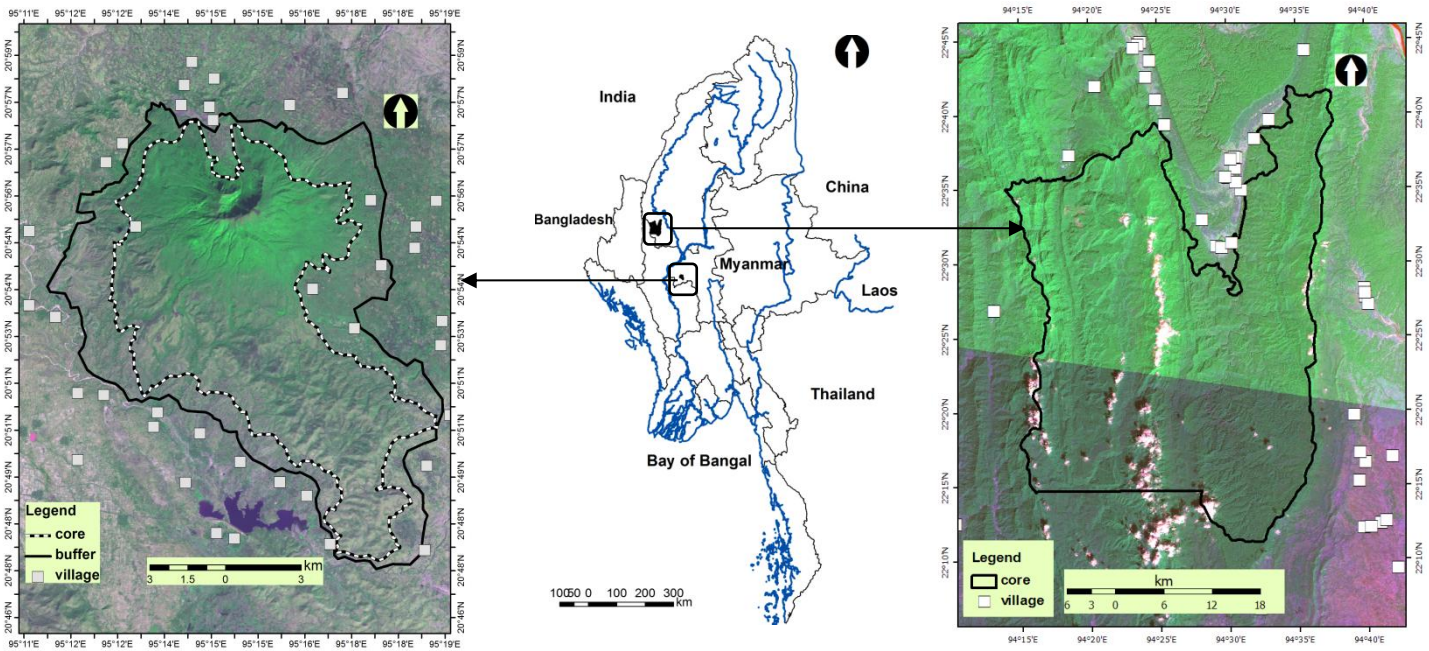


Figure 1.3 True color composite of ASTER image (15 x 15 m) for Popa Mountain Park (a), and Landsat 7 TM (30 x 30 m) for Alaungdaw Kathapa National Park (b) in Myanmar. The study sites were surrounded many villages (□) and consequently human interferences were observed in both sites during the study.

1.4.2 Data collection and structure of the dissertation

Data collection schedule

The intensive field study was conducted between 2010 and 2012. DNA samples were collected in July and August 2012 at PMP and AKNP, respectively (chapter II). Behavior study (chapter III) and observation of habitat uses were conducted in June and November 2010 for rainy and winter, respectively and April 2012 for summer. The ASTER satellite image data (path/row: 133/ 46) was acquired for 2010 January 17 scene and ground vegetation data was collected during June and November 2010 in PMP (chapter IV). Moreover, chapter IV was supplemented by the secondary data such as reference sites, topographic map, and the existing shape files for roads, villages and park boundary of PMP. A group of Phayre's leaf monkey in PMP was selected for behavior study.

Structure of the dissertation

This dissertation comprises five chapters. The introduction chapter followed by main three research chapters and each chapter has specific objectives and hypotheses. Chapter II concerns the taxonomic status of Phayre's leaf monkey from two study sites, PMP and AKNP. However, the contents for chapter III (habitat use and behavior study) and IV (modelling habitat suitability) focus the population of PMP only. Chapter V is the last part of dissertation and the integration of key findings, conclusion and conservation implications is

presented. References cited throughout the manuscript is listed at the end of dissertation and followed by appendices, Korean and Myanmar abstracts.

Chapter II. Molecular Phylogenetic Status of Phayre's Leaf Monkey

2.1 Introduction

Phayre's leaf monkey is phylogenetically embedded within the family Cercopithecidae and closely related to the genus *Semnopithecus* (Perelman *et al*. 2011, Wang *et al*. 2012). Three subspecies (*T. p. phayrei, T. p. crepusculus, and T. p. shanicus*) inhabit Bangladesh, northeastern India, Myanmar, Southwestern China, Thailand, Laos, and northern Vietnam (Bleisch *et al.* 2008). Recent genetic analyses have demonstrated that subspecies *T. p. crepuculus* as a distinct species (Geissmann *et al.* 2004, He *et al*. 2012) while the other two species, *T. p. phayrei* and *T. p. shanicus* form as a sister taxa (He *et al*. 2012). At a species level, Phayre's leaf monkey forms as Southeast Asia clade with the closely related taxa: Silver leaf monkey (*T. cristatus*), Francois' leaf monkey (*T. francois*), and Dusky leaf monkey (*T. obscurus*). Within the clade, the affiliations of *T. phayrei* and *T.obscurus* are disputed and the phylogenetic position of these two species is still in controversy. Geissmann *et al.* (2004) put *T. phayrei* and *T. obscurus* as a sister group based on the results of mitochondrial cytochrome b (*Cyt-b*) gene. They also reported that the similar facial depigmentation of both species showing conspicuous white eye-patches and a large white patch around the mouth, extending up to the base of the nose. Similarly, Karanth *et al.* (2008) reported that the mtDNA phylogeny tree revealed the *T. phayrei* from India is more closely related to

the *T. obscurus* while *T. phayrei* from Vietnam is more closely related to the *T. francois.*

In Myanmar, the distribution of all three subspecies of *T. phayrei* had been recorded throughout the country (Pocock 1939). But no scientific documents have been published for its molecular status yet. As a result, the taxonomic status of *T. phayrei* in Myanmar is still controversial particularly the remnant population of Popa Mountain Park (PMP) located as an isolated mountain in central plains. The existence of *T. phayrei* in PMP was first recorded in 1913 (FD 1981) and subsequent records were given by Pocock (1939), Yin (1993) and Min *et al*. (2009). However, there was erroneous identification of Phayre's leaf monkey in PMP as Dusky leaf monkey (*T. obscurus*) (Oo 1995) due to similar morphological characteristics of these two species (**Figure 2.1a-b**). No molecular studies have been conducted yet to confirm the identity of Phayre's leaf monkey in PMP both at species and subspecies levels.

Moreover, there were some suspicious reports for the existence of Phayre's leaf monkey in Alaungdaw Kathapa National Park (AKNP) which is located about 200 km north of PMP. The country longest and biggest river, the Ayeyarwaddy River, is flowing between the PMP and AKNP. Pocock (1939) reported that the existence of Phayre's leaf monkey at the Kin village close to the AKNP but there was no record for this monkey inside the park yet. However, one monkey species showing the similar morphological characters of Phayre's leaf monkey (**Figure 2.1c-d**) was recently reported. It is interested to investigate molecular status of the populations from AKNP to clarify the

species identity. The results could provide the basic information for park management and further considerations of species conservation.

This study was conducted to assess phylogenetic status of Phayre's leaf monkey in Myanmar particularly in PMP and AKNP. This was also the first molecular work using non-invasive (feces, hair) samples which were collected without making any pains to the animals by incision or injection. In addition, the non-destructive (bone) samples were also used and these bones were the remains of a dead monkey killed in land slide in PMP. Although DNA extracted from these kind of samples tend to be of low quantity and quality, it has been used to produce reliable results (Bradley *et al.* 2001, Rutledge *et al.* 2010) and could especially be preferred for the wild primates which are endangered, elusive, or both (Vigilant *et al.* 2012).

Two DNA markers, nuclear protamine P1 (*Prm1*) and mitochondrial cytochrome b (*Cyt-b*) gene, were used to infer the phylogenetic status of *T. phayrei*. In general, mitochondrial gene can be a useful source of information for both phylogenetic and biogeographic studies (Irwin *et al*. 1991, Templeton *et al*. 1995). However, to draw firm conclusions, the result of genetic analyses based on mitochondrial variation alone is to risk repudiation by later research on nuclear variation (Melnick and Hoelzer 1993). Therefore, it is good to use both nuclear and mitochondrial genes for phylogenetic status of a species taking the most interesting information yielded by mitochondrial gene with careful consideration of the disagreements between the results of genetic analyses for these two genes.

Habitat fragmentation results in reduced population sizes and it may cause significant genetic drift, inbreeding, and susceptibility to further stochastic demographic contraction (Goossens *et al.* 2011). For example, the mtDNA genetic diversities of golden-brown mouse lemurs (*Microcebus ravelobensis*) were significantly different among the isolated forest fragments in Madagascar (Guschanski *et al.* 2007). On the other hands, the main geographical barriers such as impassable rivers are expected to negatively affect gene flow and thus result in genetic differentiation of populations (Vigilant and Guschanski 2009). For instance, the genetic variations were significantly different between the two populations of Bornean orang-utans (*Pongo pygmaeus*) from either side of the Kinabatangan River in Sabah, Indonesia (Jalil *et al.* 2008).

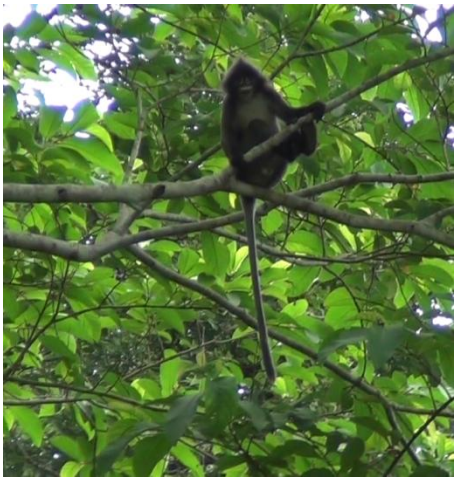
Wildlife management aimed at species conservation generally needs the adequate information of species identities and their phylogenetic positions among species, subspecies as well as closely related taxa (Issac *et al.* 2007, Kuntner *et al.* 2010, Medina-Romero *et al.* 2012). This study aimed to clarify the molecular status of Phayre's leaf monkey in PMP and also to investigate the species identity of the populations from AKNP both at species and subspecies level. Additionally, the phylogenetic relationship between the two populations of Phayre's leaf monkey from PMP and AKNP was examined with the aim of discovering whether the habitat fragmentation and geographical barriers affect the phylogenetic status of a species. Understanding the taxonomic status of a threatened species and its adaptation

to source habitats is vital to conserving biological resources and designing the effective conservation strategies.

(a) Photo: Thaung Win

(b) Photo courtesy: Geissmann *et al.* 2004

(c) Photo: Thant



(d) Photo: Thant

Figure 2.1 Morphological features of the Phayre's leaf monkey and Dusky leaf monkey. (a) Phayre's leaf monkey inhabits in Popa Mountain Park, (b) Dusky leaf monkey occurred at Zurich Zoo in Switzerland, (c) so called Phayre's leaf monkey found at KaBaing village close to Alaungdaw Kathapa National Park, and (d) a monkey found in the natural forests of Alaungdaw Kathapa National Park

2.2 Materials and methods

2.2.1 Study sites

This study was mainly focused on the populations of Phayre's leaf monkey in Popa Mountain Park (PMP) and Alaungdaw Kathapa National Park (AKNP).

PMP is located in the central dry area and was established in 1989. It is about 50 km away from east of the Ayeyarwaddy River flowing between the western Rakhine and central Bago Ranges (**Figure 2.2**). Although the PMP is an isolated extinct volcanic mountain rising abruptly out of the plains, there might have been some past vegetative connectivity with Bago Range to the south, and Shan Plateau to the east (**Figure 2.2**). There is no precise evidence as to the age of the Mt. Popa but it was estimated at approximately 2-3 million years ago (FD 1981). The existence of Phayre's leaf monkey in the Mt. Popa was first recorded by Shortbridge during his faunal survey in 1913 (FD 1981). Subsequent observations were reported in Pocock (1939), Yin (1958, 1993), and Min *et al.* (2009). However, little is known about the migration of this monkey from such an isolated mountain to other regions.

Alaungdaw Kathapa National Park is located about 200 km far from PMP in northwest direction and at the west bank of the Chindwin River which is a secondary to the Ayeyarwaddy River. It is connected with the ranges of northeast India (**Figure 2.2**). Alaungdaw Kathapa National Park was established in 1989 and then it became the first national park in Myanmar (MoFA 1989). In AKNP, mixed-deciduous forest predominates with some riverine evergreen forests along the water courses (FD 1984). Although one

species of leaf monkeys, Capped leaf monkey (*Trachypithecus pileatus*) had been reported, there was no information about the existence of Phayre's leaf monkey (*T. phayrei*) in the AKNP (FD 1984). There is no any known study on Phayre's leaf monkey in AKNP.

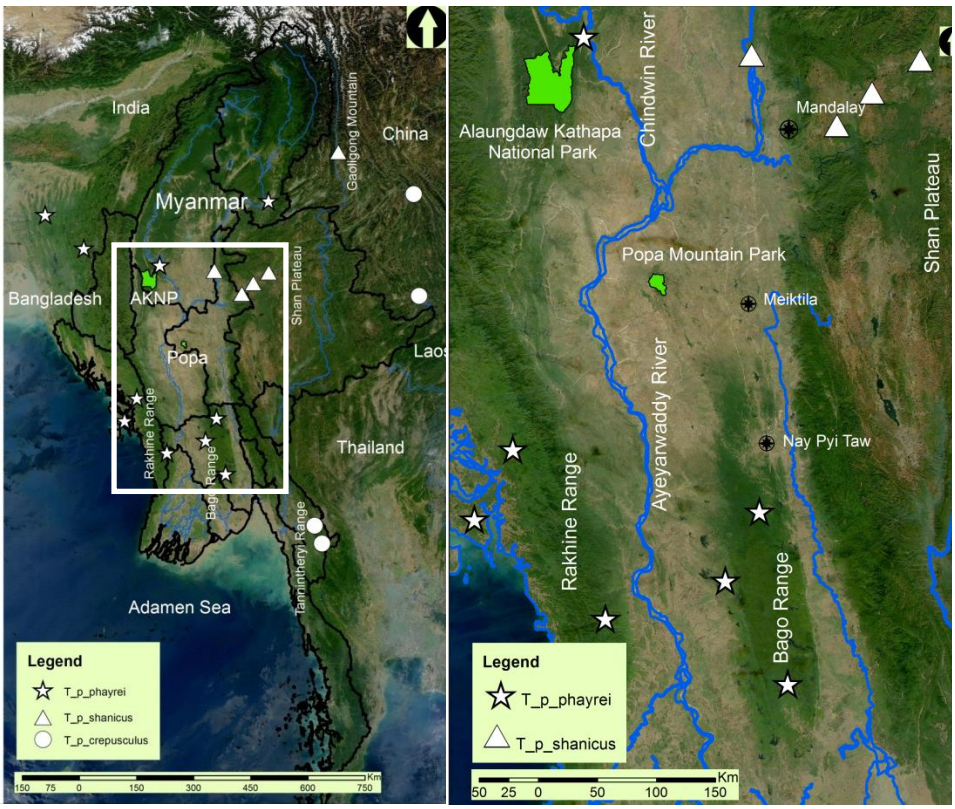


Figure 2.2 Distribution of Phayre's leaf monkey in Myanmar and neighbouring countries, India and China (left). The study sites (Popa Mountain Park and Alaungdaw Kathapa National Park) where DNA samples were collected (right). The main river, Ayeyarwaddy, is flowing through north to south between the two study sites. Maps were created according to existing information for localities of Phayre's leaf monkey (Pocock 1939, Gupta 2001, He *et al*. 2012, this study 2012).

2.2.2 Sample collection

Two kind of non-invasive samples (feces and hair) and one non-destructive sample (bones from a dead monkey) were collected in July and August 2012, from Popa and AKNP, respectively. A total of 50 fecal samples (n = 32 from PMP and n = 18 from AKNP) were collected. About 15 hair samples (**Figure 2.3a**) were collected from a captive animal at Kabaing village close to AKNP. The hairs were collected by combing to the back of animal and then transferred to a clean envelope. The bones (head, femur and tibia, **Figure 2.3b**) were obtained from local people who had stored the bones of a monkey killed in landslide at PMP. The bone samples were stored in a clean plastic bag in ambient temperature. Fecal samples were collected within one hour post-defecation at both study sites and then stored in 2 ml tubes containing 99.9 % ethanol (**Figure 2.3c**). All samples were stored at -20˚C until DNA extraction. The sample number, sample ID and types of samples used in this study were provided in **Appendix 2.2**.

(a) (b) (c)

Figure 2.3 Three types of DNA samples were used to analyse the phylogenetic status of Phayre's leaf monkey in this study. (a) hair cutting for DNA extraction; (b) Bone (head, femur, tibia) from a dead monkey in land slide; (c) feces stored in the 2 ml tube with 99.9% ethanol

2.2.3 Deoxyribonucleic acid (DNA) markers

Nuclear protamine P1 (Prm1)

Protamines are small, arginine-rich, nuclear proteins that replace histones late in the haploid phase of spermatogenesis and they believed essential for sperm head condensation and DNA stabilization (Kasinsky 1989). They occur in all mammals (Oliva and Dixon 1991) and found inside the nucleus of animal cell (**Figure 2.4**). The nuclear genes are transmitted by both sexes (Melnick and Howlzer 1993). There are two different types of mammalian protamine, called protamine P1 (*Prm1*) and protamine P2 (*Prm2*) (Oliva *et al.* 1989). *Prm1* gene has been used for the phylogeny status of primate (Retief *et al.* 1993) and other mammalian groups (Blacket *et al.* 1999, Krajewski *et al.* 1997). Protamine forms more stable complexes with DNA and are more efficient at displacing histones and transition proteins from DNA (Ohtsuki *et al.* 1996).

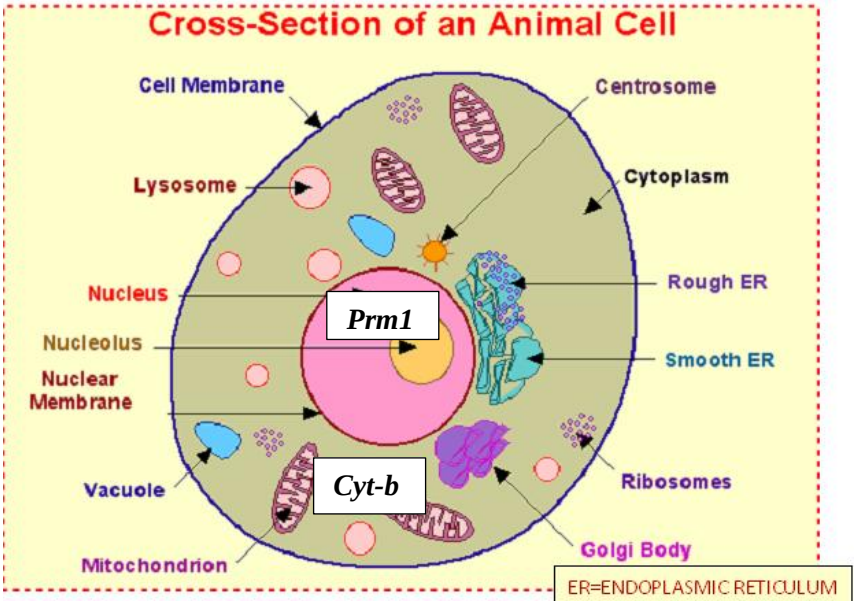


Figure 2.4 Cross-section of an animal cell (Adopted from http://dbscience5.wikispaces.com/Jillian). Protamine P1 (*Prm1*) genes are transmitted by both sexes and found inside the nucleus. Mitochondrial cytochrome b (*Cyt-b*) genes are maternally inherited and located in the fluid (the cytoplasm) that surrounds the nucleus.

Mitochondrial cytochrome b (Cyt-b)

Mitochondria are endocellular cells that convert the energy from food into a form that cells can use. Each cell contains hundreds to thousands of mitochondria, which are located in the fluid (the cytoplasm) that surrounds the nucleus (**Figure 2.4**). The average rate of substitution is approximately five to ten times faster than in the nuclear genome (Brown *et al.* 1979). Accordingly, comparisons of closely related populations or species generally show much more variation in mitochondrial DNA than in nuclear DNA. In addition, the clonal-maternal inheritance (maternal gene), lack of recombination, and economical organization of *mt*DNA (Brown 1983) simplify its quantitative analysis (Melnick and Howlzer 1993). Moreover, *mt*DNA is the simpler source of genetic data and yields more information for the analysis of animals' population and phylogeny studies, for instance in the tiger salamander (*Ambystoma tigrinum*) (Templeton *et al.* 1995), in galliformes (Armstrong *et al.* 2001), in the rocky intertidal barnacle (Sotka *et al.* 2004). The *mt*DNA gene is also widely used for genetic study of primates (Melnick and Howlzer 1993). Among 37 different regions of primate *mt*DNA molecule (**Figure 2.5**), the cytochrome *b* (*Cyt-b*) gene is preferable to use for phylogenetic analysis in most langurs and leaf monkeys in Asia (Geissmann *et al.* 2004, He *et al.* 2012, Karanth 2010, Karanth *et al.* 2008).

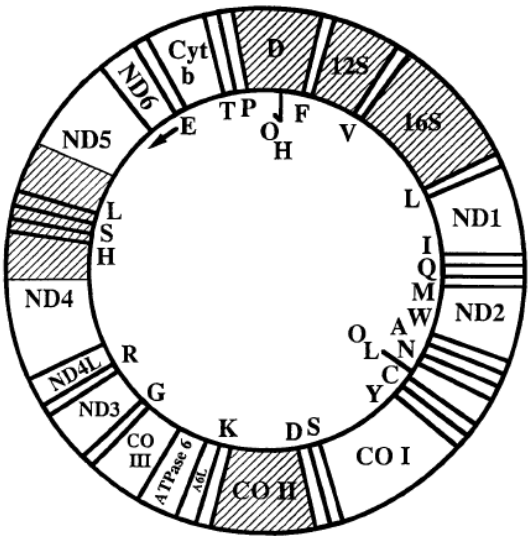


Figure 2.5 Gene organization of a primate *mt*DNA molecule. The molecule contains 13 protein-coding genes [three cytochrome oxidase subunits (COI-III), seven NADH-dehydrogenase subunits (ND1-6, ND4L), two ATPase subunits (6 and 6L), and cytochrome b (*Cyt b*)]; 2 ribosomal RNA genes [12s and 16s]; 22 transfer RNA genes [upper case letters along the inside rim of the circle]. Figure adopted from Melnick and Howlzer (1993).

2.2.4 DNA extraction, Polymerase Chain Reaction (PCR) amplification and sequencing

DNA extraction

The protocol used for hairs DNA extraction was modified from Karanth *et al.* (2008). Using a sterile sissor, five to six hairs were cut 5 mm from root (follicle) and all shafts to a 2 ml tube. The resulting hairs were decomposed in 200 μl of digestion buffer (Buffer TL) and 20 μl of Proteinase-K (LaboPass^{TM} Tissue Mini Kit, COSMO GENETECH, Korea) in a incubator for 21 hours at 60˚C. After the hairs were completely digested, 400 μl of buffer TB was added to the sample and mixed thoroughly. The supernatant was transferred into a spin column and centrifuged at full speed (20000 g) for 1 min. The supernatant was replaced into a new spin colum and 700 μl of buffer BW were added. The tube was centrifuged again at full speed. After DNA precipitation, the resulting pellet was replaced to a new 2 ml tube and dried for 5 min at room temperature. The pellet was re-dissolved in 200 μl Buffer AE and then incubated for 10 min at room temperature. The tube was centrifuged again at full speed for 1 min. The protocol described above was also applied for 20 mg of bones.

Fecal DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen: Cat No. 51504) with slight modification of the user mannual. 200 mg of feces were weighed in a 2 ml tube and added 1.6 ml of Buffer ASL. The mixture were spinned on the vortex until the feces were thoroughly homogenized. The mixture was centrifuged for 1 min at full speed. Then 1.4 ml of the supernatant was removed to a new 2 ml tube. Added 1 InhabitEX

Tablet to the solution and spinned until the tablet was completely dissolved in the solution. The mixture was incubated for 1 min at room temperature and then centrifuged for 6 min at full speed. All supernanant was removed to a 1.5 ml tube and centrifuged for 3 min at full speed. This process was duplicated. Then 500 μl of the supernatant was removed to a new 2 ml tube and added 25 μl of proteinase K and 600 μl of Buffer A. After spinning the mixture, it was incubated at 70 ˚C for 10 min and then brifely centrifuged to remove drops from the inside of the tube lid. The lysate was added 600 μl of 99.9% ethanol and briefly vortexed. Applied 700 μl lysate to a QIAamp spin coulmn followed by centrifuging at full speed for 1 min. The rest of lysate was centrifuged again. Replaced the pellet to a new 2 ml tube and the filtrates were discarded. The pellet was washed with 500 μl Buffer AW1 and centrifuged at full speed for 1 min. Added 500 μl Buffer AW2 for second round washing and centrifuged at full speed for 3 min. Dried the pellet for 5 min as some residues of ethanol was still have on the pellet. Transferred the pellet to a new 1.5 ml tube followed by adding 200 μl Buffer AE to elute DNA and centrifuged at full speed for 1 min.

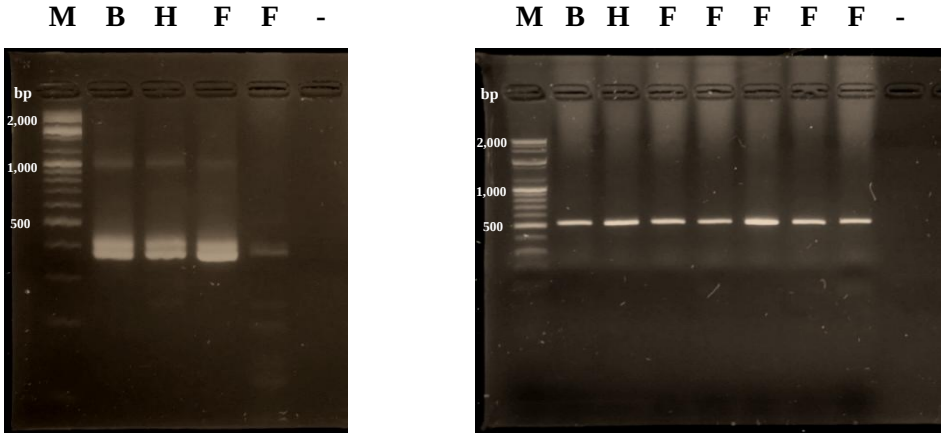
All genomic DNA were electrophoresed in a 1% agarose gel containing 0.3 μg Ez-Vision Three™ DNA Dye Loading Buffer 6x in 5x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM sodium EDTA) at 150 V for 30 min. All samples were assessed DNA concentration under an UV illuminator.

PCR amplification and sequencing

The *Prm1* gene was used to infer the nuclear DNA (nDNA) phylogeny of Phayre's leaf monkeys (Karanth *et al.* 2008, He *et al.* 2012). A nested PCR approach was used because high DNA quality was not available particularly for the the fecal samples. Full-length of *Prm1* sequences (**Figure 2.6a**) was obtained by PCR as follows: 25 μl total reactions containning 1 μM each of primers, 1X PCR buffer (iNtRON Inc., Korea), 2.5 μg of BSA (Promega Inc., USA), 1.5 mM Mg^{2+} (iNtRON Inc.), 0.2 mM of each dNTP (iNtRON Inc.), 1 μl DNA templates and 2 U of *i-Star Taq* polymerase (iNtRON Inc.). PCR recipe for *Prm1* was given in **Appendix 2.1a**. A primer pair, Quer1 and ProtR, was used for the first PCR. The second PCR was conducted using a newly designed primer set, ProtL1 and ProtR1 (**Table 2.1**) with 1μl of the first PCR template. The first PCR was carried out using the following protocol: initial denaturation for 5 min at 94˚C, followed by 30 cycles (94˚C for 40 s, 56˚C for 30 s and 72˚C for 1 min), and a final extension at 72˚C for 5 min. The second PCR followed the same protocol except for running 50 cycles. DNA concentration for both nuclear and mitochondrial genes was tested using 100 bp Plus DNA Ladder (Bioneer Corp., Korea) before sequencing.

Cyt-b gene was used to infer the mitochondrial DNA (mtDNA) phyolgeny (Geissmann *et al.* 2004, Karanth *et al*. 2008, He *et al*. 2012). Two sequential PCRs were carried out by a nested PCR approach. The partial length of *Cyt-b* gene sequences (**Figure 2.6b**) was obtained by PCR as

follows: 25 µl total reactions containing 1 µM each of primers, 1X PCR buffer, 2.5 mM Mg^{2+}, 0.2 mM of each dNTP, 1 µl of DNA templates and 2 U of *i-Star Taq* polymerase. PCR recipe for *Cyt-b* was given in **Appendix 2.1b**. The first PCR was conducted using a set of primers, Cytb-F and Cytb-R. A set of newly designed species-specific primers, CytbTP-Fand CytbTP-R (**Table 2.1**) was used for second PCR with 1 µl of the first PCR template. Both PCRs were carried out using the following protocol: initial denaturation for 3 min at 94˚C, followed by 50 cycles (94˚C for 1 min; 50˚C for 45 s, and 72˚C for 1 min), and a final extension at 72˚C for 5 min.



(a) (b)

Figure 2.6 A gel electrophoresis of PCR products: (a) 343 bp length of nuclear protamine P1 (*Prm1*) gene; and (b) 514 bp length of mitochondrial cytochrome *b* (*Cyt-b*) genes. DNA concentration for both nuclear and mitochondrial genes was measured using 100 bp Plus DNA Ladder (Bioneer Corp., Korea). M: marker; B: bone; H: hair; F: feces; –: negative control

The PCR was performed in a PTC-100 PCR Thermal Cycler (MJ Research, Inc., U.S.A). The PCR products were purified using the Zymoclean^{TM} Gel DNA Recovery Kit (ZYMO RESEARCH Corp., Korea). The purified *Prm1* PCR products were bi-directionally sequenced using the forward ProtL1 and reverse ProtR1 primers. The *Cyt-b* PCR products were bi-directly sequenced using the primer pair (CytbTP-F and CytbTP-R) but they showed little success for most of samples suggesting the presence of numts (nuclear-mitochondrial) which arise due to random transfer and incorporation of mitochondrial DNA(mtDNA) frgaments into the nuclear genome (Karanth 2010). Instead of the CytbTP-R, a newly designed species-specific inner primer (CytbTP-IF) was used for sequencing. PCR and sequencing success were shown in **Appendix 2.2**. The sequences of *Prm1* and *Cyt-b* genes for both PMP and AKNP populations were described in the **Appendix 2.3a-b**.

Table 2.1 Primers for amplifying and sequencing DNA

Primer	Direction	Sequence (5' - 3')	Annealing temperature	Reference
Protamine P1 (*Prm1*)				
Quer1	Forward	ACC TGC TCA CAG GTT GGC	62˚C	1
ProtR	Reverse	TTG ACA GGT CGG CAT TGT TC	60˚C	1
ProtL1-F	Forward	TGG TGC CCT GCT CTG AGC	60˚C	3
ProtR1-R	Reverse	GGA TGG TGG CAT TTT CAA GA	56˚C	3
Mitochondrail cytochrome *b (Cyt-b)*				
Cytb-F	Forward	CTC CTC ATT GAA ACA TGA AAT AT	55˚C	2
Cytb-R	Reverse	CTT TGT TGT TTG GAT TTG TG	56˚C	2
CytbTP-F	Forward	TTA CTC ATA ACC ATA GCA ACA G	56˚C	3
CytbTP-IF	Inner Forward	TTC ATT ATT GCA ACC CTA ACA	50˚C	3
CytbTP-R	Reverse	GCT AAG ATA AGA ATG GAT AGA	57˚C	3

Note. 1: Karanth *et al.* (2008), 2: Geissmann *et al.*(2004), 3: this study

2.2.5 Phylogenetic analysis

A total of twelve sequences were analyzed in this study. All sequences of *Prm1* and *Cyt-b* genes were aligned by AlignIR program version 2.1 (LI-COR Inc., U.S.A.) and manually examined in detail (**Figure 2.7**). Since the nuclear *Prm1* gene is inherited from both sexes (Melnick and Howlzer 1993), the appearances of heterozygote were carefully check by naked eyes for both the forward and reverse sequences. After aligning *Prm1* sequences from each sample, all genotypes were assigned to homozygotes. Basic genetic polymorphism data such as haplotype (H), haplotype diversity (*h*), nucleotide diversity (π), and genetic distance between nucleotide sequences (p_s) were analyzed using MEGA version 5.1 (Tamura *et al.* 2011).

In total, 20 *Cyt-b* and 16 *Prm1* gene sequences of closely related taxa including all three subspecies of Phayre's leaf monkey were used in this analysis (**Table 2.2**). Other reference sequences of Baboon (*Papio cynocephalus*), Guereza colobus (*Colobus guereza*), Red colobus (*Piliocolobus badius*) and Rhesus monkey (*Macaca mulatta*) were used as out-groups. All reference sequences were assessed at GenBank (http://www.ncbi.nlm. nih.gov/genbank/). Phylogenetic tree reconstructions were carried out using three algorithms, neighbor-joining (NJ) (Saitou and Nei 1987), maximum-parsimony (MP) (Felsenstein 1985) and maximum-likelihood (ML) (Kimura 1980), included in MEGA version 5 (Tamura *et al.* 2011). Support of internal branch lengths were determined by bootstrap analyses performed with 1,000 replications for all algorithms.

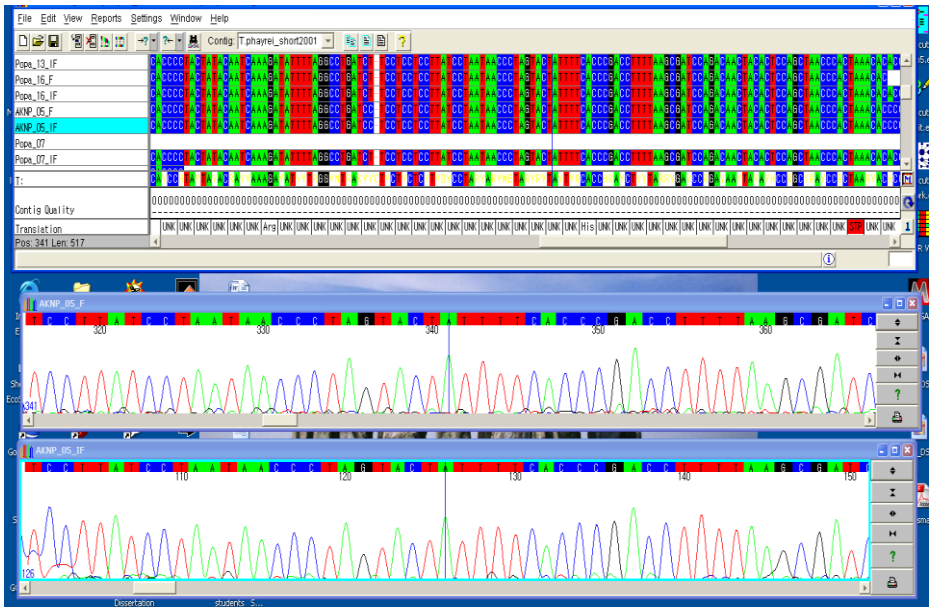


Figure 2.7 All sequences were aligned by the AlignIR Program version 2.1 and manually examined in detail. The purified PCR products of protamine P1 (*Prm1*) and mitochondrial cytochrome b (*Cyt-b*) were bi-directly sequenced using the forward and reverse primers (see **Table 2.1**).

Table 2.2 Sequences used for the phylogenetic analysis, their accession numbers, and sources of the samples

Common name	Scientific name	Code	Sample type/Reference	Source	Accession no.	
					Cyt-b	*Prm1*
Hanuman langur	*S. entellus*		Hair	HZ	AF293952	AF294852
Nilgiri langur	*T. johnii*		Hair	W	AF294619	AF294853
Golden leaf monkey	*T. geei*		Hair	HZ	AF294618	AF294857
Phayre's leaf monkey	*T. phayrei*	I	Tissue	W	AF294621	AF294858
	T. phayrei	V	DNA	EPRC	AF294622	AF294860
	T. p. phayrei	M	Museum skin	ZMB	AY51946	-
	T . p. crepusculus	V	Hair	EPRC	AY519461	-
	T. p. crepusculus	M	He *et al.* (2012)	-	KC285863	KC285882
	T. p. crepusculus	C	He *et al.* (2012)	-	KC285864	KC285884
	T. p. shanicus	C	He *et al.* (2012)	-	KC285868	-
	T. p. shanicus (this study)	M	Bone	Popa	KC751745	KC833502
	T. p. shanicus (this study)	M	Feces	Popa	KC833499	KC833501
	T. p. shanicus (this study)	M	Feces	Popa	KC833500	-
Tenasserim lutung	*T. barbei*		Hair	BZ	AY519462	-
Capped leaf monkey	*T. pileatus*		Hair	SZ	AF294626	AF294856
Purple-faced langur	*T. vetulus*		Karanth *et al.* (2008)	-	AF295577	AF119236
Francois'leaf monkey	*T. francois*		Karanth *et al.* (2008)	-	AF295578	AF119234
Dusky leaf monkey	*T. obscurus*		Karanth *et al.* (2008)	-	AF295579	AF119238
Silvered leaf monkey	*T. cristatus*		Blood	NYZS	AF295580	AF294861
Guereza colobus	*C. guereza*		Karanth *et al.* (2008)	-	U38264	AF119233
Red colobus	*P. badius*[a]		Tissue	LSU	AF294625	AF29485O
Rhesus monkey	*M. mulatta*		Karanth *et al.* (2008)	-	U38272	AF119240
Baboon	*P. cynocephalus*[b]		Karanth *et al.* (2008)	-	Y16590	AF119239

Note. *S* = *Seminopithecus*, *T* = *Trachypithecus*, *C* = *Colobus*, [a] P = *Procolobus*, [b] P = *Papio* , M = *Macaca*

Phayre's langurs samples, C from China (K. He), I from India (K. P. Karanth), V from Vietnam (K. P. Karanth) and M from Myanmar (T. Geissmann). HZ = Hyderabad Zoo, South India (K. P. Karanth); EPRC = Endangered Primate Rescue Center, Vietnam (C. Roos); SZ = Shipahijala Zoo, Northeast India (K. P. Karanth); BZ = Bangkok Zoo, Thailand (T. Geissmann); W(Nilgiri langur) = Wild from Anamalai Hills, South India (M. Singh); W (*T. phayrei*) = Wild from Shipahijala national park, Northeast India (K. P. Karanth); LSU = Louisiana State University, USA (P.A. Marx and P. Telfer); NYZS = New York Zoological Society, USA (D. Wharton); ZMB: Zoologisches Museum der Humboldt Universität Berlin (T. Geissmann); Popa = Popa Mountain Park, Myanmar

2.3 Results and discussion

2.3.1 PCR, sequencing and genetic polymorphism

Out of 52 collected samples, 33 % (17/52) for PCR products and 23 % (12/52) for sequencing were successful. In this analysis, overall success rate of PCR and sequencing were relatively low. I subject this to the low DNA quality of fecal samples. Although the feces were collected at one hour post defecation, these were directly stored in 99.9 % ethanol at ambient temperature and the handling time were long before keeping the samples under freezing conditions. Another possibility may be due to the high humidity (over 70 %) of the sample collection period (July – August) in Myanmar. The environmental factors such as rain and moisture were major difficulties in the field for fecal samples to have successful analysis due to easily DNA degradation in these environments (Vigilant and Guschanski 2009). A total of 12 sequences were analyzed for the two study sites and the genetic polymorphism data of haplotype (H), haplotype diversity (*h*), nucleotide diversity (π), genetic distances between haplotypes (p_s) were shown in **Table 2.3**.

Table 2.3 Number of collected samples, PCR and sequencing success, haplotype (H), haplotype diversity (*h*), nucleotide diversity (π), genetic distance between haplotypes (p_s) for the Phayre's leaf monkey in this study. Values given are mean ± standard deviation.

Samples	***Prm1***	***Cyt-b***
Overall population from both study sites		
collected samples (bone: n =1, hair = 1, feces: n = 50)	52	52
PCR success (%)	33 (17/52)	33 (17/52)
sequencing success (%)	23 (12/52)	23 (12/52)
haplotype (H)	5	6
h	0.9 ± 0.16	1.0 ± 0.09
π	0.47 ± 0.14	1.1 ± 0.21
p_s (%)	0.5 ± 0.2	1.2 ± 0.3
Population from Popa Mountain Park (PMP)		
collected samples (bone: n =1, feces: n = 32)	33	33
PCR success (%)	39 (13/33)	39 (13/33)
sequencing success (%)	24 (8/33)	24 (8/33)
haplotype (H)	2	3
h	0.429	0.61 ± 0.16
π	0.25	0.13 ± 0.04
p_s (%)	0.6	0.3 ± 0.2
Population from Alaungdaw Kathapa National Park (AKNP)		
collected samples (hair: n =1, feces: n = 18)	19	19
PCR success (%)	21 (4/19)	21 (4/19)
sequencing success (%)	21 (4/19)	21(4/19)
haplotype (H)	3	3
h	0.83 ± 0.22	0.83 ± 0.22
π	0.29 ± 0.10	0.29 ± 0.10
p_s (%)	0.4 ± 0.3	0.4 ± 0.2

2.3.2 Nuclear protamine P1 (*Prm1*) gene

The sequences of *Prm1* were highly similar to those of close related colobines deposited in GenBank. Out of a total of twelve *Prm1* sequences analyzed, 2 haplotypes (Popa 1 and 2) and 3 haplotypes (AKNP 1, 2 and 3) were detected for PMP and AKNP, respectively. Both of haplotype diversity (*h*) and nucleotide diversity (π) were low (overall $h = 0.9 \pm 0.16$ and $\pi = 0.47 \pm 0.14\%$) across individuals. Genetic distance (p_s) between haplotypes was also low (overall $p_s = 0.5 \pm 0.2\%$). The consensus NJ, MP and ML trees showed a similar topology (**Figure 2.8**). The *Prm1* gene trees revealed that all subspecies, *T. p. phayrei*, *T. p. shanicus*, and *T. p. crecupulus* form a clade with the study populations from PMP and AKNP but with very low bootstrap values less than 50 % (**Figure 2.8**). Moreover, Dusky leaf monkey (*T. obscurus*) was also clustered within the group of *T. phayrei* ($p_s = 0.0$ - 0.3%, **Figure 2.8 and Table 2.4**). This result was concluded that the nuclear protamine P1 (*Prm1*) gene could not resolve the phylogenetic position of *T. phayrei*. It is possibly due to the slow fixation time for *Prm1* gene evolution (Ting *et al.* 2008, Wilson *et al.* 1985) and it shows little or no differences among closely related taxa (Ruvolo 1997). Additionally, similar to the previous studies (Karanth *et al.* 2008, Karanth 2010), Hanuman langur, Nilgiri langur, and Purple-faced langurs form a sister clade with Silvered leaf monkey, while Golden leaf monkey, Capped leaf monkey and Francois leaf monkeys were clustered as a separated group (**Figure 2.8**).

Table 2.4 *Prm1* gene; pairwise genetic distances (p_s) among the analyzed sequences of Phayre's leaf monkey and Dusky leaf monkey. Genetic distances among the taxa are relatively low (p_s = 0 – 0.9%).

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
T. p. shanicus (1)		0.000	0.000	0.000	0.000	0.000	0.003	0.004	0.004	0.003	0.003
*T. phayrei*_India (2)	0.000		0.000	0.000	0.000	0.000	0.003	0.004	0.004	0.003	0.003
*T. phayrei*_Vietnam (3)	0.000	0.000		0.000	0.000	0.000	0.003	0.004	0.004	0.003	0.003
*T. p. crepusculus*_China (4)	0.000	0.000	0.000		0.000	0.000	0.003	0.004	0.004	0.003	0.003
*T. p. crepusculus*_Myanmar (5)	0.000	0.000	0.000	0.000		0.000	0.003	0.004	0.004	0.003	0.003
Dusky leaf monkey (6)	0.000	0.000	0.000	0.000	0.000		0.003	0.004	0.004	0.003	0.003
AKNP 1 (7)	0.003	0.003	0.003	0.003	0.003	0.003		0.003	0.003	0.004	0.003
AKNP 2 (8)	0.006	0.006	0.006	0.006	0.006	0.006	0.003		0.004	0.005	0.003
AKNP 3 (9)	0.006	0.006	0.006	0.006	0.006	0.006	0.003	0.006		0.005	0.003
Popa 1 (10)	0.003	0.003	0.003	0.003	0.003	0.003	0.006	0.009	0.009		0.004
Popa 2 (11)	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.003	0.003	0.006	

Note: Number of base substitutions per position (below diagonal) and standard error (above diagonal) obtained by a bootstrap procedure (1,000 replicates). Analyses were conducted using the Kimura 2-parameter model. AKNP 1, 2, and 3: nucleotide sequences of Phayre's leaf monkey from Alaungdaw Kathapa National Park (AKNP); Popa 1 and 2: nucleotide sequences of Phayre's leaf monkey from Popa Mountain Park

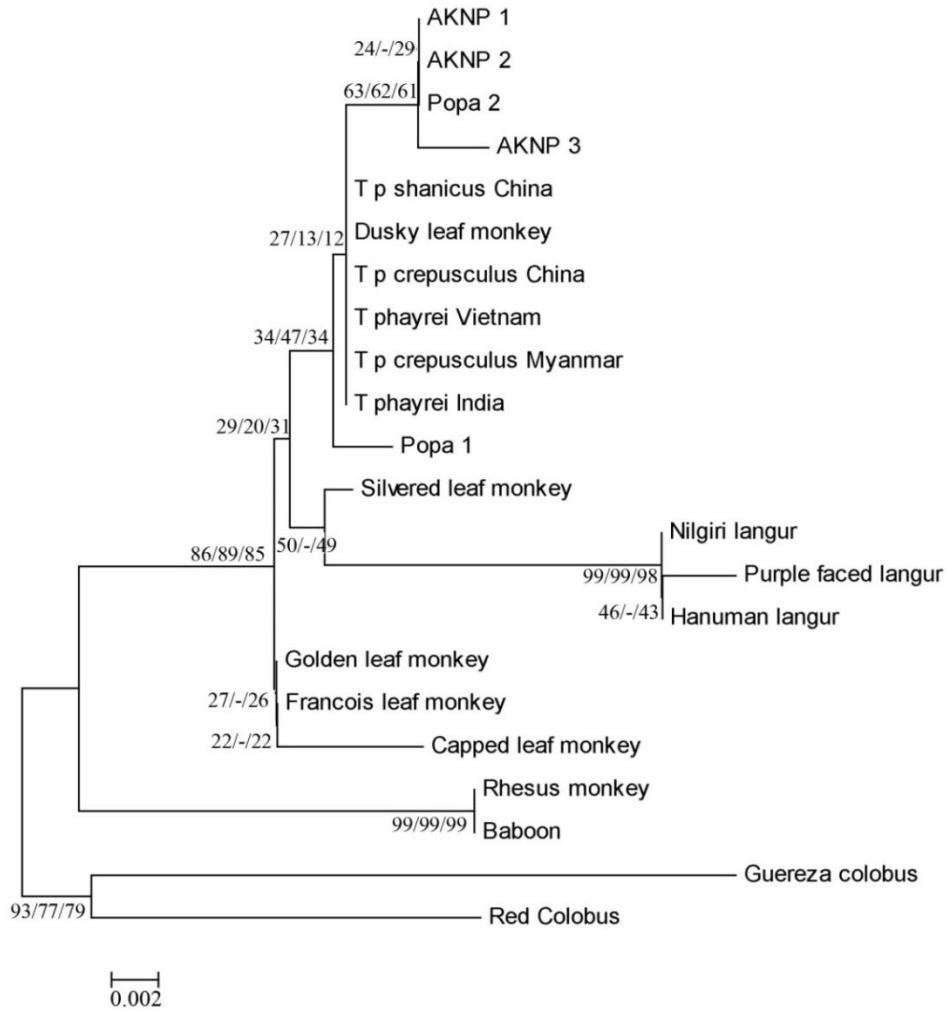


Figure 2.8 50%-majority rule consensus nuclear protamine P1 (*Prm1*) phylogeny trees. Numbers on the branches indicate bootstrap values (1,000 replications) obtained from the neighbor-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) analyses. The populations from Popa Mountain Park (Popa 1 and 2) and that from Alaungdaw Kathapa National Park (AKNP 1, 2, and 3) form a clade with the subspecies of *T. phayrei* group but with very low bootstrap value. Dusky leaf monkey also clusters together with all the subspecies *T. phayrei* group.

2.3.3 Mitochondrial cytochrome *b* (*Cyt-b*) gene

Out of twelve sequences, 3 haplotypes (Popa 1, 2 and 3) and (AKNP 1, 2 and 3) of *Cyt-b* gene were assessed for each study site. In contrast to *Prm1* gene, both haplotype diversity and nucleotide diversity were relatively high (overall $h = 1.0 \pm 0.09$ and $\pi = 1.1 \pm 0.21\%$) across individuals. Genetic distance between haplotypes was also relatively high (overall $p_s = 1.2 \pm 0.3\%$). The consensus NJ, MP and ML trees showed a similar topology (**Figure 2.9**). *Cyt-b* gene trees revealed that Phayre's leaf monkey from PMP is more closely related to the subspecies *T. p. shanicus* from China (genetic distance: $p_s = 1.8 – 2.0\%$, **Table 2.5**) rather than the subspecies *T. p. phayrei* from the southwest Rakhine Range in Myanmar ($p_s = 2.0. – 2.2\%$). Although PMP is isolated in the recent decades, there was possibly past vegetative connectivity between the PMP and the eastern Shan Plateau where the distribution of the subspecies *T. p. shanicus* was recorded (Pocock 1939, **Figure 2.2**). Consequently, there might have been past migrations as well. Large scale deforestations might have occurred throughout Southeast Asia during Pleistocene glaciations letting formerly widespread species into refugia (Meijaard and Groves 2006). Moreover, most of tropical forests including PMP, have been highly threatened by human activities (Htun *et al.* 2011) resulting into the isolation of some populations in the recent past. Based on the genetic structure and habitat isolation process, the Phayre's leaf monkey from the PMP should be considered at least management unit which is important for conservation implications (Moritz 1994). To confirm this scenario, however, further

detailed studies on morphological, demographic, behavioral and genetic information of the population from PMP are recommended (Eo *et al.* 2010).

On the other hand, the populations from AKNP cluster together with the subspecies *T. p. phayrei* from the southwest Rakhine Range in Myanmar and India with low genetic distance (genetic distance: $p_s = 0 – 1.0\%$). This is possibly due to the result of contiguous geographical range because population along the mountain ranges showed little genetic differences and shared common alleles for mitochondrial gene (Tennessen and Zamudio 2008). In Myanmar, *T. p. phayrei* distributes along the western Rakhine range (**Figure 2.2**) extending to northeast India where distribution of *T. phayrei* have been previously reported (Gupta 2001, Karanth *et al*. 2008, Bleisch *et al.* 2008). The current study suggested that the genetic variations between the two populations from PMP and AKNP may possibly due to the effect of impassable river, the Ayeyarwaddy River flowing between the two study sites (**Figure 2.2**). Previous studies had also reported about some primates for which river barriers seem to be important include gorillas (*Gorila spp*.) (Anthony *et al.* 2007), chimpanzees (*Pan troglodytes*) (Gonder *et al.* 2006) and mouse lemurs (*Microcebus ravelobensis*) (Guschanski *et al.* 2007). However, this is not always the case, and an effect of rivers was not detected in the study of long-tailed macaque (*Macaca fascicularis*) having capability for swimming (de Ruiter and Geffen 1998). Interestingly, the facial features of the two populations from PMP and AKNP are slightly different showing the white circular rings around the eyes for PMP population (**Figure 2.1a**) but

only half of the ocular rings bend toward the nose are clearly seen for AKNP population (**Figure 2.1c**).

Cyt-b gene supported monophyletic group of *T. phayrei* from Vietnam and the subspecies *T. p. crepusculus* from Myanmar, Vietnam and China with high bootstrap values (**Figure 2.9**) and low genetic distance (p_s = 0.2 – 1.0%). Thus *T. phayrei* from Vietnam may have a similar genetic status to subspecies *T. p. crepusculus*. This result is consistent with a previous study reporting the monophyletic group of *T. p. crepusculus* for these three countries (He *et al.* 2012). Although *T. p. crepusculus* has been identified as a subspecies of *T. phayrei* (Pocock 1939, Bleisch *et al.* 2008), the *Cyt-b* gene trees showed that *T. p. crepusculus* was more closely related to Francois leaf monkey (*T. francois*) which was not analyzed in this study. Previous studies had also reported on the sister grouping of Francois and Phayre's leaf monkeys (Geissmann *et al.* 2004, Karanth *et al.* 2008, He *et al.* 2012). Due to this incongruence, Liedigk *et al.* (2009) suggested that *T. p. crepuculus* should be regarded as a distinct species as *T. crepusculus* in the group of the genus *Trachypithecus*.

In contrast to *Prm1* gene trees, the *Cyt-b* gene trees showed no clustering of Dusky leaf monkey (*T. obscurus*) together with *T. phayrei* groups including the populations from PMP and AKNP (**Figure 2.9**). Genetic distances between the Dusky leaf monkey and the study populations are relatively high (p_s = 3.6 – 3.8 % for PMP, p_s = 3.6 – 3.9 % for AKNP).

Table 2.5 *Cyt-b* gene; pairwise genetic distances (p_s) among the analyzed sequences of Phayre's leaf monkey and Dusky leaf monkey. Genetic distances among the taxa vary 0 to about 10% (p_s = 0 – 9.8%).

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
T. p. shanicus (1)		0.006	0.005	0.014	0.014	0.014	0.015	0.009	0.006	0.005	0.006	0.006	0.006	0.006
*T. p. phayrei*_Myanmar (2)	0.020		0.003	0.014	0.014	0.015	0.015	0.008	0.004	0.003	0.004	0.006	0.006	0.006
*T. phayrei*_India (3)	0.016	0.004		0.014	0.014	0.014	0.015	0.008	0.003	0.000	0.003	0.005	0.006	0.006
*T. phayrei*_Vietnam (4)	0.096	0.089	0.094		0.007	0.007	0.007	0.015	0.015	0.014	0.015	0.014	0.015	0.015
*T. p. crepusculus*_Vietnam (5)	0.096	0.091	0.091	0.024		0.002	0.004	0.015	0.015	0.014	0.014	0.015	0.015	0.015
*T. p. crepusculus*_China (6)	0.098	0.094	0.094	0.022	0.002		0.004	0.015	0.015	0.014	0.015	0.015	0.015	0.015
*T. p. crepusculus*_Myanmar (7)	0.103	0.098	0.098	0.026	0.010	0.008		0.016	0.015	0.015	0.014	0.014	0.016	0.015
Dusky leaf monkey (8)	0.045	0.036	0.036	0.101	0.101	0.103	0.103		0.009	0.008	0.009	0.008	0.009	0.009
AKNP 1 (9)	0.022	0.010	0.006	0.096	0.094	0.096	0.101	0.039		0.003	0.002	0.006	0.006	0.006
AKNP 2 (10)	0.016	0.004	0.000	0.094	0.091	0.094	0.098	0.036	0.006		0.003	0.005	0.006	0.006
AKNP 3 (11)	0.020	0.008	0.004	0.094	0.091	0.094	0.098	0.036	0.002	0.004		0.006	0.006	0.006
Popa 1 (12)	0.018	0.020	0.018	0.096	0.096	0.098	0.103	0.036	0.018	0.016	0.016		0.002	0.002
Popa 2 (13)	0.020	0.022	0.020	0.098	0.098	0.101	0.105	0.038	0.020	0.018	0.018	0.002		0.003
Popa 3 (14)	0.020	0.022	0.020	0.098	0.098	0.100	0.105	0.038	0.020	0.018	0.018	0.002	0.004	

Note: Number of base substitutions per position (below diagonal) and standard error (above diagonal) obtained by a bootstrap procedure (1,000 replicates). Analyses were conducted using the Kimura 2-parameter model. AKNP 1, 2, and 3: nucleotide sequences of Phayre's leaf monkey from Alaungdaw Kathapa National Park; Popa 1, 2 and 3: nucleotide sequences of Phayre's leaf monkey from Popa Mountain Park

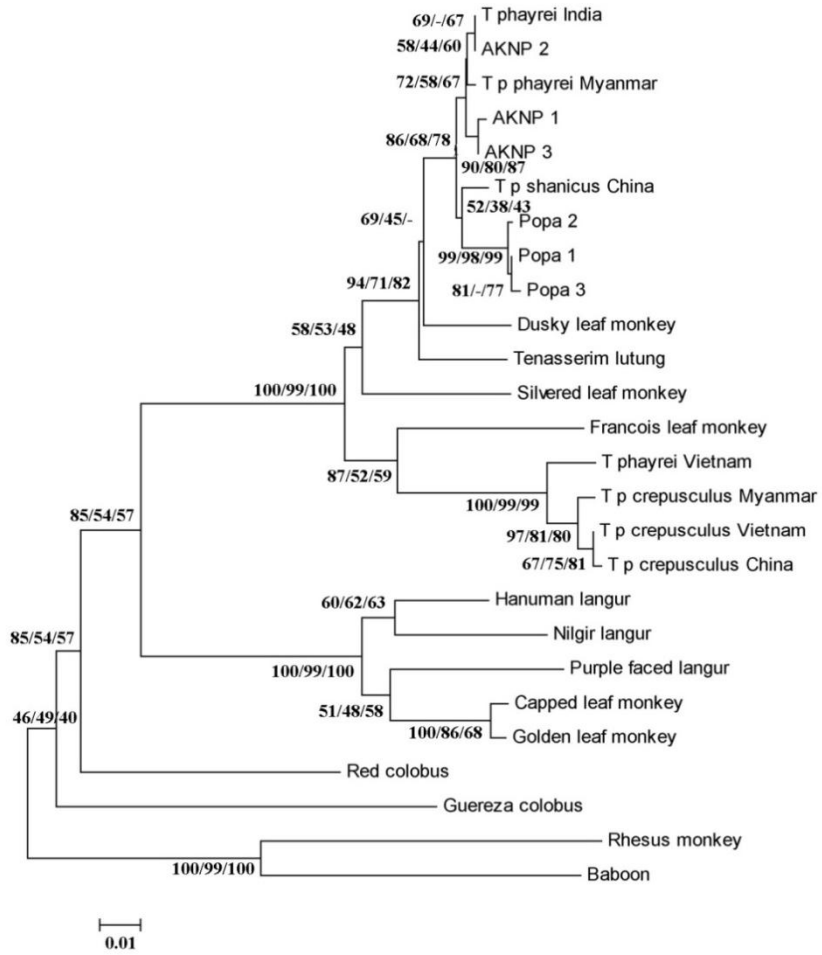


Figure 2.9 50%-majority rule consensus mitochondrial cytochrome b (*Cyt-b*) phylogeny trees. Numbers on the branches indicate bootstrap values (1,000 replications) obtained from the neighbor-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) analyses. The population of Phayre's leaf monkey from Popa Mountain Park (Popa 1, 2, and 3) is more closely related to the subspecies *T. p. shanicus* while that from Alaungdaw Kathapa National Park (AKNP 1, 2, and 3) clusters together with the subspecies *T. p. phayrei*. Dusky leaf monkey shows no clustering together with *T. phayrei* group.

2.3.4 Comparison of the results from *Prm1* and *Cyt-b* analyses

The *Prm1* trees revealed that the study populations of Phyare's leaf monkey from PMP and AKNP clustered together with all subspecies of *T. phayrei* groups as well as *T. obscurus*. In contrast, the *Cyt-b* trees showed that the population from PMP is more closely related to the subspecies *T. p. shanicus* while that from AKNP clusters together with the subspecies *T. p. phayrei* and showed no clustering with *T. obscurus*. This incongruence between nuclear and mitochondrial phylogenetic trees might reflect the result of different gene flows of male and females of the species. Maternally inherited mtDNA genes tend to be highly substructured between populations due to female natal philopatry in mammals (Melnick and Howlzer 1993, Karanth *et al*. 2008) and also mtDNA genes evolve rapidly (Ruvolo 1997). However, the evolution of nuclear genes is very slow (Ting *et al*. 2008) and consequently little or no variation of *Prm1* gene has been assessed among closely related taxa (Ruvolo 1997). The results of phylogenetic incongruence between two DNA markers in this study supported that the previous phylogenetic works using the nuclear and mitochondrial markers for langurs and leaf monkeys (Ting *et al*. 2008), common vampire bat (Martins *et al*. 2009), and macaques (Tosi *et al*. 2000).

2.4 Conclusion

This study is the first molecular phylogenetic analysis on Phayre's leaf monkey using non-invasive and non-destructive samples in Myanmar. The results confirmed the population from the Popa Mountain Park as Phayre's leaf monkey. Moreover, the species existence and identification of Phayre's leaf monkey in Alaungdaw Kathapa National Park has been recorded for the first time. The mitochondrial *Cyt-b* gene trees showed that population from the PMP is more closely related to the subspecies *T. p. shanicus* while that from the AKNP clusters together with the *T. phayrei* from India and *T. p. phayrei* from southwest Myanmar. This genetic variation between the two populations is possibly due to the effect of major geographical barrier, the Ayeyarwaddy River, between two study sites. Based on genetic structure and habitat isolation process, the remnant population of *T. phayrei* in PMP should be considered at least management unit which is important for conservation implication (Moritz 1994, Eo 2010). However, further studies on morphological, demographic, behavioral and genetic information of the population from PMP are highly recommended to confirm whether it is a distinct unit for management and conservation. Moreover, the current distribution and taxonomic status of all *T. phayrei* populations within Myanmar should be investigated to resolve the whole spectrum of phylogenetic position as well as migration among all subspecies.

Chapter III. Population, Habitat Use and Behavior of Phayre's Leaf Monkey in Popa Mountain Park

3.1 Introduction

Tropical forests are rich in biodiversity and world biodiversity hotspots are predominated by the tropical forests, appearing in 15 hotspots out of 25 (Myers *et al.* 2000). About 90 percent of primates live in the tropical forests which are disappearing at an alarming rate due to human interferences. Consequently over 40 percent of the total 634 primates are threatened to extinction (PCI 2013). Rowe and Myers (2013) have reported that 69 of the world's primates species (11%) are critically endangered (CR), 137 (22%) are endangered (EN), and further 97 (15%) are vulnerable (VU) in the IUCN Red List. Among them, a medium size primate, Phayre's leaf monkey is categorized as EN (Bleisch *et al.* 2008) and it has been confined to tropical forests in Asia particularly in the Southeast Asia. Likewise other primates in the tropical forests, the population of Phayre's leaf monkey is decreasing with threatening from human disturbances. The authentic population status of this monkey is available only in India and Bangladesh which host about 1,000 individuals each (Gupta 2001, Gittins and Akonda 1982) but data are deficient for other countries including Myanmar.

Since the primates are the most diverse mammals, there is a great variation of ecological requirements among primates (Crandlemire-Sacoo 1988). For instance, the Sichun Snub-nosed Monkeys (*Rhinopithecus*

roxellana) in China selected the habitats comprised with the tree species with abundant lichens, as well as dead trees which also provided lichens (Yiming 2006). The conservation implications came out with the recommendations to conserve the top priority tree species with lichens and to prohibit the dead-tree harvesting from the habitats where the monkeys lived in. Similarly, Schwarzkopf (1989) studied the three primate species richness in relation to habitat structure in Amazonian rainforests. He found out that Red howler monkeys (*Alouatta seniculus*) persisted in the forest fragments with high numbers of large trees while White-faced sakis (*Pithecia pithecia*) associated with the presence of the streams, highest numbers of lianas, highest numbers of trees and lowest percentages of large trees, and Golden-handed tamarins (*Saguinus midas*) that lived in the tall secondary growth forest fragments. Since all wildlife species need specific habitat requirements, species adaptation (behaviors) to the source habitats could not be separated in the effectiveness of species conservation plan (Morris *et al.* 2008).

Despite its fragmented and declining population, the Phayre's leaf monkey is probably the least studied primates of Asia and thus very little is published about this monkey. However, the available ecological studies come from some its range countries. In India, Gupta and Kumar (1994) studied about feeding ecology of Phayre's leaf monkey inhabited the secondary vegetation after shifting cultivation and the monkey spent much feeding time in winter rather than in summer and monsoon. Food sources varied from bamboo species *Meloconna bambusoides* to tree species belonged to family Leguminosae. In Bangladesh, Aziz and Feeroz (2009) reported that Phayre's

leaf monkey preferred the semi-evergreen forests and selected a variety of plant species comprised 29 species belonged to 14 families. Of these, family Leguminosae and Graminae were ranked as top preferred species. Whereas in Lao, Phayre's leaf monkey often resides in the forests with broken canopy and extensive tall bamboo as well as limestone karst areas (Timmins *et al.* 2011). Ecological and behavior studies are less known for other range countries including Myanmar.

In Myanmar, the Phayre's leaf monkey was found in more than five sites (**Figure 3.1**), however, the habitats have been isolated because of human activities such as farming and human settlements. Although the monkey has been listed as a completely protected animal by the national wildlife law (MoF 1994), its current status and habitat use throughout the country remain unknown. Only one remnant population (about 100 to 150 individuals) is confined to Popa Mountain Park (Min 2006) but little is known about the actual population, habitat use and its behaviors.

This study aimed at understanding the habitat use and ecology of the Phayre's leaf monkey by studying the remnant populations in PMP. Knowledge on the distribution of Phayre's leaf monkey and habitat use can provide the park management with the recommendations for the critical areas that should be protected and the tree species that should be conserved as food resources. In this study, it was expected that habitats with more food plant species will be selected by the animal over those with fewer food plant species. Secondly, the habitat (forest structure) utilization of Phayre's leaf

monkey would be affected by food distribution. Third, it was expected that the activity budget of Phayre's leaf monkey changed in seasons and times of day.

3.2 Materials and methods

3.2.1 Study area

Popa Mountain Park (PMP) is located in central Myanmar and it hosts one of the Phayre's leaf monkey subpopulations in the country. The park is a relatively small (about 150 km^2 in area) and isolated extinct volcanic mountain. The vegetation types of PMP are considerably diverged along elevational gradient (Thant *et al.* in press) and major forest types are dry forest, mixed deciduous forest and semi-evergreen (dry hill) forests (Htun *et al.* 2011). The mountain was opened up to northern side during the eruption and the crater became a deep valley opening to the north, enclosing to the east, south and west by the remaining steep rims (**Figure 3.1a-b**). The vegetation types in the crater is dominated by the semi-evergreen (dry hill forest) consisting of *Quercus serrata, Carallia brachiata, Trevia nudiflora* and other species.

There has been long relationship between local communities and the PMP. The original vegetation of Mt. Popa had almost disappeared once due to cutting, clearing for cultivation, burning and other destructive human influences in the early 20^{th} century (FD 1981). But the vegetation covers have restored again under the Forest Department's reforestation efforts during

1957 -1972. The area surrounding the PMP is somewhat densely populated and there are 45 villages (population about 55,000 in 2010) around the foot of the mountain (Htun *et al.* 2011). Of these, 4 villages are in the buffer area and one pilgrim site locally called Taung-kalat (volcanic plug) is located at western foothill in the core area. This volcanic plug is a prominent landmark as well as one of the famous religious sites in Myanamar and several thousand visitors visit it each year.

Almost all villages are loacted just beside the buffer boundary and human inteferences on the park management were always expected (Rao *et al.* 2002). The minimum distance between the villages and the buffer boundary is less than 1,000 m (**Figure 3.2**). The local people are mainly farmers and their main crops are rice, sesame, maize, and tomato. Bananas are extensively cultivated in the eastern part of the park and some are in the buffer area. Sugar palms (*Borassus spp.*) are an important tree crops for people living in the western and southern part of the PMP. The jaggery (black candy) production from the sugar palms is a main business for local people and this production needs large quantities of fuel woods. The flower of *Michelia champaca* (Family: Magnoliaceae) is an important income for people living in Popa village at western part and they collect these flowers mostly inside the park.

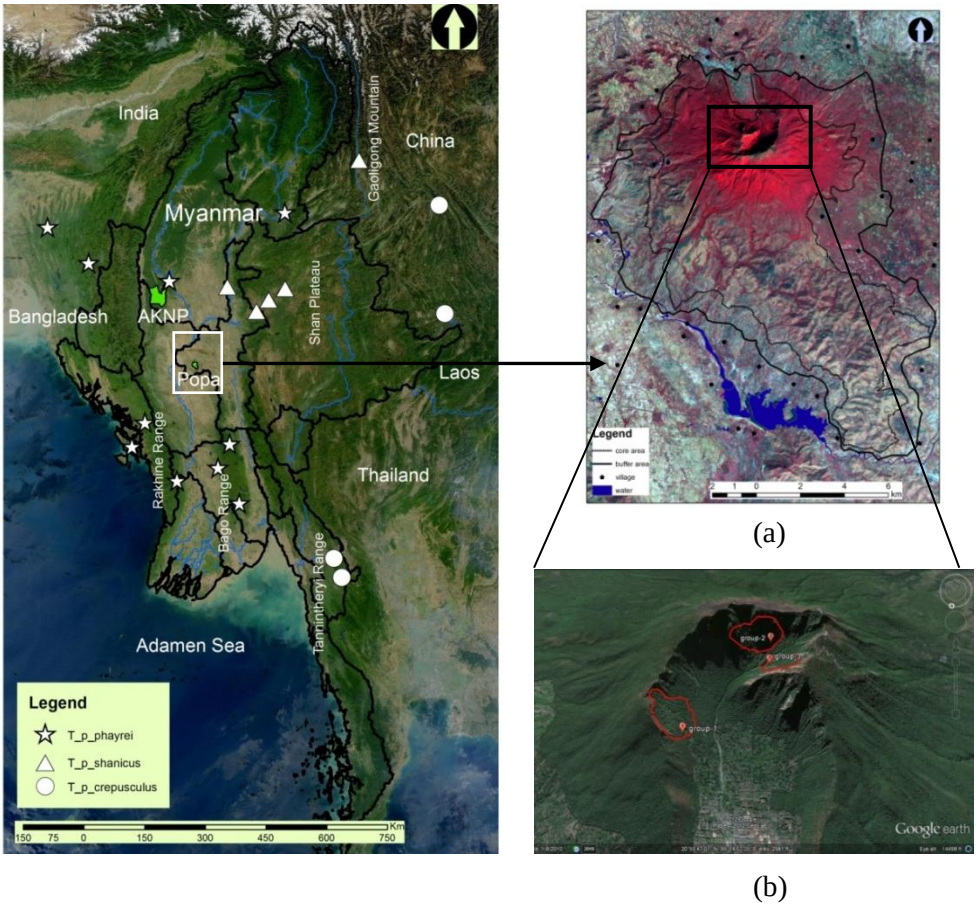


Figure 3.1 Map showing the distribution of Phayre's leaf monkeys in Myanmar and neighboring countries (Pocock 1939, Gupta 2001, He *et al.* 2012, this study 2012). (a) study site, Popa Mountain Park, false color composite of ASTER image acquired on 17th January 2010. (b) distribution of the monkeys (3 groups were assessed in this study) in the crater of Mt. Popa viewed from northern side (Google earth image, 2012)

3.2.2 Data collection

Vegetation and disturbances

In order to investigate the vegetation structure of PMP and habitat use of Phayre's leaf monkey, a total of 46 sample plots (**Figure 3.2**) were randomly set up using Hawth's tool extension in ArcGIS 9.3. The sample plot size was 900 m^2 (plot A, 30 m x 30 m), a modification of the nested pixel plot design by (Kalkham, n.d). Subplots B (10 m x10 m) and subplots C (5 m x 5 m) were established at two opposite corners in each sample plot A (**Figure 3.3**). In sample plot A, all trees with ≥10 cm diameter at breast height (dbh)—1.3 m aboveground— were recorded. Total individual number of saplings (dbh < 10 cm and height ≥ 130 cm) was recorded at subplots B. All seedlings (height < 130 cm) were recorded at subplots C for trees, sapling and seedling categorization (Deb and Sundriyal 2008, Htun *et al.* 2011, Parthasarathy and Karthikeyan 1997). Measuring tape was used for setting up the sample plots and the diameter of trees was measured at 1.3 m above ground (diameter at breast height) by using diameter tape (Forestry Suppliers, Inc. Germany). Location and elevation of each sample plot was recorded by using global positioning system (GPS 60 CS, Garmin International, Thaiwan). Tree species were identified according to Kress *et al.* (2003) and TNRS (2012). In each sample plot, disturbance data of cutting (stem/plot) and farming (area percentage/plot) were recorded. Distance to village (m) was also noted and then checked with the topographical map in ArcGIS 9.3.

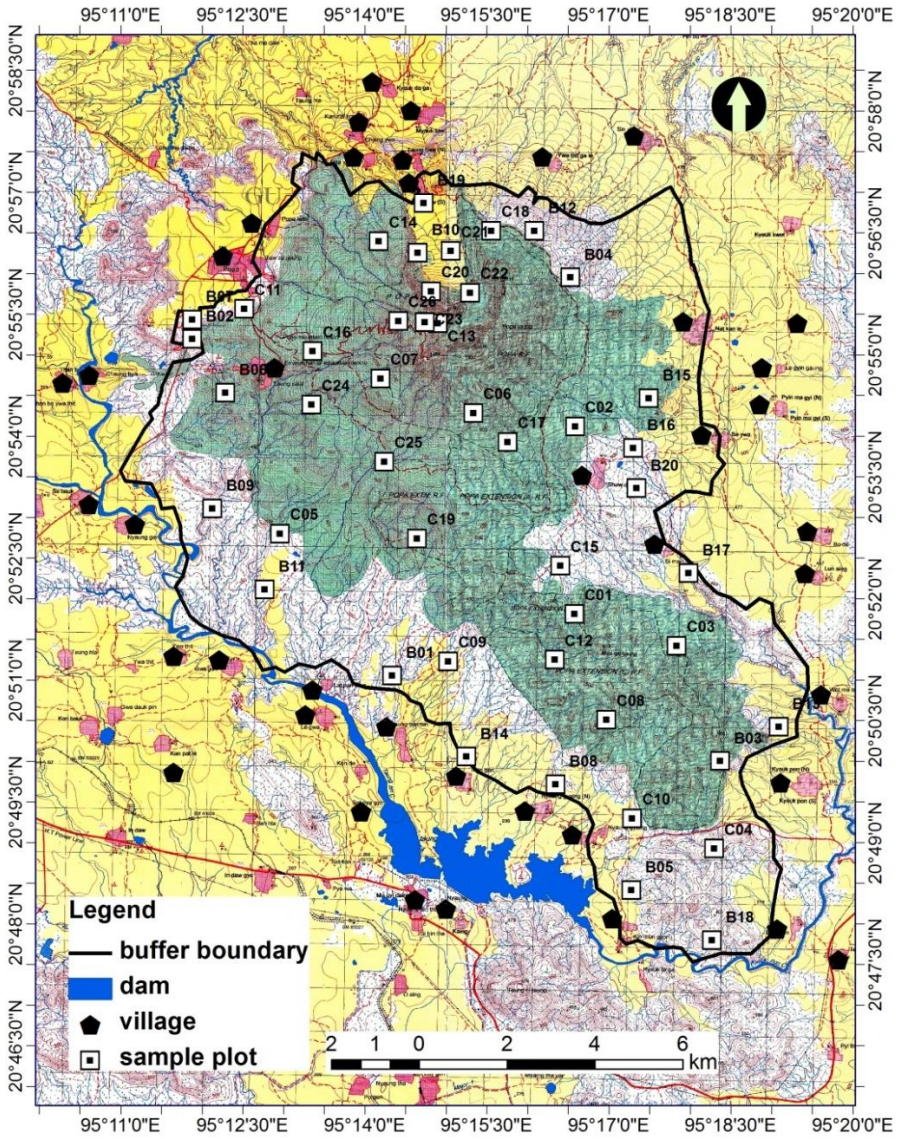


Figure 3.2 Map showing the randomly distribution of sample plots (n = 46, ⊡) that were divided into 26 (C01 – C26) plots in the core area and 20 (B01 - B20) plots in the buffer area. Vegetation information (tree density, tree height, basal area) and disturbance data (tree cutting, farming percentage) were recorded at each sample plot.

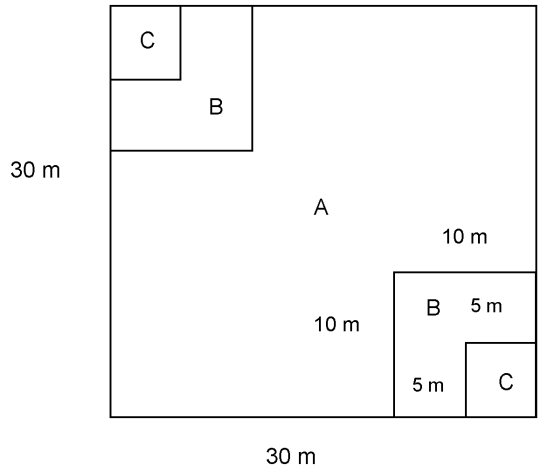


Figure 3.3 Vegetation sampling design: Sample plot A (30 x 30 m) for trees (dbh ≥ 10 cm); Subplot B (10 x 10 m) for sapling (dbh < 10 cm and height ≥ 130 cm) and Subplot C (5 x 5 m) for seedling (height < 130 cm). Disturbance data of cutting (stem/plot), farming (area percentage/plot), and distance to village (m) were also recorded at every sample plot.

Population and occupancy

Population

Due to the restricted distribution of Phayre's leaf monkey in PMP, direct counting was conducted. Three groups of monkeys (group-1, group-2, group-3) were identified in the northern crater (**Figure 3.1b**) and they were about 1.5 - 2 km far from each. Follow-up were made for each group for at least three days to identify suitable locations for total counts. Phayre's leaf monkeys seemed to keep in line while moving one place to another (Pocock 1939). Two observers waited at the selected points and counted the monkeys as they crossed from one point to another using the same path. Each group was counted three times. Telescope (30 x WF, NIKON, JAPAN) and binocular (8 x 40 MINOX, JAPAN) were used for the direct counts.

Occupancy

The occurrence of Phayre's leaf monkey was assessed in November 2010 using randomly line transects to cover the study area. Due to logistic constraints such as steep terrains in the study site, spatial correlation method with replication of segments was used for occupancy survey (Hines *et al.* 2010). Multiple sampling units (a total of 78 transect lines with at least 1 km interval) were randomly established to reach all 46 vegetation sample plots (**Figure 3.4**) and then surveyed at once (MacKenzie *et al.* 2006). Two observers walked along transect and looked for the presence of the monkeys (i.e. live, track and signs). The distance covered was subdivided into segments

of equal length (200 m) that were then treated as replicates in the occupancy analysis. Species detection history: '1' for detection and '0' for nondetection was created for every segment (Hines *et al.* 2010). Moreover, forest types and human disturbances (tree cutting, farming) were recorded at each segment and these data were used as covariate factors in occupancy analysis (Mackenzie *et al.* 2002, Tyre *et al.* 2003).

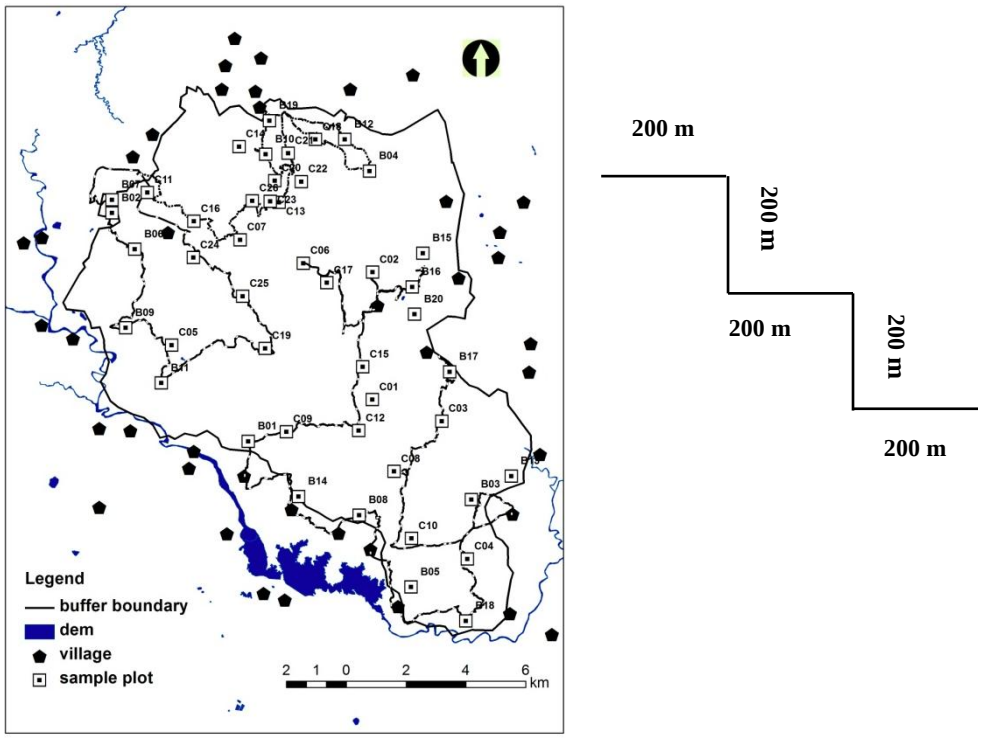


Figure 3.4 Distribution of 1 km transect lines (n = 78) for occupancy survey (left) and each transect was subdivided into segments of equal length in 200 m (right). The right figure represented the sampling design (1 km transect with 5 of 200 m segments) employing spatial replication to draw inference about occupancy and detection probabilities. Detection of Phayre's leaf monkey (i.e. live, track and signs) was recorded at every segment and detection history (1: detection, 0: non-detection) was created for each transect. At each segment, vegetation types and human disturbances (i.e tree cutting, farming) were also recorded as covariate factors. Occupancy value (ψ) was calculated for each transect line and then linked with the vegetation sample plots (n = 46). C01 – C26: sample plots in core area, B01- B20: sample plots in buffer area

Seasonal and daily behavior

The behavioral survey was conducted during three different seasons: June 2010 for rainy season, November 2010 for winter, and April 2012 for summer. Additional behavioral study was made in July 2012 to record ground and vertical tree strata use of Phayre's leaf monkey. A total of 80 sampling sessions was conducted with 17,954 records during the group scan with an average of about 6,000 records / season. A total of 15 working days and 57 hours were spent for behavioral study. One group (group-1) was selected and a team consisted of at least two people observed the monkeys from dawn until dusk for five consecutive days in each season. The scan sampling with instantaneous recording method was used (Altmann 1974) and a whole group of monkeys was rapidly scanned at regular time-intervals. Each sampling session lasted 20 min, with 10 min rest intervals between sessions that was established with slight modification of Silver (1998). Since the Phayre's leaf monkey is an incredibly active animal, a visible individual was looked at for 5 sec to determine behavioral types. Plant species eaten by the monkey were recorded during the scanning. Four types of behaviors were identified (Gupta and Kumar 1994) in this study:

1. Feeding: procuring and/or handling food items with the hands or mouth, chewing, or other obvious signs of ingestion

2. Resting: standing or sitting motionless or sleeping at noon

3. Travelling: locomotion, including walking, running, climbing and jumping

4. Others: grooming oneself, calling and sentry

Ground and vertical tree strata use

Factor influencing the ground and tree stratum use of a species can be divided into ecological factors and morphological characteristics (Yiming 2006). The ecological factors are predator pressure, sympatric primate species competing for resources, resources distribution, and habitat structure while the morphological characteristics include body size, tail length, and limbs (Campbell *et al.* 2005, Cant 1992). In this study, resource distribution and habitat structure were considered as ecological factors that influenced the behavioral types of Phayre's leaf monkey in PMP.

To understand the ground and tree strata use of Phayre's leaf monkey, two categories of ground and three categories of vertical tree strata were divided as follows because the habitat selected for behavioral study comprises open rock areas and individuals trees (**Figure 3.5**). Their behaviors associated with the strata were recorded in July 2012:

1. Ground-1 : 0.0 m (open rocky area)
2. Ground-2 : 0.0 m (rocky area with climbers)
3. Low stratum: 1.0 - 3.0 m
4. Middle stratum : 3.1 - 6.0 m
5. Upper stratum: 6.1 - 9.0 m

A total of 66 scans were conducted for 4,507 records during the 5 consecutive days that covered 56,100 sec in total. Each scan last for 5 sec and vertical distribution of the monkeys per scan ranged from ground or one tree strata to ground and all tree strata. The monkeys rarely climbed into a

canopy of > 9 m , only about 1 % (n = 42) of total activities was recoreded at that height. These data were excluded for statistical analysis.

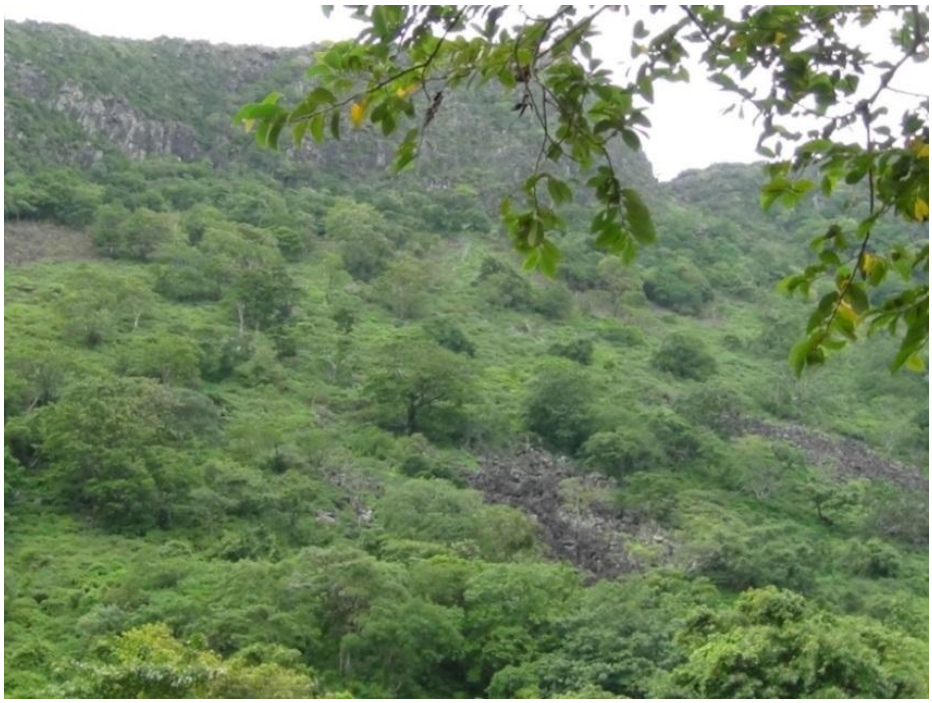


Figure 3.5 Habitat selected for behavioral study in Popa Mountain Park and the area is locally called NwaLaBo Taung stands as the eastern crater rim. The area comprises open rock areas where monkeys spent for resting. Most of individual trees in this picture provide food resources and the climbers underneath the trees were also consumed by Phayre's leaf monkey.

3.2.3 Statistical analysis

Vegetation

To investigate the vegetational characteristics of the PMP, sample plots were grouped into three elevation ranges: low elevation (LE); 301-600 m, mid elevation (ME); 601-900 m and high elevation (HE); > 900 m. Descriptive statistics of plant species diversity, density and basal area were calculated for all three forest stands. Species diversity of tree, sapling and seeding were calculated using EstimateS software version 8.2.0 (Colwell 2005) and Fisher's α-diversity index (Fisher *et al*. 1943), S = a * ln (1 + n/a), where *S* is number of taxa, *n* is number of individuals and *a* is the Fisher's alpha. The Fisher's α-diversity index can be used for an existing dataset that consists of sample plot at any scale and it reduces the impact of sample variation (Htun *et al.* 2011, Magurran 2004). Changes of species diversity, density and basal area of three forest stands at each elevation range were compared by one-way analysis of variance (ANOVA) in SPSS version 16. Games-Howell *post-hoc* test was used in order to reduce the impact of sample size variation (Htun *et al*. 2011). Data were analysed for 5 sample plots at HE (fewer because PMP is cone shaped and surface area decrease at the mountain top), 21 plots at ME stands and 20 plots at LE stands.

Vegetation characteristics and food resources of Phayre's leaf monkey were investigated to compare between the forest stands occupied by the monkeys and those that were not used by the monkeys. Sample plots were divided into 10 plots that fell in the monkey's habitats and 18 plots for

non-habitats. To compare the food availability between the two forest stands, data were analysed only for plant species eaten by Phayre's leaf monkey. Because of unequal sample size (number of plots), data were managed using the non-parametric test (Kruskal-Wallis Test) provided in SPSS for Windows Release 16.0.

Dominant food tree species in two forest stands were identified on the basis of importance value index (IVI) of each tree species (Curtis and McIntosh 1951, Sagar *et al.* 2003, Yeom and Kim 2011, Zhu *et al.* 2007). The species having highest importance value was defined as dominant. The importance value index was calculated by using the following equation:

$$IVI = (RD + Rf + Rd)/3$$

where, *IVI* is the importance value index, *RD* is relative density (*RD* = number of individuals of a species x 100)/total number of individuals of all species, *Rf* is relative frequency (*Rf* = number of plots containing a species x 100)/total plots and *Rd* is relative dominance (*Rd* = basal area of a species x 100)/total basal area of all species.

Dominance (rank-abundance) curve was constructed to display relative species abundances of each forest stand. Using Microsoft Excel 2007, the relative abundance of each species was plotted on a logarithmic scale against the species' rank, ordered from the most abundant to the least abundant species (Benitez-Malvido and Martinez-Ramos 2003).

Disturbance impacts

The disturbance level of each sample plot (n = 46) were defined as the sum of relative values of tree cutting per plot, area percentage of farming per plot, and distance between sample plots to villages (Zhu *et al.* 2007). The maximum tree cuts, maximum farming percentage and minimum distance to village were given the impact factors as '1' respectively. For cutting and farming intensity, the impact factors were calculated as the proportion to their maximum values while for distance from village, a proportion of inverse of minimum value was used. It was assumed that the closer the village the higher the disturbance. According to the field data of this study, the criteria, i.e. the maximum values for the cutting impact was 20 stems per plot and the farming impact was 100% that represented all area was transformed to cultivated land. The minimum distance to village was 1,000 m. For example, the cutting intensity of Plot A was 5 stems, farming intensity was 50 percent and distance to village was 1,500 m. The cutting impact was 0.25 (5/20 = 0.25) and the farming impact was 0.5 (50/100 = 0.5). The impact of distance to village was 0.67 (1000/1500 = 0.67). Thus, the disturbance factor of the Plot A was 1.42 (the sum of the impact factors; 0.25 + 0.5 + 0.67 = 1.42).

Sample plots were categorized into three groups of disturbance levels using the cluster analysis provided in PC-ORD version 4 (McCune and Mefford 1999). Since the distribution of monkey was not in normality, the non-parametric Spearman's rank correlation was used to know the relationship between the occurrence of Phayre's leaf monkey and habitat characteristics. Data were managed using Microsoft Excel for Window 2007

and SPSS for Windows Release 16.0 (SPSS Inc., Chicago, Illinois). The data used in this analysis was provided in **Appendix 3.1.**

Population and occupancy

Mean population of Phayre's leaf monkey was calculated using Microsoft Excel for Window 2007. Occupancy modelling was based on several assumptions for estimating occupancy (MacKenzie *et al.* 2006). Assumptions made for this study were summarized as follows:

1. Occupancy variation of leaf monkey can be modelled with site covariates.
2. Detection of leaf monkey was independent from detection of other species at a site (i.e. call and drops of Phayre's monkey are different from that of Rhesus macaque, *Macaca mulatta,* which is a close relative taxa found in PMP)
3. Sites covariates identified were independent of each other (i.e. forest types and human disturbances were selected as independent covariates that influence the presence of Phayre's leaf monkey)

Terminologies and notations used in occupancy modelling are defined below:

Terminology

Sampling unit: a unit used in sampling process and statistical analyses (in this study, 1 km track lines are referred as sampling units)

Replicate: a basic segment for collection of detection or non-detection data of species of interest (in this study, 200 m segments are referred as replicates)

Notation for spatial correlation method

ψ = probability that a species is present at a sampling unit

P' = detection probability of a species at a replicate being sampling unit occupied and species present on replicate

θ = probability of species present on a replicate being not present on previous replicate and sampling unit occupied

θ' = probability of species present on a replicate being present on previous replicate and sampling unit occupied

Akaike Information Criterion (AIC) and Second-order Akaike Information Criterion (AICc)

A total of 78 sampling units (1 km transect) with 375 replicates (200 m segments) were included in occupancy analysis. Using Program PRESENCE ver.3.0 (Beta) (Hines 2006), the best model was fitted and occupancy value (ψ) was calculated for each transect line (n = 78). These occupancy values were linked with the respective vegetation sample plots (n = 46) to analyse the relationship between the occurrence of monkeys and vegetation characteristics as well as disturbance factors.

Usually the model with minimum AIC value is selected for the best model (Burnham and Anderson 2002, Hines 2006, MacKenzie *et al.* 2006) using the following mathematical equation:

$$AIC = -2\ln [L (\theta/x)] + 2\delta$$

where, x is the value of covariate and δ is the number of parameters estimated in the model. However, Burnham and Anderson (2002) recommended the use of the second-order information Akaike criterion (AIC_c) when the sample size divided by the number of variables is less than 40, which applied in this study. The criterion of AIC_c can be expressed mathematically as:

$$AIC_c = AIC + \frac{2K(K+1)}{(n-K-1)}$$

where, K is the number of estimable parameters in the approximating model, n is the sample size. In this analysis, three plausible models with covariates and one without were fitted to the data (**Table 3.1**). The first model concerned with the forest types [ψ (habitat),θ, θ', $p'(.)$] with minimum AIC_c value (AIC_c = 88.08) was selected as the best.

Table 3.1 Criteria of plausible models for occupancy of Phayre's leaf monkey in Popa Mountain Park

Models	**AIC**	**AIC_c**	**AICwgt**	**K**
Model-1: ψ (habitat), θ, θ′, p′(.)	87.76	88.08	0.707	3
Model-2: ψ (habitat + disturb) θ, θ′, p′(.)	90.26	91.09	0.202	5
Model-3: ψ (disturb), θ, θ′, p′(.)	91.88	92.20	0.090	3
Model-4: ψ (.), θ, θ′, p′(.)	97.32	97.48	0.000	2

Notes: **AIC** is Akaike Information Criterion, **AIC_c** is the second-order Akaike information criterion, **AICwgt** is the AIC model weight, **K** is estimable parameters in the approximating model, **ψ** is probability that a species is present at sampling unit, **p′** is detection probability of a species at a replicate being sampling unit occupied and species present on replicate, **θ** is probability of species present on a replicate being not present on previous replicate and sampling unit occupied, **θ′** is probability of species present on a replicate being present on previous replicate and sampling unit occupied, (**habitat**) is vegetation types (semi-evergreen or other forests), and (**disturb**) is human disturbances (disturbed or non-disturbed)

Phayre's leaf monkey behavior

Scanned individual number of each behavioral category was converted to percentage of the total scanned behavioral records. Percent time spent on an activity during the day was estimated according to Gupta and Kumar (1994) as follows:

$$T_a = n_a \times 100 / N$$

where T_a = % day time spent on activity; n_a = number of records with activity a; and N = total number of records for the day.

Non-parametric tests were used for behavioral analysis between seasons, times of the day, terrestrial and tree strata use of Phayre's leaf monkey. Kruskal-Wallis H Test was selected because it is much less sensitive when data do not follow a normal distribution pattern (McDonald 2009).

3.3 Results and discussion

3.3.1 Vegetation and disturbances

Tree species composition along the elevational ranges

A total of 92 tree species belonging to 43 families were recorded in this study. Tree density (no./ha), basal area (m^2/ha) and importance value index (IVI) of tree species in three forest stands of Popa Mountain Park were shown in **Appendix 3.2**. The IVI calculation gives a total picture of the social structure of species in a community and can be used to form an association of dominant species (Parthasarathy and Karthikeyan 1997). The most dominant species in HE stands was *Pittospotum napaulense* (25.8 %) whereas *Jatus hamiltonianus* dominated in both ME (19.5%) and LE (25.8%) stands (**Figure 3.6**).

Out of the five top dominant species in HE stands, three species *Carallia brachiata, Trevia nudiflora* and *Quercus serrata* are typical semi-evergreen species (Kermod 1964) which provide food resources for Phayre's leaf monkey (Min 2006). In this study, ME stands were predominated by the mixed deciduous species *J. halmitonianus, Shorea obtusa* and *Pentacme siamensis*. Whereas in LE stands, the highest IVI were recorded for *J. halmitonianus, Ziziphus jujube* and *Terminalia oliveri* which are common for dry forest mostly occupied along the southern foothills of PMP. The most part of the southern range consists of andesitic larva with traces of manganese and copper (FD 1981). In this study, the forest types classification mirrors that of Kermod (1964) who describes the species composition and dominance of

different forest types such as dry forests, mixed deciduous forests and dry hill forests in Myanmar.

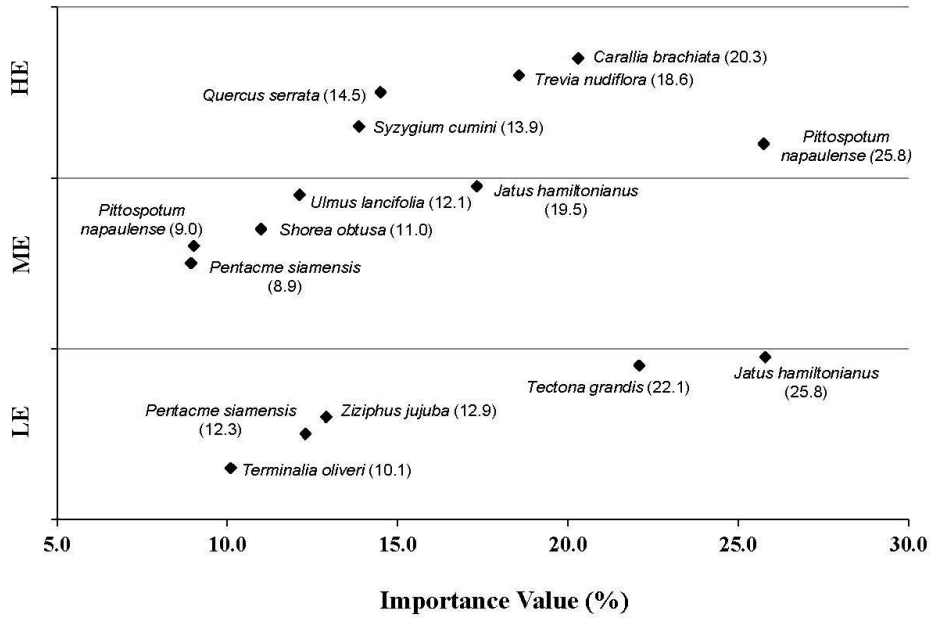


Figure 3.6 Most dominant species (top five) in three forest stands of Popa Mountain Park. Value in the bracket is species importance value (IVI in percentage). HE stands were dominated by typical semi-evergreen species *Carallia brachiata*, *Trevia nudiflora* and *Quercus serrata* which are often consumed by Phayre's leaf monkey. LE = low elevation stand (301-600 m), ME = mid elevation stand (601-900 m), HE = high elevation stand (> 900 m)

Species diversity, density and basal area along the elevational ranges

Species diversity for all tree, sapling and seedling were recorded as the highest in ME stands followed by LE stands, and the HE stands had the lowest diversity (**Table 3.2**). One-way ANOVA results showed that species diversity were significantly different among three forest stands (tree diversity; $F_{2,43}$ = 65.43, $P < 0.0001$, sapling diversity; $F_{2,43}$ = 295.4, P = 0.002, seedling diversity; $F_{2,43}$ = 133.62, P = 0.022). Fisher's diversity indices for trees, saplings and seedlings showed that diversity was lower in HE stands which occupied only a few percentage of the mountain area. Area decreasing toward the top of mountain probably trims down the species diversity. Rahbek (1995) and Adams (2009) reported that the upper part of the mountain might be considered as an isolated and restricted area with limitation to species adaptation.

Tree density was highest in high elevation (HE) stands followed by mid elevation (ME) stands and the lowest density was recorded in low elevation (LE) stands (**Table 3.2**). In contrast, sapling and seedling density were recorded as lowest in HE stands. Among three forest stands at different elevation, tree and seedling densities were significantly different whereas sapling density was not significantly different (ANOVA result: tree density; $F_{2,43}$ = 6.21, $P = 0.004$, sapling density; $F_{2,43}$ = 0.334, P = 0.718, seedling density; $F_{2,43}$ = 3.304, P = 0.046). Tree basal area varied from 5.00 to 20.78 m^2/ha in the three forest stands. The mean basal area was higher in HE stands followed by ME stands and the lowest basal area was recorded in LE stands

(**Table 3.2**) and it was significantly different among three forest stands ($F_{2, 43}$ = 17.90, P < 0.0001).

Tree density and mean basal area was much higher in HE stands rather than that of ME and LE stands. This may reflect to the fertility of volcanic soil at the higher part of mountain particularly at the crater that opens to the northern side of mountain and looks like a valley (FD 1981, Zin 1998). Although tree density was higher in HE stands, seedling and sapling density were relatively low in these stands. This may possibly due the effect of dense canopy that absorbs most of daylight and there may have limited regenerations underneath. Adams (2010) urges that, in most dense tropical rain forests, photosynthesis is usually slow due to insufficient sunlight and there is a sparse layer of plants on the forest floor.

Table 3.2 Species diversity, density and basal area along the elevational ranges of Popa Mountain Park. LE = low elevation stand (301-600 m), ME = mid elevation stand (601-900 m), HE = high elevation stand (> 900 m)

	LE (n = 20)	**ME (n = 21)**	**HE (n = 5)**
Number of species	50	72	31
Number of families	28	32	26
Tree diversity (Fisher's α)*	12.67 ± 1.00^{a}	22.39 ± 0.25^{b}	9.88 ± 0.10^{c}
Sapling diversity (Fisher's α)*	10.11 ± 1.37^{a}	14.59 ± 1.58^{b}	3.10 ± 0.39^{c}
Seedling diversity (Fisher's α)*	7.84 ± 1.19^{a}	11.93 ± 1.34^{b}	5.31 ± 2.19^{c}
Tree density (no. / ha)*	220 ± 35^{a}	336 ± 44^{b}	517 ± 72^{c}
Sapling density (no. / ha)	$1{,}257 \pm 272^{a}$	$1{,}204 \pm 151^{a}$	870 ± 259^{a}
Seedling density (no. / ha)*	$7{,}940 \pm 1374^{a}$	$8{,}638 \pm 1262^{a}$	$1{,}440 \pm 685^{b}$
Tree basal area (m^2 / ha)*	5.00 ± 0.73^{a}	9.13 ± 1.39^{b}	20.78 ± 3.12^{c}

Note: Values given are mean ± standard error. Those within a row not followed by the same letter are significantly different within forest stands (Games-Howell *post-hoc* test: $P < 0.05$). * indicates significant differences ($P < 0.05$) among three forest stands

Stand diameter distribution along the elevational ranges

Diameter distribution of three forest stands at different elevations was shown in **Figure 3.7**. Trees with diameter at breast height (dbh) between 10 and 15 cm were abundant in all forest stands. All forest stands showed the same trend with lower density of higher diameter classes and higher density of lower diameter classes. The diameter distribution of trees has often been used to represent the population structure of forests (Rao *et al.* 2002, Htun *et al*. 2011) and the type of curves are used to interpret the vegetation characteristics. In this study, stand structures in three forest stands showed reverse *J*-shaped curves indicating that the stands had growing and healthy population structure (Parthasarathy and Karthikeyan 1997, Htun *et al*. 2011).

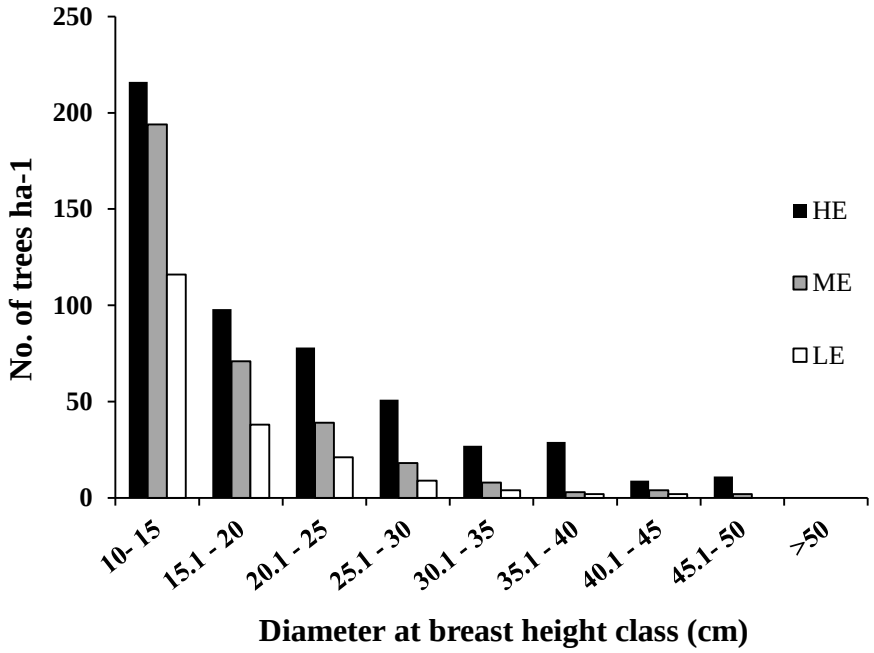


Figure 3.7 Stand diameter distribution of three forest stands in Popa Mountain Park. All forest stands reveal the reverse J-shape curves indicating healthy population structure in the park. LE = low elevation stand (301- 600 m), ME = mid elevation stand (601-900 m), HE = high elevation stand (> 900 m)

Disturbances

All sample plots were categorized into three forest stands associated with disturbance impacts; high disturbance stand (n = 18), intermediate disturbance stand (n = 14), and low disturbance stand (n = 14) (**Figure 3.8**). Disturbance impacts were calculated for each sample plots based on the intensity of tree cutting, farming and distance to villages (**see session 3.2.3**).

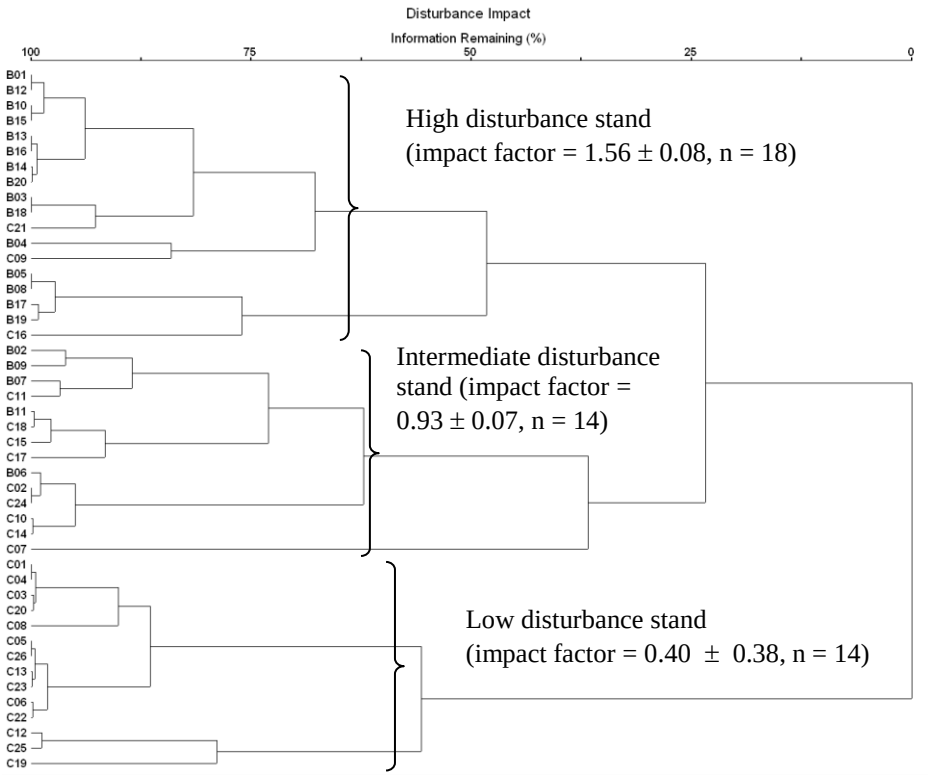


Figure 3.8 Clustering the sample plots into three groups of disturbance level. High disturbance stand (impact factor: 1.56 ± 0.08, n = 18); Intermediate disturbance stand (impact factor: 0.93 ± 0.07, n = 14); and Low disturbance stand (impact factor: 0.40 ± 0.38, n = 14). C01 - C26: sample plots in core area, B01 - B20: sample plots in buffer area

3.3.2 Population and occupancy of Phayre's leaf monkey

Population

Three groups of Phayre's leaf monkey (mean: 41 ± 0.67, 41 ± 0.58 and 21 ± 0.67 individuals for group-1, group-2, and group-3, respectively, **Figure 3.9**) were recorded in the study area (about 150 km^2). Male and female could not be identified because Phayre's leaf monkey is not sexual dimorphic as well as they are of similar size (about 7 and 6 kg for male and female, respectively) (Cantwell 2011) and all active (Davies and Oates 1994). However, sex could be determined by observing differences between ocular markings (Bhattacharya and Chakraborty 1990) which could be difficult with the unhabituated groups especially in steep terrains in this study area. The young composed 14% (n = 6) in a group of monkey (group-1) that was selected for behavior study. The identification for age could not be done for other groups (group-2 and group-3) due to the steep terrains at the habitats they live in.

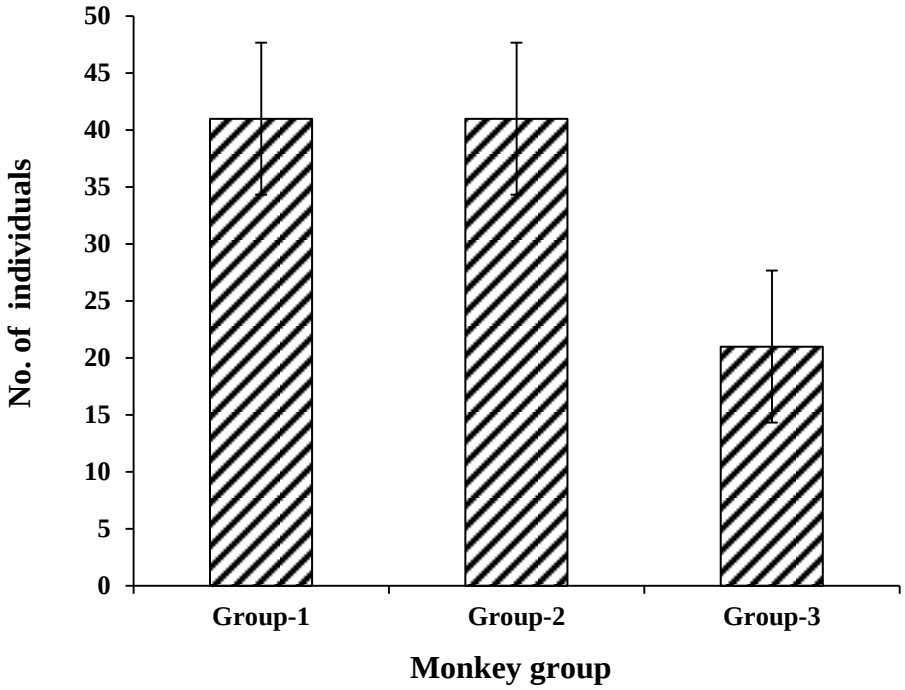


Figure 3.9 The average number of individuals for three monkey groups (mean: 41 ± 0.67, 41 ± 0.58 and 21 ± 0.67 individuals for group-1, group-2 and group-3, respectively)

Occupancy

The occupancy results were pooled into four habitat types: 1) semi-evergreen and non-disturbed, 2) semi-evergreen and disturbed, 3) other forests and non-disturbed, and 4) other forests and disturbed (**Table 3.3**). The highest occupancy value (ψ = 0.7679, SE = 0.182) was recorded for the semi-evergreen forest with little or no disturbances. This result is consistent with the previous study that revealed that Phayre's leaf monkey prefers to inhabit semi-evergreen forest in Bangladesh (Aziz and Feeroz 2009). In this study, the result showed that Phayre's leaf monkey possibly survives at the places affected by natural disturbances such as landslides that allow the areas to have vegetation of early succession stages. This result is concordant with the findings from India, whereby, Gupta and Kumar (1994) reported that Phayre's leaf monkey had not been affected by shifting cultivation and they suggested that the regeneration of fallow lands possibly provided foods to Phayre's leaf monkey. However, in this study, no evidence was gathered for the presence of the Phayre's leaf monkey at the forest stands disturbed by human (other forests and disturbed: ψ = 0.0306, SE = 0.021). It could be concluded that Phayre's leaf monkey tend to avoid areas which are frequently visited by human.

Table 3.3 Occurrence of Phayre's leaf monkey in the Popa Mountain Park. Semi-evergreen forest with no human disturbances was highly occupied by Phayre's leaf monkey (ψ = 0.7679, SE = 0.182). Occupancy values among habitats are significantly different (Kruskal-Wallis *H* Test: $H = 77$, $df = 3$, $P < 0.0001$).

Covariates	**Occupancy value (ψ)**	**SE**	**UL_CI**	**LL_CI**
Semi-evergreen forest and non-disturbed	0.7679^{a}	0.182	0.9606	0.3099
Semi-evergreen forest and disturbed	0.4503^{b}	0.421	0.9584	0.0299
Other forests and non-disturbed	0.1591^{c}	0.240	0.8645	0.0056
Other forests and disturbed	0.0306^{d}	0.021	0.1144	0.0076

Note: Other forests represent deciduous, indaing, than-dahat forests. **ψ**: probability that a species is present in a given area, **SE**: standard error, **UL_CI**: upper limit confidence interval, **LL_CI**: lower limit confidence interval.

3.3.3 Habitat use of Phayre's leaf monkey

Food resources for Phayre's leaf monkey

Out of the 92 tree species recorded in this study, 10 tree species belonged to 10 families that were consumed by Phayre's leaf monkey. The pictorial report of plant species consumed by the monkey is provided in the **Appendix 3.3**. Of these food trees, *Quercus serrata* occupied about 75% of the total IVI whereas *Carallia brachiata* and *Protium serratum* occupied about 40% and 20%, respectively. Three climbers: *Cissus vitiginea, Piper attenuatum* and *Smilax perfoliata*, and one shrub *Cordyline fruticosa* were also consumed by Phayre's leaf monkey (**Table 3.4**). Food plants for the monkey came from fourteen different plant families including Moraceae and Myrtaceae which provide most of its food in other range countries, Bangladesh and India (Aziz and Feeroz 2009, Gupta and Kumar 1994). Moreover, Phayre's leaf monkey in PMP also eats climbers (Min 2006, this study) which have not been recorded elsewhere in its range. Aziz and Feeroz (2009) reported that Phayre's leaf monkey in Bangladesh consumed a variety of plant species (29 species in total) which mostly were different taxa. The result of this study supports that Phayre's leaf monkey likely to select a variety of plant species as well as to inhabit a wide range of habitats (Gupta 2001).

Table 3.4 Availability of food tree species consumed by Phayre's leaf monkey in Popa Mountain Park. Tree density (DEN: no./ha), basal area (BA: m^2/ha) and importance value index (IVI in percentage). Ten tree species, three climbers, and one shrub were recorded as food resources for Phayre's leaf monkey in this study.

No.	Species name	Local name	Family name	Edible plant parts	DEN (no./ha)	BA (m^2/ha)	IVI (%)	Form
1	*Carallia brachiata*	Mani-awga	Rhizophoraceae	Leave	300	7.8	36.7	Tree
2	*Cissus vitiginea*	Yinnaung-new	Vitaceae	Leave, bud	5,333	0.0	0.0	Climber
3	*Cordyline fruticosa*	Kun-line	Asparagaceae	Leave	8,400	0.0	0.0	Shrub
4	*Diospyros mollis*	Thin twe nyo	Ebenaceae	Leave, bud	11	0.3	4.8	Tree
5	*Eugenia contracta*	Tha bye	Myrtaceae	Leave, bud	44	2.0	18.6	Tree
6	*Ficus obtusifolia*	Nyaung gyat	Moraceae	Leave, bud, fruit	22	2.5	10.5	Tree
7	*Flacourtia indica*	Naywe	Salicaceae	Leave, bud, fruit	39	1.8	14.3	Tree
8	*Piper attenuatum*	Sayo	Piperaceae	Leave	19,733	0.0	0.0	Climber
9	*Protium serratum*	Thadi	Burseraceae	Fruit	78	5.8	22.3	Tree
10	*Quercus serrata*	Thit-e	Fagaceae	Leave, bud	456	20.6	74.7	Tree
11	*Smilax perfoliata*	Sein-nabaw	Smilacaceae	Leave, bud	3,733	0.0	0.0	Climber
12	*Terminalia bellirica*	Thit-seint	Combretaceae	Fruit	11	0.1	4.5	Tree
13	*Trema orientalis*	YethaKhwar	Cannabaceae	Leave, bud, fruit	11	0.1	5.2	Tree
14	*Trevia nudiflora*	Sakadu	Euphorbiaceae	Fruit	11	0.3	7.7	Tree

Correlation between the occurrence of Phayre's leaf monkey and habitat characteristics

There was a positive relationship between the occurrence of Phayre's leaf monkey and forest stand characteristics (Spearman's rank correlation: tree density (no./plot): $\Upsilon_s = 0.305$, $P = 0.039$; basal area (m^2/plot): $\Upsilon_s = 0.469$, $P < 0.0001$; mean tree height (m): $\Upsilon_s = 0.583$, $P < 0.0001$). These results support that Phayre's leaf monkey is predominantly arboreal species (Molur *et al.* 2004) and mostly inhabits the forested areas (Cantwell 2011). However, Phayre's leaf monkey prefers to select a wide range of habitats. For instance, in India, Phayre's leaf monkey distributes dense natural patches of evergreen forests, semi-evergreen forest, deciduous forests and mixed forestry plantations (Gupta 2001). But in Lao, this monkey often resides in the forests with a heavily broken canopy and much tall bamboo (Timmins *et al.* 2011).

In this study, Phayre's leaf monkey preferred to live at higher elevation (Spearman's rank correlation: $\Upsilon_s = 0.509$, $P < 0.0001$) but with less human disturbances ($\Upsilon_s = -0.439$, $P < 0.002$) (**Figure 3.10**). This study supports that the typical behavior of *T. phayrei* groups which show shyness and avoid human interferences (Gupta 2001, Robert *et al*. 2011). Moreover, the result is also consistent with the previous study reported that the Phayre's leaf monkey was not associated with gentle terrains and mainly distributed along the ranges of 500 up to 1,000 m in Laos (Timmins *et al.* 2011).

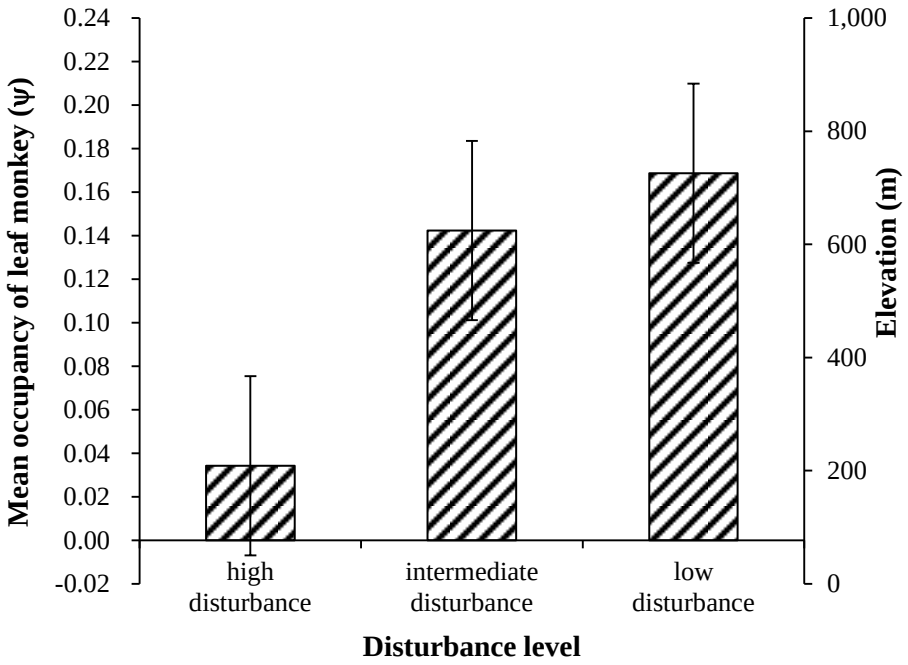


Figure 3.10 Occurrence of Phayre's leaf monkey related to human disturbances and elevation. The monkeys prefer to live at higher elevation with less human disturbances. High disturbance stand (impact factor: 1.56 ± 0.08, n = 18); Intermediate disturbance stand (impact factor: 0.93 ± 0.07, n = 14); and Low disturbance stand (impact factor: 0.40 ± 0.38, n = 14)

Comparison of vegetational characteristics between two forest stands occupied and unoccupied by Phayre's leaf monkey

Vegetational characteristics (tree density, species diversity, diameter at breast height, and basal area) were significantly different between the forest stands inhabited by the monkey and where monkeys avoided to live (**Table 3.5**). The occurrence of Phayre's leaf monkey was positively related with food tree species, a scenario which has not been reported elsewhere for Phayre's leaf monkey. However, these results could be compared with those reported for red colobus (*Procolobus rufomitratus*) in Kenya (Mbora and Meikle, 2004) and vervet monkey (*Cercopithecus aethiops*) in South Africa (Willems and Hill 2009). Both of monkeys belong to the same family (Cercopithecidae) as Phayre's leaf monkey and they spent more time in the habitats in which food was abundant.

Sapling and seedling density of food trees in the occupied forests were higher than that in the unoccupied forests (Kruskal-Wallis *H* Test: sapling; 906 ± 22.74 *versus* 308 ± 42.64 no./ ha, $H = 39.20$, $df = 1$, $P = 0.0001$, seedling; 6051 ± 522.74 *versus* 1593 ± 163.50 no./ ha, $H = 16.17$, $df = 1$, $P = 0.0001$). Because the monkey selected the northern crater which had more edible plant species for this monkey, this could suggest that food availability is probably the most determinant factor for habitat selection. As expected, Phayre's leaf monkey used forest stands with tall trees (Kruskal-Wallis *H* Test: 11.50 ± 0.58 m *versus* 6.81 ± 0.38 m, $H = 18.32$, $df = 1$, $P = 0.0001$). This result supports that Phayre's leaf monkey is an arboreal

species, mostly occupied the high canopy layer (Gupta 2002) although it could spent a significant time on the ground especially in forest gaps and rocky hills.

Moreover, the results revealed that Phayre's leaf monkey inhabited the forest stands with high species richness and evenness (**Figure 3.11**). This suggests that Phayre's leaf monkey in PMP selected their habitats with high abundance of food resources and the results are consistent with that of Aziz and Feeroz (2009) who reported that the continued availability of food trees supports the survival of Phayre's leaf monkey in Bangladesh. Similarly, in the primate study of Amazon (Tapajos) National Park in Brazil, abundance of primates revealed significant correlation with the fruiting and insect abundance of riverine forests during the four months of inundation (Branch 1983). In his new approach to assess the habitat utilization of neotropical primates, Warner (2002) also reported that primates are more likely to spend time in the preferred habitats those provide important feeding resources and/or safe resting sites.

Table 3.5 Vegetational characteristics between the two forest stands occupied and unoccupied by Phayre's leaf monkey in Popa Mountain Park. Values given are mean ± standard error. Habitat characteristics between two forest stands are significantly different (Kruskal-Wallis *H* Test: at all $df = 1$, $P < 0.0001$).

Variables	**Occupied forest (n = 10)**	**Unoccupied forest (n = 18)**	***H***
Tree density (no. / ha)	283 ± 14.78	50 ± 14.78	47.82
Tree species diversity index (H′)	1.81 ± 0.05	1.67 ± 0.11	36.01
Tree height (m)	11.50 ± 0.58	6.81 ± 0.38	18.32
Diameter at breast height (cm)	21.50 ± 0.83	15.70 ± 1.01	8.34
Basal area (m^2 / ha)	21.14 ± 0.63	13.16 ± 0.79	25.56
Sapling density (no. / ha)	906 ± 22.74	308 ± 42.64	39.20
Seedling density (no. / ha)	6,051 ± 522.74	1,593 ± 163.50	16.17

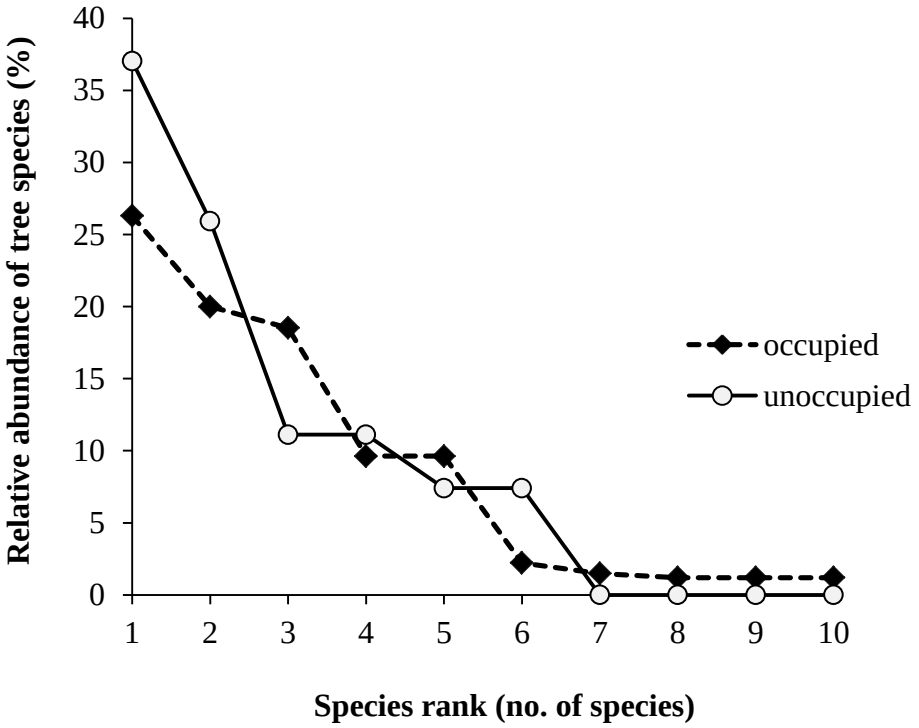


Figure 3.11 Species dominance (rank-abundance) curve for two forest stands occupied and unoccupied by Phayre's leaf monkey in Popa Mountain Park. Occupied forests represent more diverse food tree species because of the gentle slope line (high evenness) with high species richness. Tree species were ranked ordering from the most abundant to the least abundant species.

3.3.4 Ground and vertical tree strata use

The mean activity of Phayre's leaf monkey for using ground and tree strata was shown in **Table 3.6**. The monkeys travelled randomly in each stratum (Kruskal-Wallis *H* test: $H = 3.527$, $df = 4$, $P = 0.474$) but they displayed non-randomly for feeding, resting, and other behaviors in each of the tree stratum and on the ground (Kruskal-Wallis *H* test: feeding: $H = 23.214$, $df = 4$, $P < 0.0001$; resting: $H = 23.291$, $df = 4$, $P < 0.0001$; others: $H = 11.107$, $df = 4$, $P = 0.025$).

The monkey spent 60% (n = 2,712) of their time in trees and 40% (n = 1,795) on the ground. The ground use of Phayre's leaf monkey was relatively high compare with other arboreal primates (Yiming 2006). In this study, Phayre's leaf monkey used the grounds (ground-1 and ground-2) for feeding (24%), resting (45%), travelling (38%) and other behaviors such as grooming and playing (62%), respectively (**Figure 3.12**). This is possibily the results of unique topography of the study area particulary where the monkey inhabits. The monkey inhabits the steep slopes comprised with rocky area partially covered with climbers (**Figure 3.13a**). They spend most of their time for resting including sunbath in winter season on the rocks associated with feeding climbers (**Figure 3.13b)**.

Table 3.6 Ground and vertical tree strata use of Phayre's leaf monkey in Popa Mountain Park. The monkeys travelled randomly in each stratum (H = 3.527, df = 4, P = 0.474) but they displayed non-randomly for feeding, resting, and other activities (grooming, playing). Kruskal-Wallis H test was used at significant level of 0.05.

Strata	**Activity records (n)**	**Mean activity**			
		feeding	**resting**	**travelling**	**others**
ground_1 (open)	1,507	1.90 ± 1.90	53.67 ± 9.16	9.57 ± 2.68	6.62 ± 2.56
ground_2 (climber)	288	38.50 ± 18.20	5.33 ± 4.94	3.33 ± 2.16	0.83 ± 0.83
low stratum (1.0 - 3.0 m)	196	12.71 ± 6.94	3.86 ± 2.25	11.43 ± 7.83	0.00 ± 0.00
middle stratum (3.1 - 6.0 m)	1,291	17.58 ± 4.87	38.84 ± 4.94	9.42 ± 3.13	2.11 ± 1.16
upper stratum (6.1 - 9.0 m)	1,225	26.47 ± 6.38	36.82 ± 7.82	5.88 ± 1.51	2.88 ± 1.15
df		4	4	4	4
***H* value**		23.214	23.291	3.527	11.107
***P* value**		< 0.0001	<0.0001	0.474	0.025

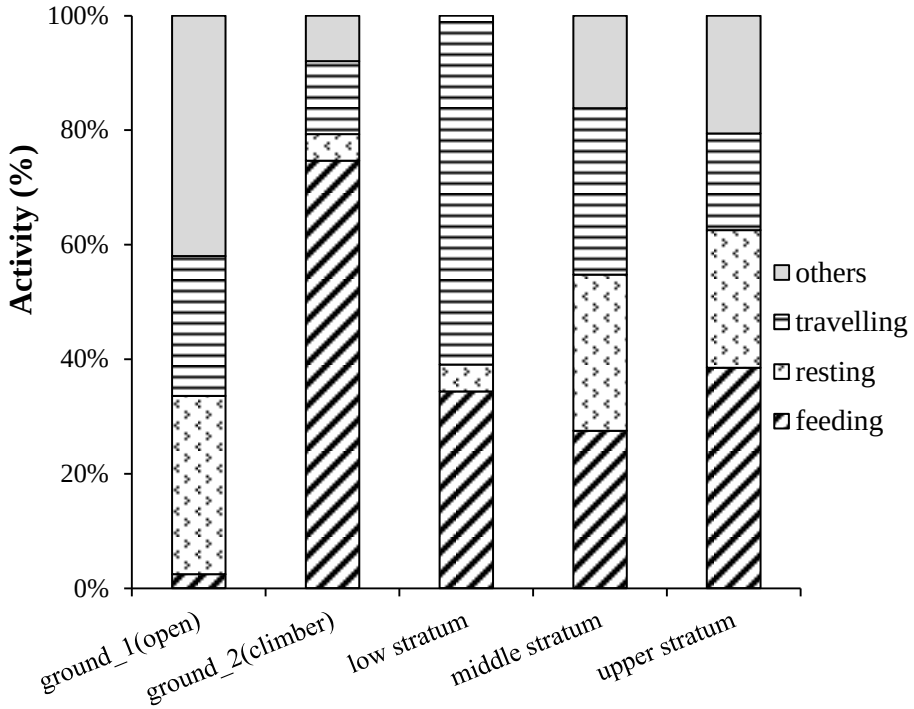


Figure 3.12 Composition of behavioral types on the ground and in the tree strata for Phayre's leaf monkey in the Popa Mountain Park. The monkeys spent much feeding time on the ground covered with climbers (ground_2) and also in the trees strata. They spent much resting time on the exposed rock areas (ground_1) as well as middle and upper tree strata. Monkeys mostly travelled through the lower branches of the trees.

(a)

(b)

Figure 3.13 Habitat of Phayre's leaf monkey in Popa Mountain Park: (a) Hmanpya Taung at the eastern rim of Mt. Popa and (b) a group of Phayre's leaf monkey taking sunbath on the rocky area

As an arboreal species, the Phayre's leaf monkey in PMP also prefered the middle and upper strata for feeding about (69%), resting (53%), travelling (48%), and 38 % for other activities such as grooming and playing (**Figure 3.12**). The monkeys rarely spend their time at low stratum in trees except for travelling. A high degree of arboreality of the monkeys in the park may be attributed to 1) vertical distribution of food resources in the trees, 2) food distribution on the ground, and 3) vegetation types. The vertical distribution of food items in the diet may largely reflect the distribution of food resources in the forests. The diet of the monkeys was mainly composed of trees as well as climbers on the rocks and trees. The main food resources for the monkeys in the study area were in the trees, almost all of which range from above 3 m tall. The monkeys on the ground mainly fed on climbers. Resting was a main component of ground use for the monkeys in PMP. Habitat with a high proportion of open areas may probably increase ground use of the monkey. It can be concluded that Phayre's leaf monkey prefers the habitat associated with exposed rock faces and this result is consistent with the previous study of closed relative Lao leaf monkey (*Trachypithecus (francoisi) laotum*) which preferred the exposed lime stone karst in Laos (Steinmetz *et al*. 2011).

The most preferred climbers—*Cissus vitiginea* (Family: Vitaceae) and *Piper attenuatum* (Family: Piperaceae), that have medicinal values (Charles *et al*. 2009, Naz *et al*. 2009), and their annual availability were also high (**Table 3.4**). The leaves of herbal plants contain a higher proportion of proteins than the leaves, bark, and buds of most trees (Milton 1979). Thus, the

monkeys may feed on these herbal plants on the ground possibly because these herbs can provide important food proteins. The primates usually prefer higher protein food and not higher fiber or secondary compounds, such as tannin and alkaloids (Milton 1979). Low stratum usually has fewer branches and trunks than middle and upper strata. Therefore, the monkeys use this low stratum less often for all behavioral types except travelling associated with searching foods than other strata.

The vegetation types have an effect on tree strata and ground use. There may be a difference in the distribution of food resources, the proportion of open areas, and the differences in vegetation height and structure, which results in the monkeys using tree strata differently in different behaviors.

3.3.5 Seasonal and daily behaviors

Phayre's leaf monkey spent most of their day time for feeding (54 %) following by 32 % of resting (**Figure 3.14**). Travelling was recorded 7% of total daily activity and other activities like grooming and playing accounted for 7% either. The result of this study was consisted with that of previous studies (Gupta and Kumar 1994, Gupta 2005) who found the Phayre's leaf monkey in India spent much time for feeding (Gupta 2005). This result suggested that the survival of Phayre's leaf monkey mainly depends on the habitats with abundance of food resources.

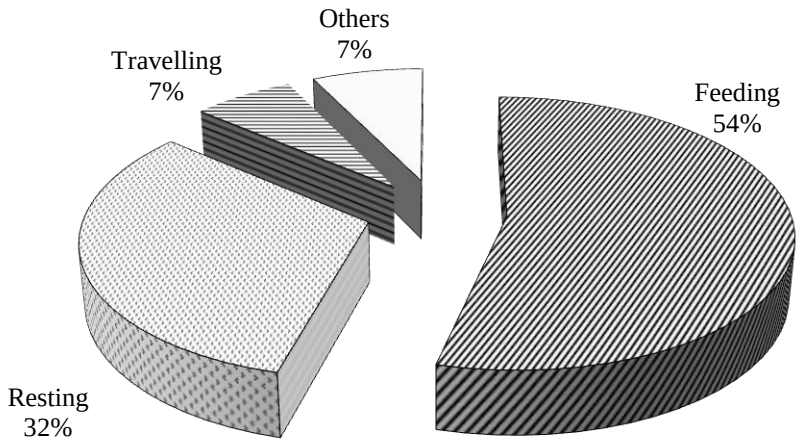


Figure 3.14 Total activity budgets (frequency) of Phayre's leaf monkey in Popa Mountain Park. Phayre's leaf monkeys spent most of their time for feeding (n = 12,884) and it was followed by resting (n = 7,817), travelling (n = 1,476), and other activities (n = 1,597).

Seasonal behavioral changes

Resting activity of Phayre's leaf monkey in this study was slightly different among seasons (Kruskal-Wallis *H* test: $H = 6.44$, $df = 2$, $P = 0.04$). But the monkeys spent equal time for feeding ($H = 2.36$, $df = 2$, $P = 0.308$), travelling ($H = 0.47$, $df = 2$, $P = 0.792$) and others behaviors such as grooming and playing ($H = 0.937$, $df = 2$, $P = 0.626$). Phayre's leaf monkey in PMP spent time equally for all behaviors for the whole year with slight higher in time spent resting in winter (31.65%) rather than resting in summer (28.73%) and rainy (27.63%) (**Figure 3.15**). This increase in resting period during winter was probably due to the effect of sunbathing on the exposed rock areas. Myanmar has three distinct seasons: the cold and dry season (winter: November- February) with average monthly temperatures of between 20℃ and 24℃, the hot-dry season (summer: March – mid May) with average monthly temperatures between 30℃ and 35℃, and the wet season (rainy: mid May - October) with average temperature between 25℃ and 30℃. However, in PMP, the average temperature is lower than other regions and it is minimized about 14 °C (57 °F) in winter (Popa Agricultural Office 2010). Thus, the Phayre's leaf monkeys possibly spent more resting time for sunbathing in winter. However, this result was contrary from activity time budget of Phayre's leaf monkey in India, where it spent less time for resting in winter compared to monsoon rainy season (Gupta and Kumar 1994, Gupta 2005). The difference could be because of due to different in habitat structure and topography of the study areas. In India, the Phayre's leaf monkeys inhabit the fallow lands within a range of 150 m to 380 m altitude (Gupta and Kumar

1994) while the monkeys in this study preferred the semi-evergreen forests found at the steep slopes above 600 m a.s.l.

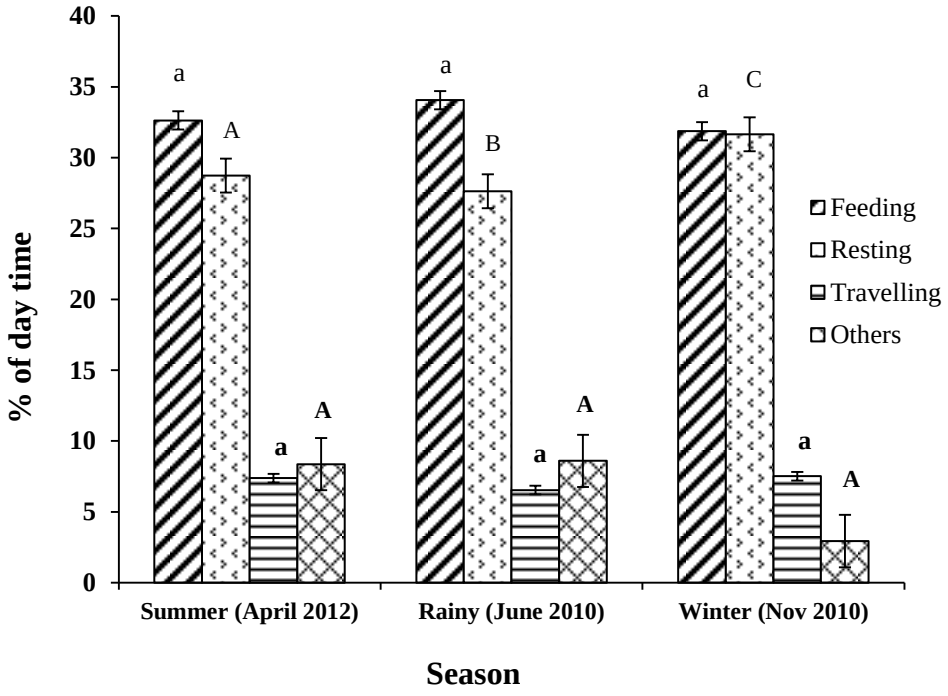


Figure 3.15 The percent daytime spent (in seasons) on the major four categories of activities by the study group of Phayre's leaf monkey. Data were analysed for total scan numbers in summer (n = 17), rainy (n = 33), and winter (n = 24). There was a slightly difference between seasons in time spent resting (Kruskal-Wallis *H* Test: $H = 6.44$, $df = 2$, $P = 0.04$) and no differences for feeding, travelling, and other behaviors (i.e. grooming, playing). The mean difference is significant at the 0.05 level.

Daily behavioral changes

Six intervals of time variation were categorized starting from 0600 h until 1800 h. The graphical illustration revealed that the Phayre's leaf monkey had two feeding peaks (**Figure 3.16**): one in the early morning (0600 - 0800 h) and another in the late evening (1600 - 1800 h). The monkeys spent much time for resting from 1000-1600 h. Other studies (Srivastava 1999, Aziz and Ferroz 2009) also reported that the monkey prefers to feed in the early morning and late evening. But for all time intervals, there were no significant differences except for other behaviors including grooming or playing (Kruskal-Wallis H Test: $H = 13.28$, $df = 5$, $P = 0.021$).

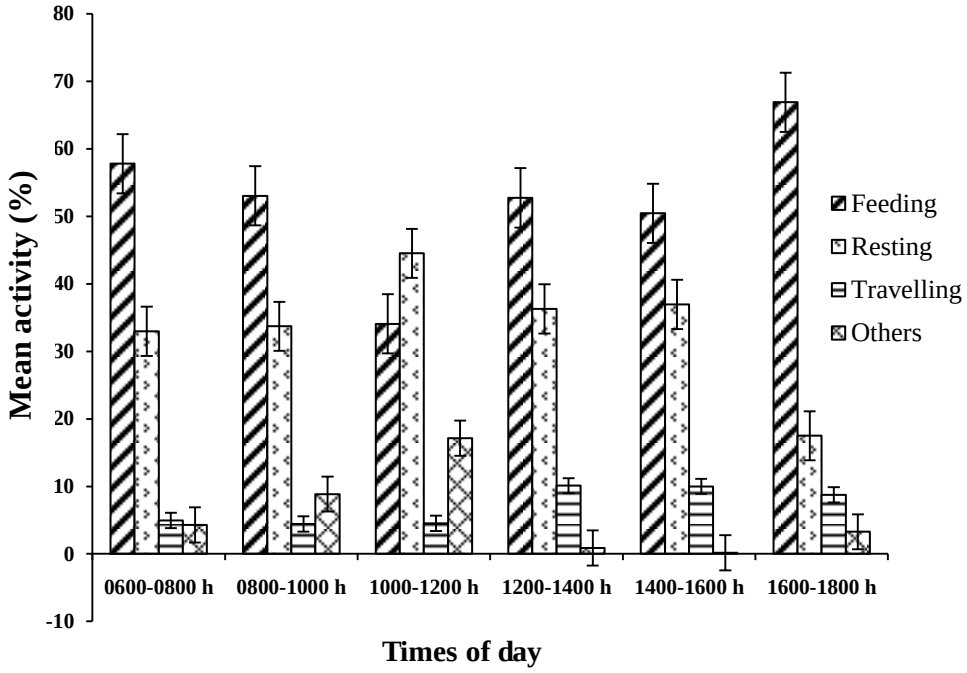


Figure 3.16 Daily time variation on behavioral types for the Phayre's leaf monkey in Popa Mountain Park. Two feeding peaks showed in early morning (0600 – 0800 h) and in the late evening (1600 -1800 h). The monkeys spent much time for resting from 1000 -1600 h. For all time intervals, there were no significant differences for behaviors except others such as grooming or playing (Kruskal-Wallis *H* Test: $H = 13.28$, $df = 5$, $P = 0.021$).

3.4 Conclusion

The vegetation types of Popa Mountain Park vary along elevational ranges and show healthy forest stands. In PMP, about 100 individuals of Phayre's leaf monkey were recorded and they mostly inhabited northern part where semi-evergreen forests predominated but with less human disturbances. The unique topographical features of steep terrain comprising open rock areas and relatively high altitude are likely to influence the habitat selection of Phayre's leaf monkey. For being a folivore, the Phayre's leaf monkey highly depends on the foods since plant species density, diversity, basal area of forest stands occupied by the monkey were significantly higher than that of the areas where no monkey was found. Moreover, in this study, Phayre's leaf monkey selected a wide variety of plant species including climbers and shrubs which have not been recorded elsewhere in its range countries. In terms of behavior ecology, the day time activities such as feeding, resting and travelling of Phayre's were not be influenced by the seasonal and daily time changes. Given the small population of Phayre's leaf monkey in Myanmar, the effective conservation actions are recommended to protect the remnant population of PMP by protecting its habitats and prohibiting the food trees from harvesting. Additionally, given that the abundances of food resources and availability may influence demography and social organization of the Phayre's leaf monkey (Borries *et al.* 2008), further investigation to establish the correlation of food abundance and social behavior of Phayre's leaf monkey is suggested.

Chapter IV. Modelling Habitat Suitability of Phayre's Leaf Monkey in Popa Mountain Park

4.1 Introduction

Quantifying suitable habitat is basic requirement for wildlife conservation and management (Larson *et al.* 2003, Podchong *et al.* 2009). Habitat suitability models have been used to quantify the range of habitat conditions that will provide most of the requirements for a particular species (Turner *et al.* 1995). Moreover, management for endangered species (Palma *et al*. 1999, Wang *et al.* 2008), ecosystem restoration (Mladenoff *et al.* 1997), species re-introductions (Carroll *et al.* 2003), and human-wildlife conflicts (Le Lay *et al.* 2001) often rely on habitat suitability modelling. Integration of expert knowledge on a particular species and geographic information system (GIS) is a key step towards improving habitat suitability model (Davis *et al*. 1990, Radeloff *et al.* 1999, Johnson *et al.* 2004). Traditionally, habitat suitability models are applied to a sample of locations within land cover types or dominant overstory vegetation types (Larson *et al.* 2003). The outputs of habitat suitability models are usually simple, easily understandable and can be used for the assessment of habitat requirements or prioritization of conservation efforts to save threatened species (Kushwaha *et al.* 2004, Zarri *et al.* 2008).

Every single species has specific requirements based on its behavioral, biological, genetics, and evolutionary history to choose a habitat for survival and each habitat must provides these species requirements (Nazeri *et al.* 2010). For example, in India, tigers (*Panthera tigris tigris*) highly preferred dense

forests in which prey species muntjac (*Muntiacus spp.*) were abundant (Imam *et al.* 2009). The habitat use of velvet monkey (*Cercopithecus aethiops*) at the western mountain range of South Africa was strongly driven by the presence of predators (Willems and Hill 2009). Giant panda (*Ailuropoda melanoleuca*) in China preferred high-elevation zones dominated by coniferous, mixed coniferous and deciduous broadleaf forests (Wang *et al.* 2008). Understanding the species adaptation to source habitats can provide the modelling the habitat suitability which is widley considered for effective wildlife management nowadays.

Since most of primates evolved from arboreal animals, they used to inhabit forested areas particularly in the tropical rain forests (Reed 1995). Phayre's leaf monkey belongs to the subfamily colobinae having unique characters for leaf eating (Chivers and Hladik 1980). For biological reasons, Phayre's leaf monkeys have to eat large amount of vegetative parts daily to obtain sufficient nutrients and they spend most of their time for feeding (Gupta and Kumar 1994, Gupta 2005). Phayre's leaf monkey can be found in a wide variety of habitats (Cantwell 2011). In Bangladesh, Phayre's leaf monkey mostly occupies semi-evergreen and semi-deciduous forests (Aziz and Ferroz 2009). In India, Phayre's leaf monkey inhabits a wide range of habitats: dense natural patch of evergreen forests, semi-evergreen forest, deciduous secondary forests of biotic origin (shifting cultivation, logging, clear felling, fire), and mixed forestry plantations of both timber and non-timer species and even it can be found in the rubber plantations (Gupta 2001). In the recent report for Lao PDR, Phayre's leaf monkey often resides in the

forests with broken canopy and tall bamboo as well as limestone karst (Timmins *et al.* 2011). In Myanmar, no detailed habitat information was reported yet though Yin (1993) mentioned that Phayre's leaf monkeys are forest dwellers and rarely frequenting cultivated grounds. But they are frequently found in semi-evergreen (dry-hill) forests of Popa Mountain Park in central Myanmar. Although Phayre's leaf monkey has been given high legal protection, their population in the region is declining due to habitat degradation and anthropogenic activities (Molur *et al*. 2003)**.** Monitoring the remaining habitats of Phayre's leaf monkey is badly needed since the available data on habitat uses is very limited in the region including Myanmar.

Popa Mountain Park (PMP) is an extinct volcanic moutain emcompassed with diverse vegetations (Thant 2011) that habor the remant population of Phayre's leaf monkey in Myanmar (Pocock 1939, Yin 1993, Bleisch *et al.* 2008). The existence of Phayre's leaf monkeys in PMP was first recorded in 1913 (FD 1981) and it had been reported that they were inhabiting the steep terrains of the mountain (Pocock 1939). Until to date, little information is known about the habitat preferences of Phayre's leaf monkey in PMP and no documents for the relationship between this isolated population and their habitats have been publisehd yet. Given that high priority of habitat conservation and management of threatened species, this study aimed to determine spatial characteristics (vegetation types) of PMP and to develop a predictive habitat suitability model of Phayre's leaf monkey with integration of the expert knowledge and geographic information system (GIS). In particular, this study aimed to identify critical areas that should be given

high priority for protection and restoration in order to conserve the remaining populations of Phayre's leaf monkey in Myanmar.

4.2 Materials and methods

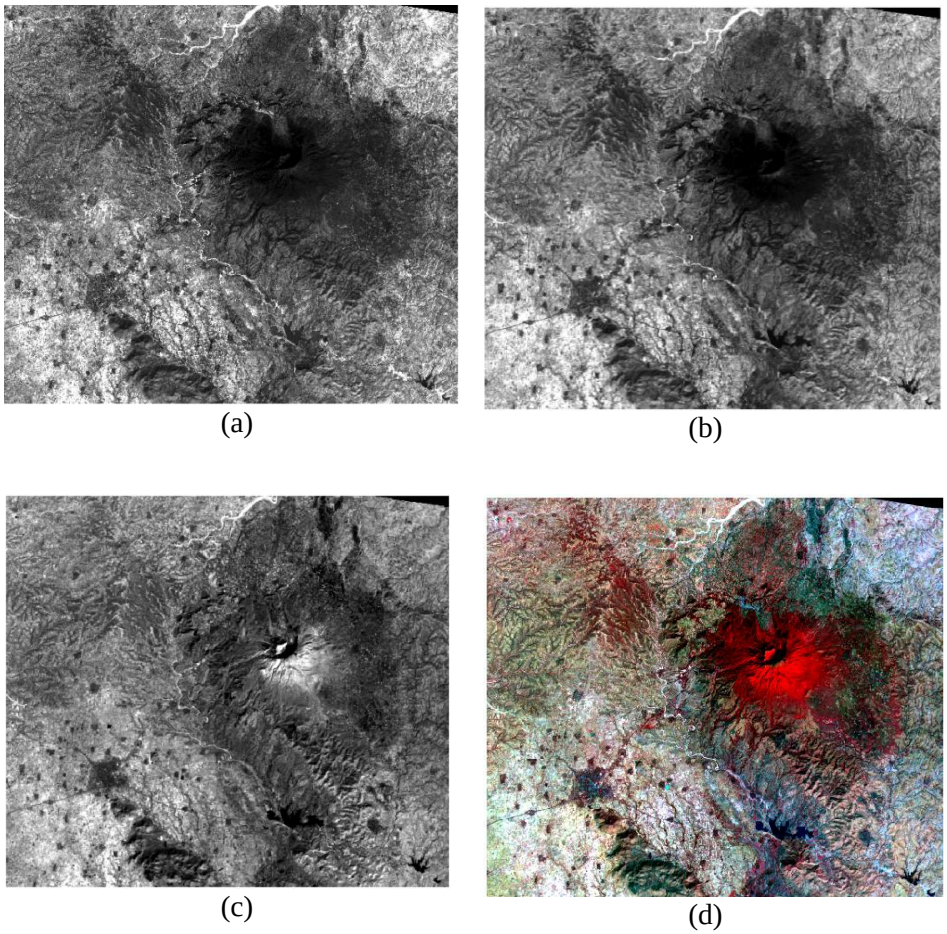
4.2.1 Vegetation types classification

Satellite data

In this study, Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) imagery (path/row: 133/46) acquired on 17 January 2010 was used and there was no cloud coverage for this image. The orthorectified ASTER bands of acquired image were provided at different resolutions. The visible and near-infrared (VNIR) bands 1, 2, and 3N were dilivered at 15 m resolution. The short-wave infrared (SWIR) bands 4, 5, 6, 7, 8, and 9 were dilivered at 30 m resolutions. In this study, only VNIR bands 1, 2, and 3N (**Figure 4.1**) were used as these bands are reflected by the vegeation and captured by the sensors of the satellite (Pettorelli *et al.* 2005a). The image was georeferenced by image-to-image co-registration using the Landsat 7 ETM+ imagery 2006 scence which had already been georeferenced to the coordiante system of the study area in universe transverse mercator-world geodetic system-84 (UTM-WGS 84, Zone 46N).

The image was georeferenced with root mean square error (RMSE) of less than 0.01 pixels by means of a first order polynomial transformation and nearest neighbour resampling. The digital elevation model (DEM) data of shuttle radar topographic machine (SRTM) was downloaded from the website

(http://www .srtm. usgs.gov) and was resampled to 15 m resolution based on a nearest neighbor algorithm in ArcMap 9.3. Eelevation and slope data were extracted from DEM. Image processing was done in ERDAS IMAGINE 9.1® (Leica Geosystems Geospatial Imaging, Wayland, MA, USA).



(a) (b)

(c) (d)

Figure 4.1 Three visible and near-infrared (VNIR) bands of ASTER image used for vegetation cover classification of Popa Mountain Park in this study. (a) Band 1, (b) Band 2, (c) Band 3N and (d) false color composite image (3N, 2, 1 in RGB)

Normalized Difference Vegeation Index (NDVI)

Normalized Difference Vegetation Index (NDVI) was used for measuring and mapping the density of green vegetation (Imam *et al.* 2009). In this study, NDVI was derived using the expression given in the following equation to analyse the forest density of PMP;

$$NDVI = (NIR-R) / (NIR+R)$$

where NIR = Near Infra Red (Band 3N); R = Red (Band 2) for ASTER image. NDVI was calculated in the raster calcular tool in ArcGIS 9 (**Figure 4.2**). The formula is based on the fact that chlorophyll absorbs Red whereas the mesophyll leaf structure scatters NIR (Pettorelli *et al.* 2005a). NDVI values thus range from -1 to +1 where negative values correspond to an absence of vegetation (Myneni *et al.* 1995).

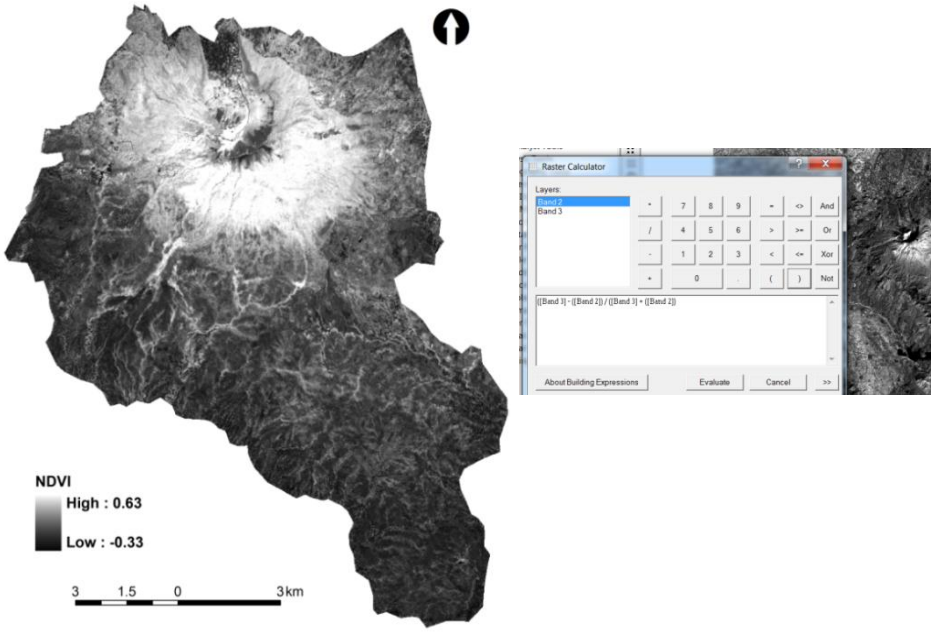


Figure 4.2 Normalized Difference Vegetation Index (NDVI) was used for measuring the forest density that was assumed for suitable habitat of Phayre's leaf monkey. This index was calculated in the raster calculator tool of ArcGIS using the standard equation: NDVI = (Band3N - Band2)/ (Band3N + Band 2). NDVI values thus range from -1 to +1 where negative values correspond to an absence of vegetation. In the map, white color shows higher NDVI value and dark represents lower NDVI value.

Ground vegetation data

Ground vegetation data were collected during June and November 2010. Vegetation types were noted at every possible places for ground reference sites in PMP (**Figure 4.3**) by using global positioning system (GPS 60CS, Garmin International, Thaiwan). The existing ground vegetation points (Thant pers.comm.) were also added for the reference sites. The vegetation information of 46 sample plots from the chapter III were also added. Total reference sites (n = 654) were compiled in this study, and those were evenly divided into training and verification data in the image classification.

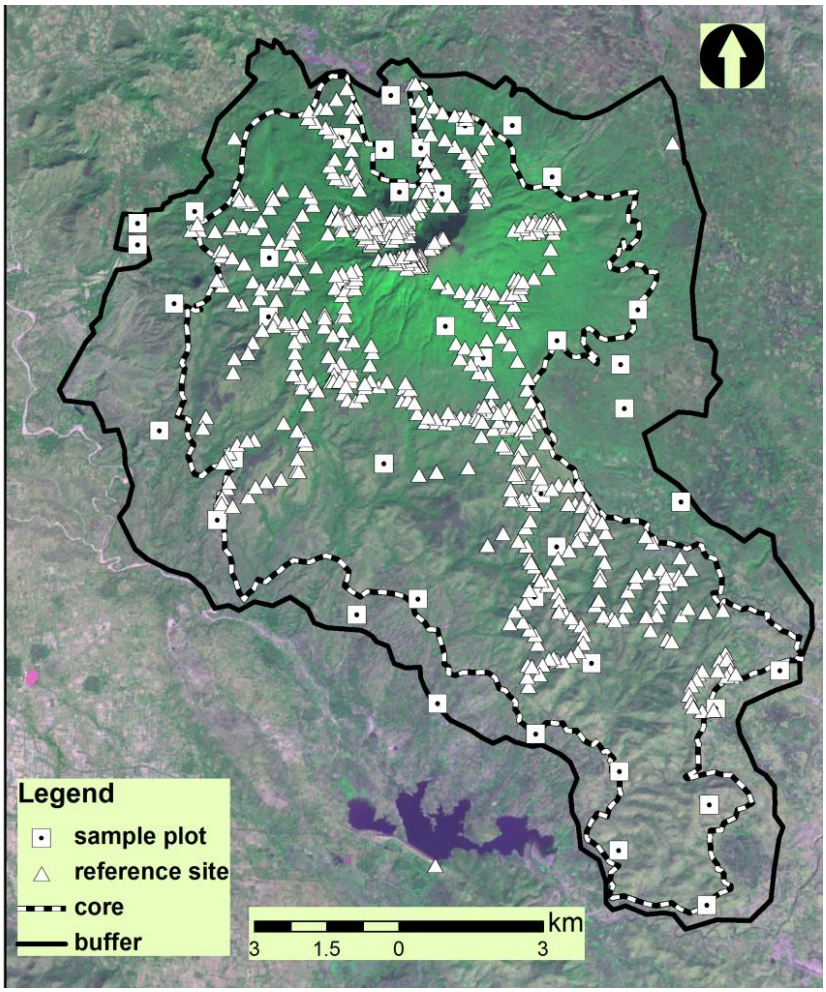


Figure 4.3 Distributin of reference sites (n = 654) in PMP. The reference sites were evenly divided into training (n = 327) and verification data (n = 327) for accuracy assessment of image classification.

Maximum Likelihood Classification (MLC)

Vegetation types of PMP were classified using the maximum likelihood classifier (MLC) which is the most widely adopted parametric classification algorithm (Shalaby and Tateishi 2007, Manandhar *et al.* 2009). The standard supervised classification with MLC was used in ERDAS IMAGINE 9.1. The supervised classification is a procedure for identifying spectrally similar areas on an image by identifying 'training' sites of known targets and then extrapolating those spectral signatures to other areas of unknown targets. Supervised classification relies on the a priori knowledge of the location and identification of land cover types that are in the image (Manandhar *et al.* 2009). This can be achieved through field works, study of aerial photographs or other independent sources of information.

Training areas (reference sites), usually small and discrete compared to the full image, are used to 'train' the classification algorithm to recognize land cover classes based on their spectral signatures, as found in the image. The MLC assumes that the training statistics for each class have a normal 'Gaussian' distribution (Liu *et al.* 2002). The classifier then uses the training statistics to compute a probability value of whether it belongs to a particular land cover category class. This allows for within-class spectral variance. The MLC usually provides more accurate results for image classification (Liu *et al.* 2002, Ismail and Jusoff 2008).

Many studies had reported that the inclusion of ancillary data can potentailly improve the accuracy of classification (Lawrence and Wright 2001,

Rogan *et al*. 2003). Htun *et al.* (2011) also used the ancillary elevation data in the classification of tropical deciduous forests. However, in this study, only standard classification method was used without elevation data as it is directly correlated with the Phayre's leaf monkey preferred habitats in PMP.

In this study, six categories of different land covers were classified (**Table 4.1**) taking into account the spectral characteristics of the satellite image and the existing records of the study area (Htun *et al.* 2011). Evaluation of the collected signatures for each forest type was performed through exploratory analysis of histogram, contingency matrix and computing signatures separability using a given transformed divergence for a distance between signatures. Signatures were recollected if not producing satisfactory results. Thresholding was also done to identify the pixels in the classified image that are most likely to be classified incorrectly. The distance image and output thematic raster layer produced by MLC were used for thresholding. The tails of histograms (pixels that are most likely to be misclassified have the higher distance file values at the tail of the histogram of the distance image) were cut off interactively and saved and the removed pixels were viewed.

Once the collected signatures were comparatively satisfactory, multiple signatures were merged into one signature for the given land cover categories and use for the classification (**Figure 4.4**). The classified image was smoothed using a 3 x 3 cell majority filter in a raster calculator tool of ArcGIS 9 to reduce noise in the data.

Table 4.1 Categories of vegetation types delineated for the supervised classification with maximum likelihood classification (MLC) approach for Popa Mountain Park

Category	Description
Semi-evergreen (Dry-hill)	Vegetation dominated by *Quercus serrata, Carallia brachiata, Trevia nudiflora*
Dry mixed deciduous	Vegetation dominated by *Tectona grandis, Xylia xylocarpa, Vitex limonifolia*
Scrub indaing	Vegetation dominated by *Dipterocarpus tuberculatus, Shorea obtusa, Pentacme siamensis*
Shrub/bush	Vegetation with shrubs, bush and farm land
Than-dahat	Vegetation dominated by *Terminalia oliveri, Jatus hamiltonianus, Acacia catechu*
Grass	Grass land

Source: FD 1981, Kress *et al*. 2003, Htun *et al.* 2011, Thant *et al.* (in press)

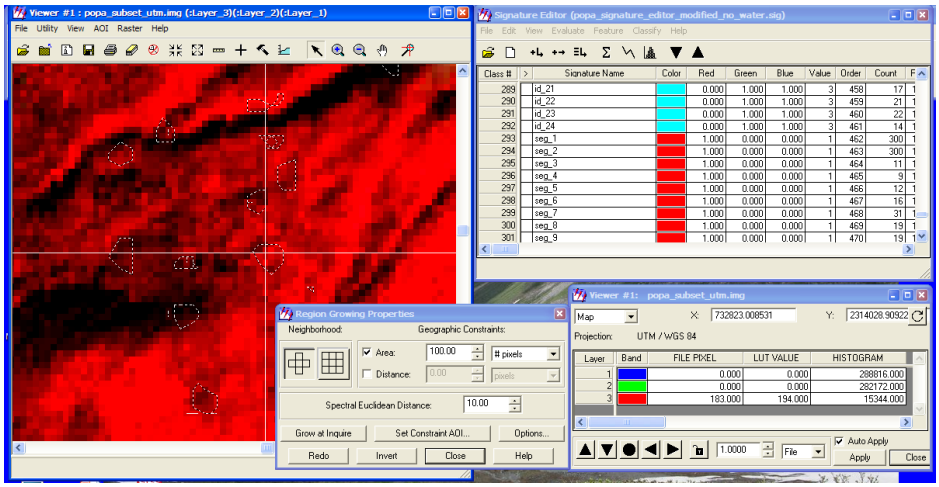


Figure 4.4 An example of taking training sets (reference sites) for different vegetation types by using the signature editor tool in ERDAS IMAGINE 9.1. Each forest types was assigned by different colors in order to be convenient for image classification procedures. In the signature editor tool box, red color represented semi-evergreen (dry hill) forest and sky blue for Indaing forest.

Accuracy assessment

The most widely promoted classification accuracy is in the form of error matrix (**Table 4.2**) which can be used to derive a series of descriptive and analytical statistics (Congalton 1991, Foody 2002). Traditionally, the total number of pixels in a category is divided by the total number of pixels of that category as derived from the reference data (i.e. the column total). This accuracy measure indicates the probability of a reference pixel being correctly classified and is really a measure of omission error. This accuracy measure is often called 'producer's accuracy' because the producer of the classification is interested in how well a certain area can be classified (Congalton 1991). On the other hand, if the total number of correct pixels in a category is divided by the total number of pixels that were classified in that category, then this result is a measure of commission error. This measure, called 'user's accuracy', is indicative of the probability that a pixel classified on the map/ image actually represents that category on the ground (Story and Congalton 1986).

In the example of deciduous forest, a 'producer's accuracy' was derived by dividing the total number of correct pixels in the deciduous category (65) by the total number of correct pixels in column (75). This division results in a 'producer's accuracy' of 87 %, which is quite good. But for calculation of a 'user's accuracy' computed by dividing the total number of correct pixels in the deciduous category (65) by the total number of pixels in the row (115) reveals a value of 57%. These can be explained that the producer of this map can claim that 87 % of the time an area that was

deciduous was identified as such, but, a user of this map will find that only 57% of the time will an area he visits that the map says is deciduous will actually be deciduous.

The other way to identify the classification accuracy is the kappa coefficient $\kappa \in [-1, +1]$ (Congalton 1991). The kappa coefficient is $\kappa = 0$ for the pure coincidence between two totally random classifications, and reaches $\kappa = 1$ for complete agreement between classification and ground truth. Paul (1991) recommended that a standard of 85% accuracy is acceptable level of digital image classification; however Story and Congalton (1986) advocated the inclusion of error matrices to enable users to compute and interpret the value on their own. On the other hand, the classified map can be reasonably used for further analysis when kappa coefficient is between 0.4 and 0.7 (Ismail and Jusoff 2008).

Table 4.2 An example of Error Matrix. Detailed explanation was given in the text

	Reference data				row
	D	**C**	**BA**	**SB**	**total**
D	65	4	22	24	115
C	6	81	5	8	100
BA	0	11	85	19	115
SB	4	7	3	90	104
column total	75	103	115	141	434

Overall Accuracy = 321/434 = 74%

Land Cover Categories	Producer's accuracy	User's accuracy
D = deciduous	D = 65/75 = 87%	D = 65/115 = 57%
C = conifer	C = 81/103 = 79%	C = 81/100 = 81%
BA = barren	BA = 85/115 =74%	BA = 85/115 = 74%
SB = shrub	SB = 90/141 = 64%	SB = 90/104 = 87%

Source: Adopted from Congalton (1991).

4.2.2 Topographic and disturbance data extraction

Geo-referencing topographic map

The topographic map of the study area (SD 2003) was scanned and exported to ERDAS IMAGINE 9.1 for geo-referencing to sub-pixel accuracy (**Figure 4.5**). The well distributed ground control points (GCP) were marked with root mean square error of one-third of a pixel and the image was re-sampled using the nearest neighborhood method. This data was re-projected into UTM-WGS 84 for further analysis. A study area of interest (AOI) was built around the park boundry to produce a rectilinear map. Information on drainages, roads, settlements and park boundaries were delineated from the topo map (**Figure 4.6**).

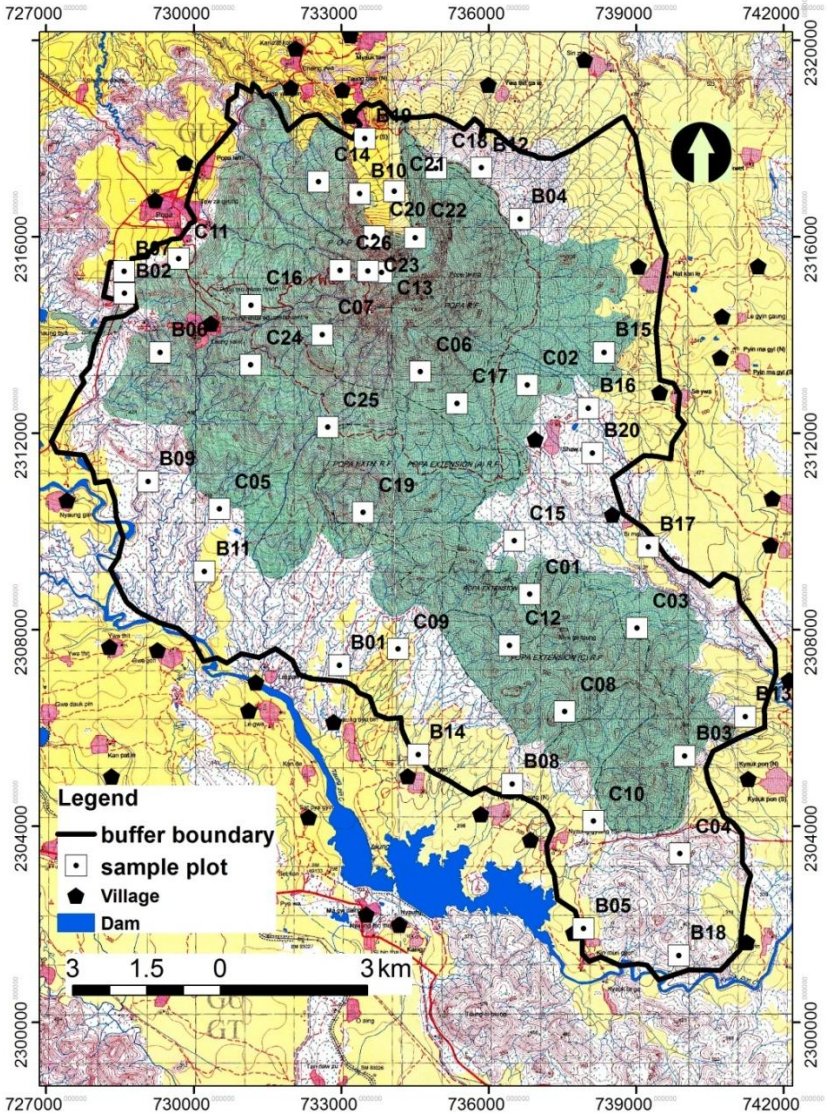


Figure 4.5 Topographic map of Popa Mountain Park. Projection in Universal Transverse Mercator (UTM), scale 1:15000. Black line is buffer boundary and the green polygon is core area. Information about human settlements, roads and park's boundaries were extracted from this topo map. Distances between the sample plots and villages were measured.

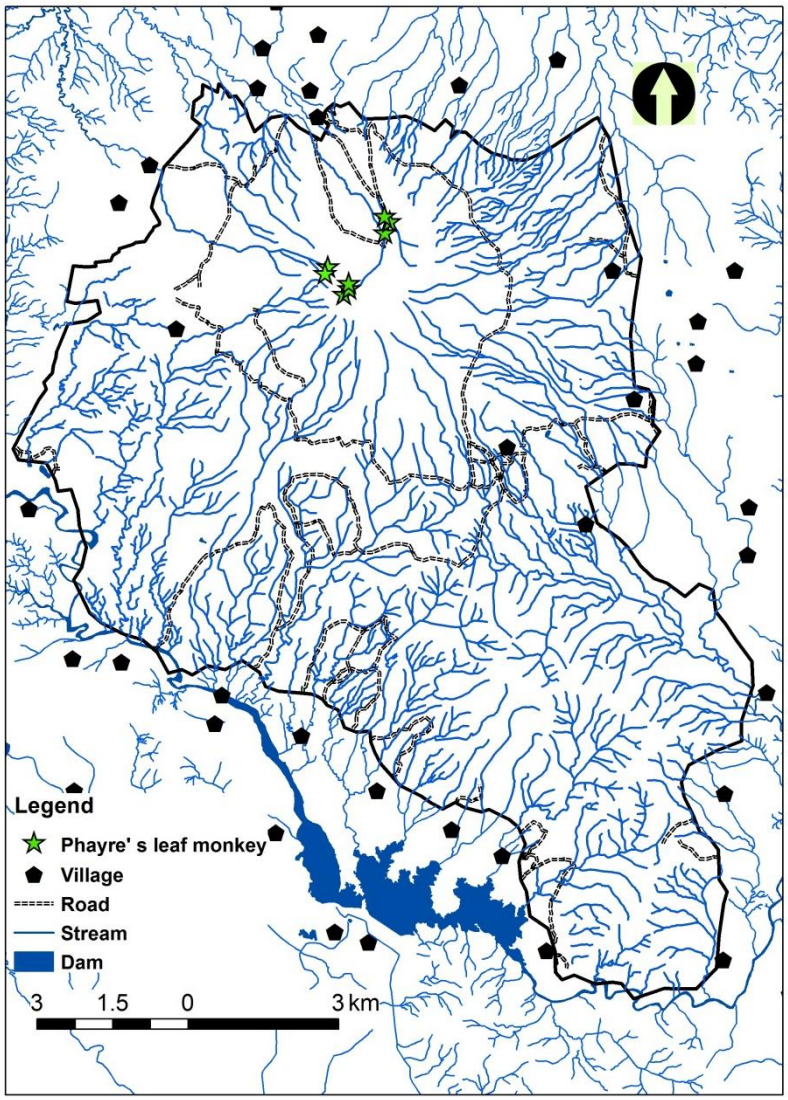


Figure 4.6 The profile of streams, roads, and villages delineated from the topographic map. Popa Mountain Park stands as main water resources for the Kyetmauk-Taung Dam in the south. Distribution of Phayre's leaf monkey restricted to the north even close to the mountain circular road which was not frequently used.

Elevation and slope

Three groups of monkeys (mean: 41 ± 0.67, 41 ± 0.58 and 21 ± 0.67 individuals for group-1, group-2, and group-3, respectively) were assessed during the study and they mainly distributed at higher elevation in the north (**Figure 4.6 and 4.7**). Distributions were loacted using GPS for places that could be assessed. However, for the monkey groups inhabited higher steep terrains, the distances between observers and monkeys were measured using the laser range finder (Bushnell, Japan) and directions were recorded by the field compass (Silva, Swedan). Then, elevation (m) and location (lat/lon) of monkeys were estimated using the geo-referrenced map of the study area in ArcGIS 9. Information about slope and aspects were not recorded during the field study since these data could be more accurately derived from the DEM (Wang *et al.* 2008).

Anthropogenic disturbances

The disturbance data (tree cutting, farming) collected at 46 vegetation sample plots (Chapter III) were used in this analysis and the distances to village were checked again with the geo-referenced topographic map (**Figure 4.5**). The sample plots associated with the diffferent disturbance intensities for high disturbance (impact factor: 1.56 ± 0.08, n = 18), intermediate disturbance (impact factor: 0.93 ± 0.07, n = 14), low disturbance (impact factor: 0.40 ± 0.38, n = 14) were plotted on the DEM elevation map (**Figure 4.7**). The distance to the sample plots represented high disturbance level was included as a variable in the habitat suitability analysis.

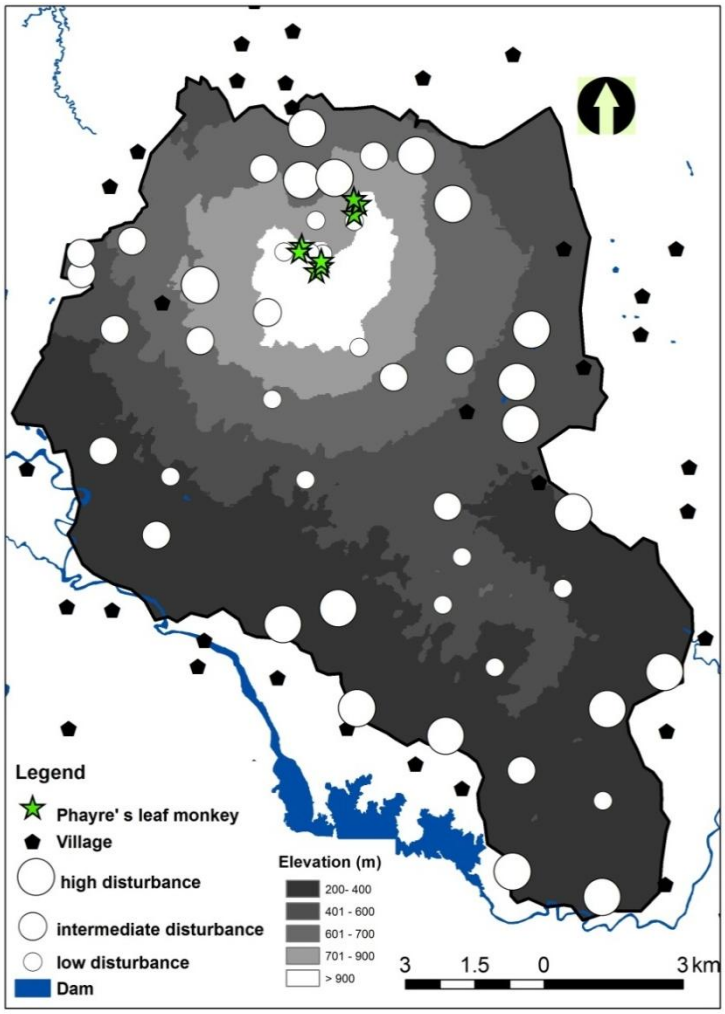


Figure 4.7 Digital elevation model (DEM) map showing the distribution of Phayre's leaf monkey in Popa Mountain Park. Three groups of monkeys were assessed in the northern site at higher elevation (> 600 m). Sample plots (n = 46) associated with different disturbance intensities (high disturbance: biggest open circle, n = 18; intermediate disturbance: medium size open circle, n = 14; low disturbance: smallest open circle, n = 14) were plotted on the map.

4.2.3 Modelling habitat suitability of Phayre's leaf monkey

Variables Selection

Environmental habitat variables of food, cover, and reproduction requirements are major components for life requisites for wildlife and its habitat (Bolen and Robinson 2003). Most of habitat modelling studies used several habitat variables for bio-geographical factors (elevation, slope, aspect, water source, vegetation, etc.,) and human interaction factors (village, roads, agriculture, hunting, etc.) (Park and Lee 2003, Wang *et al*. 2008, Podchong *et al*. 2009).

However, the selection of varaibles varies to the behavior of species and the habitat characteristics they live in. In this study, expert experiences and previous knowledge as well as literature reviews were used to quantify the relationship between habitat variables and suitability index for Phayre's leaf monkey. Seven major habitat requirements: 1) forest types, 2) elevation, 3) slope, 4) distance to disturbances, 5) distance to roads, 6) distance to streams, and 7) forest density were used for modelling habitat suitability of Phayre's leaf monkey. The required data layers were extracted using the GIS datasets (**Table 4.3**). The main human interferences such as distance to village, tree cutting, farming were inclusive in calulation of disturbance impacts (**session 3.2.3**). Thus, in this analysis, the information about distance to the sample plots associated with different disturbance levels were used. In addition, distance to the roads and streams were also considered as the suitable habitat varaibles in this analysis. Phayre's leaf monkey in PMP travelled all aspects (Thant, pers. obser.) that was not considered in this anlysis.

Table 4.3 Environmental habitat variables (n = 7) related to Phayre's leaf monkey in Popa Mountain Park

Habitat variable	GIS dataset
Vegetation types	ASTER image
Slope (degree)	DEM
Elevation (m)	DEM
Distance to disturbances (m)*	GPS, Topo map
Distance to roads (m)	Topo map
Distance to streams (m)	Topo map
Forest density (NDVI)	ASTER image

Note: *distance to disturbances were related with three disturbance factors: tree cutting, farming, and distance to villages. ASTER: Advanced Spaceborne Thermal Emission and Reflection Radiometer, DEM: Digital Elevation Model, GPS: Global Positioning System, NDVI: Normalized Difference Vegetation Index related with reflection of vegetation cover

Suitability Index (SI) variables

Phayre's leaf monkey often resides in semi-evergreen and mixed deciduous primary and secondary forests (Roonwal and Mohnot 1977, Gittins and Akonda 1982, Cantwell 2011). Thus, semi-evergreen (dry hill) and dry mixed deciduous forests were selected as food requsite suitability index (SI_1) in this study. Phayre's leaf monkeys mainly distributed at higher elevation in the north of PMP (**Figure 4.7**). Elevation and slope data related with the distribution of Phayre leaf monkeys were estimated using field data and DEM map (**Table 4.4).** The monkeys travelled at the average elevation of about 1,000 m (min = 900 m, max = 1,181 m) during this study. However, the monkeys used to come down lower altitude (approximately 600 – 700 m) to find the foods inside the valley of PMP (Thant pers.comm.). Similarly, in Laos, Phayre's leaf monkeys inhabit mostly around 500 m and often up to 1,000 m at the ranges of limestone karst (Timmins *et al*. 2011). Therefore, elevation varible was selected as habitat suitability index (SI_2) in this analysis. Phayre's leaf monkeys were historically recorded to the higher slopes (Pocock 1939) of Mt. Popa and the recent observations also confirmed it (Thant pers. obser.). The monkeys were found at higher slope of average 28.78 degree (min = 8.16°, max = 49.58°). Thus, the slope terrain was selected for habitat suitability index (SI_3) for HSI model.

Table 4.4 Elevation and slope data related to the distribution of three groups of Phayre's leaf monkey in Popa Mountain Park. Three replications were conducted to assess each group while the monkeys moved one place to another.

Monkey	Elevation (m)	Slope (degree)
group_1	909	31.91
	1,018	27.09
	978	36.23
group_2	1,011	15.22
	969	8.16
	974	38.52
group_3	1,049	49.58
	1,181	28.06
	900	27.45
Average	**1,011**	**28.78**

Note: During the survey for population count (three consecutive days in June 2010), average elevation where the monkey distributed was about 1,000 m. But monkeys used to come down lower altitude to find foods particularly fruiting seasons of *Protium serratum*, a deciduous trees found at lower altitude about 600 - 700 in the crater of PMP (Thant, pers. comm.).

Phayre's leaf monkey is extremely shy and take flight when approached by humans who are main predators (Gupta 2001, Cantwell 2011). Thus, in this study, human disturbance was considered as suitability index (SI_4) and the distances to the sample plots with highest disturbance level were included in the habitat suitability analysis. The impacts of roads were inclusive for modelling habitat suitability of a given species particularly for those associated with the human interferences (Podchong *et al*. 2009, Belda *et al.* 2012, Reza *et al.* 2013). Since the PMP is surrounded by many villages, human interferences on the park management were unavoidable (Rao *et al.* 2002). There was a mountain circular road constructed for park patrolling purpose but it was not frequently used. However, the other roads concentrated at the southwest of the PMP (**Figure 4.6**) were frequently used by the local people to collect fuel woods and non-wood forest products (Thant per. obser.). Consequently these human disturbances may affect the survival of Phayre's leaf monkey. Thus, the distance to roads was considered as suitability index (SI_5) in this analysis.

Roonwal and Mohnot (1977) reported that Phayre's leaf monkey used to inhabit bamboo forests along hillsides as well as along the banks of streams in Myanmar. Timmins *et al.* (2011) also recorded that Phayre's leaf monkey in Lao mostly inhabits in the broken canopy semi-evergreen forests near the rivers and some troops were found within 200 m of a stream. In PMP, many water drainages were found in the park but the avilability of water is not persisted year round. PMP stands as main water resources for the Kyetmauk-Taung Dam in the south (**Figure 4.6**). There are 12 significant size of natural

springs in or around the park and one perennial stream, the Kyaukpon creek flowing from the east into the dam (FD 1981). Although the monkeys in PMP rarely used the water sources (Thant per. comm.), they perhap need water sources from streams likewise other region. Thus, the stream profiles of PMP were selected as a suitability index (SI_6) in this analysis.

On the other hand, NDVI was used for measuring and mapping the density of green vegetation (Imam *et al*. 2009) and also it could be used into trophic inter-linkages (Pettorelli *et al*. 2005a). For instance, the home range of the grivet monkey (*Cercopithecus aethiops*) revealed a significantly higher average NDVI value compared with the average of the survey area in Eritrea (Zinner *et al*. 2002). Therefore, in this study, NDVI value was selected as suitability index (SI_7) to measure forest density.

Habitat Suitability Index (HSI) model

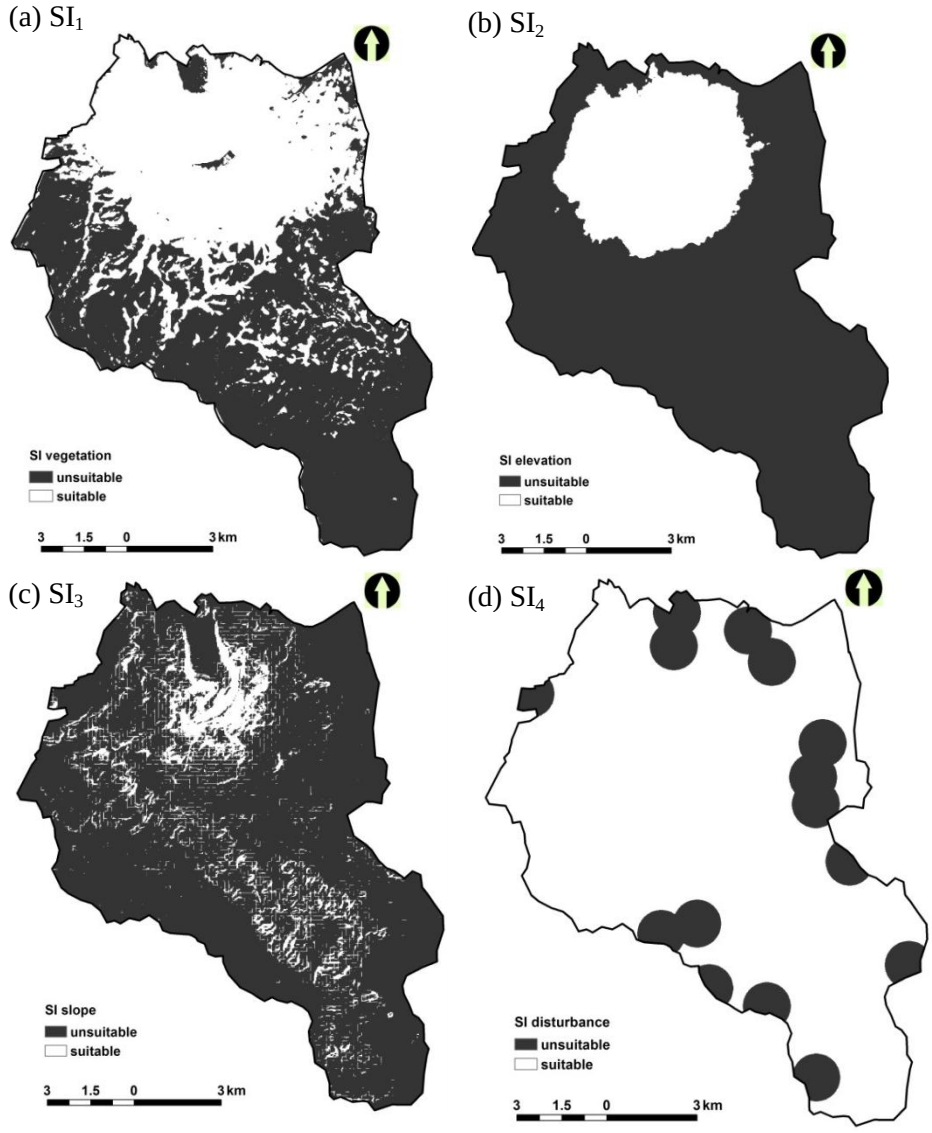
In this study, HSI model for Phayre's leaf monkey was a function of seven variables (**Table 4.5**). Using the thematic map of vegetation types, the first SI indicated that cells dominated by semi-evergreen (dry hill) and dry mixed deciduous forests provided suitable habitat (i.e., $SI_1 = 1$). All other cells were assigned $SI_1 = 0$. The second SI represented for elevetion and the value of SI_2 was 0 when a cell was $\leq$ 600 m and the other cells were assigned $SI_2 = 1$. The third SI reflected slope and a cell indicated < 20° not provide for suitable habitat (i.e. $SI_3 = 0$) and the other cells were assigned $SI_3 = 1$. The fourth SI refelected the intensity of human disturbances avoided by Phayre's leaf monkey. Assuming that Phayre's leaf monkeys were reluctant to inhabit within 800 m from a highest disturbance plot, the value of SI_4 was 0 when a cell was $\leq$ 800 m to the disturbance. The others were assigned $SI_4 = 1$. No evidence of the monkey close to the roads were recorded in PMP during the survey. Thus, Phayre's leaf monkey in PMP was assumed that not visiting within 200 m from the roads, the value of SI_5 was 0 when a cell was < 200 m from the roads and the others were assigned $SI_5 = 1$. Assuming that Phayre's leaf monkey prefers to reside within 100 m from the water drainages, the value of SI_6 was 1 when the cells indicated < 100 from the water drainages (i.e $SI_6 = 1$) and the other cells were assighed as 0. NDVI value for tropical dry mixed deciduous was assumed greater than 0.2 (Htun *et al.* 2011). In this study, the seventh suitability index (SI_7) reflected forest density and the index was 0 when a cell was < 0.2 of NDVI. The other cells were assigned $SI_7 = 1$.

Table 4.5 Habitat requisites, model parameters and implementation, source and HSI equation used in the modelling habitat suitability of Phayre's leaf monkey in Popa Mountain Park

Habitat requisites	Model parameters and implementation	Source	HSI equation
Vegetation types	SI_1: thematic land cover map (semi-evergreen, dry mixed deciduous)	Roonwal and Mohnot 1977, Gittins and Akonda 1982, Cantwell W 2011	$(SI_1 + SI_2 + SI_3 + SI_4 + SI_5+SI_6+SI_7) / 7$ (Larson *et al.* 2003).
Elevation	SI_2: elevation > 600 m	Timmins *et al*. 2011, Own observation	
Slope	SI_3: slope $\geq 20^{\circ}$	Own observation	
Distance to disturbances (tree cutting, farming, distance to village)	SI_4: distance > 800 m	Own observation	
Distance to road	SI_5: distance > 200 m	Own observation	
Distance to stream	SI_6: distance < 100 m	Timmins *et al*. 2011, Own hypotheses	
Forest density	SI_7: NDVI $\geq$ 0.20	Htun *et al*. 2011	

All raster layers of the selected variables were reclassified to SI maps using spatial analyst tool in ArcGIS 9. Vegetation suitability (SI_1) map was created using the thematic vegetation map (**Figure 4.10**). DEM was used to delineate SI_2 and SI_3 maps for suitable elevation and slope data, respectively. For human disturbances index (SI_4) map, the euclidean distance to the highest disturbances was delineated using the Euclidean tool in ArcGIS 9. Similarly, the euclidean distance to the roads (SI_5) and the water drainages (SI_6) were also created. The NDVI raster layer (**Figure 4.2**) was recalssified to forest density index (SI_7) map. All created suitable index maps (SI_1: vegetation layer, SI_2: elevation layer, SI_3: slope layer, SI_4: disturbance layer, SI_5: road layer, SI_6: stream layer, SI_7: forest density-NDVI) were shown in **Figure 4.8a-g.**

The habitat suitability map was prepared according the steps given in the **Figure 4.9.** The overall HSI map was calculated using the arithmetic means equation (i.e $SI_1 + SI_2 + SI_3 + SI_4 + SI_5 + SI_6 + SI_7 / 7$) of the generic model provided in the landscape HSI models Version 2.1 (Larson *et al.* 2003). Suitability index was set within the range of 0 to 1 from unsuitable to highly suitable, respectively (Larson *et al.* 2003, Park and Lee 2003). In the habitat suitability map, individual pixels of the index map was categorized into five classes of 1: unsuitable (0.0 – 0.2); 2: least suitable (0.2 – 0.4); 3: moderately suitable (0.4 – 0.6); 4: suitable (0.6 – 0.8); and 5: highly suitable (0.8 – 1.0).


(a) SI1
SI vegetation
unsuitable
suitable
3 1.5 0 3 km
(b) SI2
SI elevation
unsuitable
suitable
3 1.5 0 3 km
(c) SI3
SI slope
unsuitable
suitable
3 1.5 0 3 km
(d) SI4
SI disturbance
unsuitable
suitable
3 1.5 0 3 km

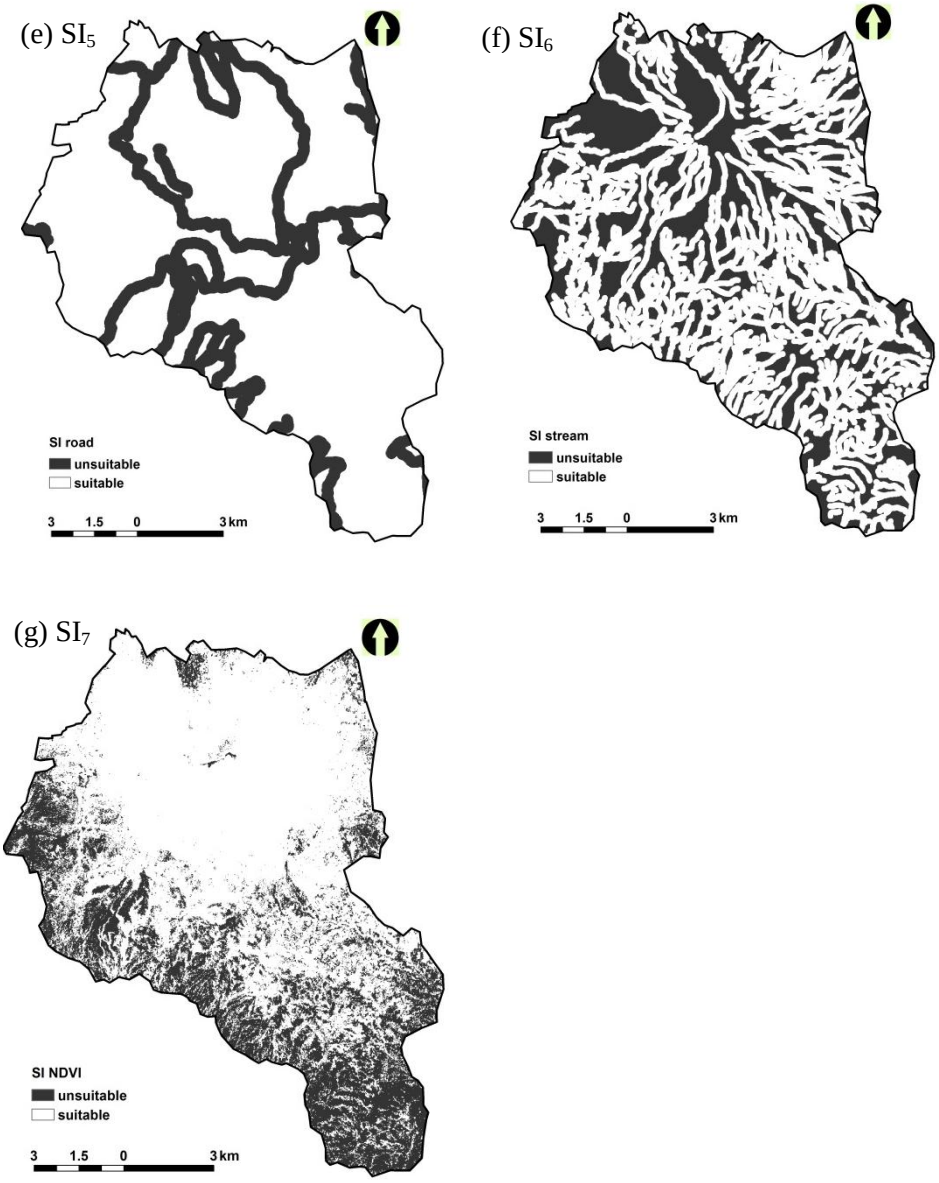


Figure 4.8 Application of the Phayre's leaf monkey Habitat Suitability Index (HSI) model to 150.81 km^2 unit of Popa Mountain Park. There are separate images for suitability habitat of vegetation types (a: SI_1), elevation (b: SI_2), slope (c: SI_3), distance to human disturbances (d: SI_4), distance to road (e: SI_5), distance to stream (f: SI_6), forest density-NDVI (g: SI_7)

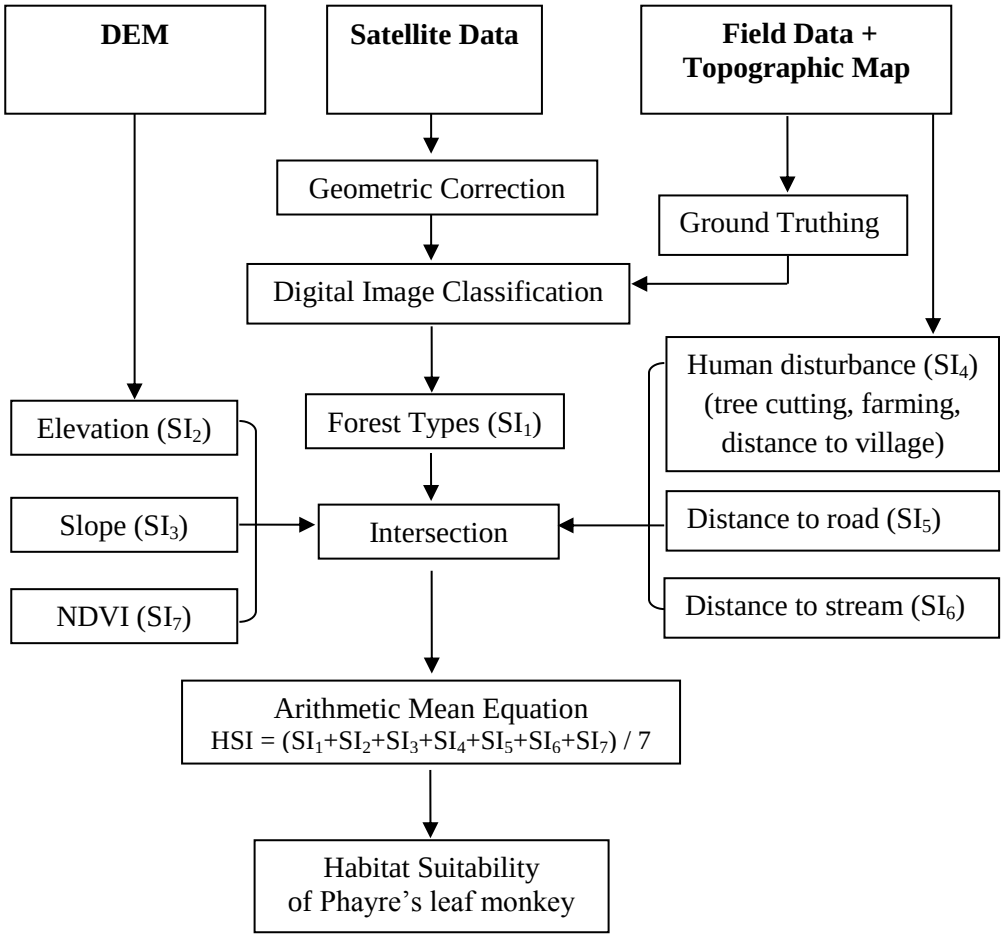


Figure 4.9 Flow chart of modelling habitat suitability of Phayre's leaf monkey in Popa Mountain Park. DEM: Digital Elevation Model, HSI: Habitat Suitability Index, NDVI: Normalized Difference Vegetaion Index, SI: Suitability Index

4.3 Results and discussion

4.3.1 Spatial extent of vegetation types generated by maximum likelihood classification (MLC) approach

Thematic vegetation-type map produced by MLC approach is presented in **Figure 4.10**. The MLC approach identified dry mixed deciduous forest occupying about 39% of the total area of the park, followed by than-dahat forest representing 34%. Scrub-indaing forest and shrub/bush equally occupied about 10% each of total area. Semi-evergreen (dry hill) and grass lands were identified as the smallest areas, 4.2% and 0.54%, respectively (**Table 4.6**). The results in the current study are supportive of the the previous study (Htun *et al*. 2011) that also reported highest occupancy of dry mixed deciduous forest while semi-evergreen and grasslands had lowest occupancy. However there was a big difference for than-dahat forest (34% versus 27% for this study and previous study, respectively). This is possibly , in this study, due to the inclusion of buffer area in which than-dahat forest is highly dominated. But, buffer area was not considered in the classification of previous study (Htun *et al.* 2011).

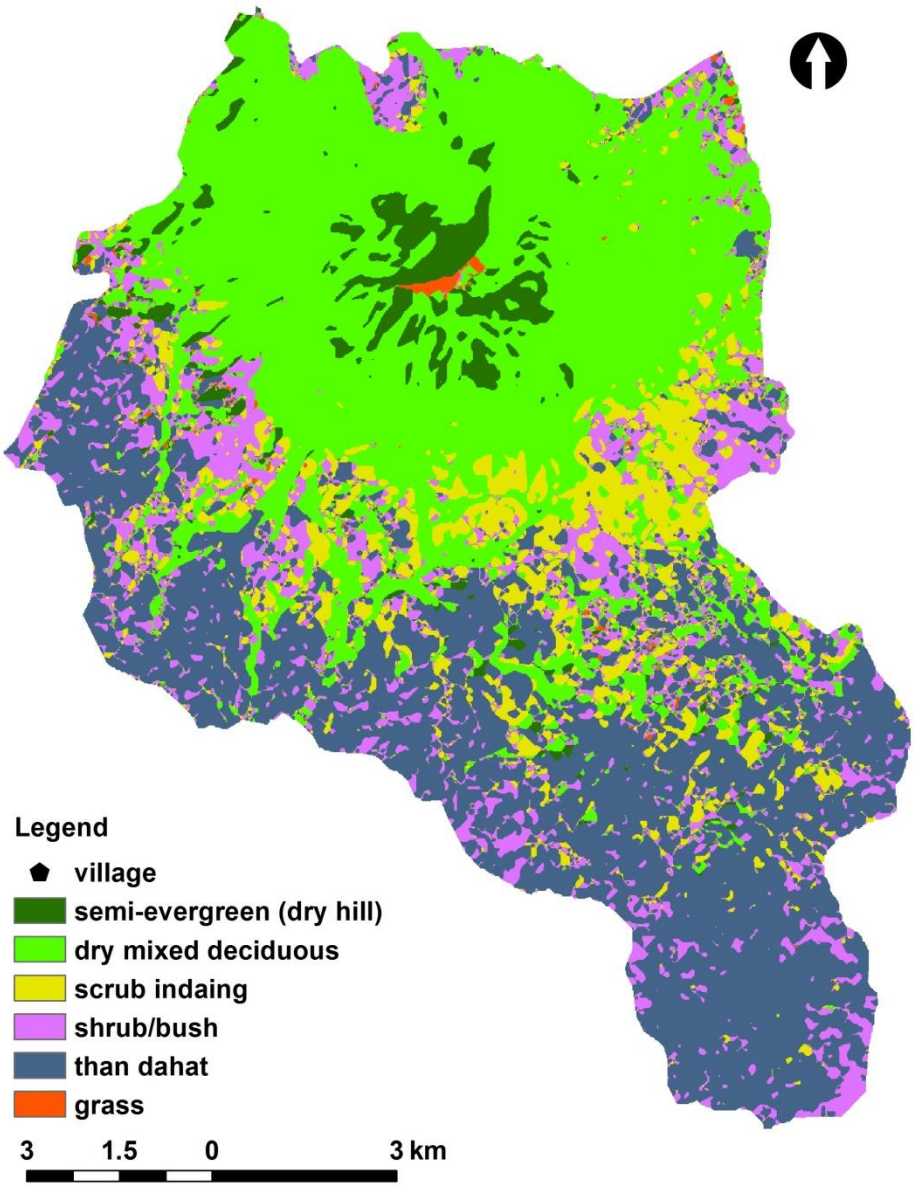


Figure 4.10 Vegetation cover map produced by maximum likelihood classification (MLC) approach for Popa Mountain Park. Vegetation covers were classifed into six types. Dry mixed deciduous forest was classified as largest area (39%) of the total area and grass lands were classified as smallest area (0.54%).

The overall accuracy of classification showed 76.6% and kappa statistics was 0.66 (**Table 4.6**). The results of current study were better than that of the previous study (Htun *et al.* 2011) who used MLC approaches using different methods including the ancillary elevation data to improve accuracy. This is possibly due to the advantages of using high resolution satellite image (ASTER image, 15 x 15 m) in this study while the previous study (Htun *et al*. 2011) had been analysed using Landsat image of 30 m resolution. The result of the current study is in agreement with the previous study that had reported a better land cover classification performance with ASTER data than Landsat TM data in the moist tropical forests of Brazilian Amazon (Lu *et al.* 2005).

Table 4.6 The results of classification accuracy, area (km^2) and area percentage of vegetation types produced by maximum likelihood classification (MLC) approach for Popa Mountain Park

Vegetation Type	**Producer's Accuracy (%)**	**User's Accuracy (%)**	**Area**	
			km^2	**%**
Semi-evergreen (dry hill)	34.48	76.92	6.34	4.20
Dry mixed deciduous	83.15	88.62	58.99	39.11
Scrub indaing	100.00	64.86	15.41	10.22
Shrub/bush	62.26	89.19	17.99	11.93
Than-dahat	96.77	65.22	51.28	34.00
Grass	50.00	54.55	0.81	0.54
			150.81	100.00
Overall Accuracy = 76.76 %		**Kappa Coefficient = 0.66**		

4.3.2 Habitat suitability of Phayre's leaf monkey

Habitat suitability map of Phayre's leaf monkey was presented in **Figure 4.11**. The results revealed that about 5.28% (7.95 km^2) of PMP was highly suitable for Phayre's leaf monkey and 12.63% (19.02 km^2) for suitable, 23.54% (35.46 km^2) for moderately suitable, and 26.31% (39.65 km^2) for least suitable, whereas 32.24% (48.58 km^2) were analysed as unsuitable habitat.

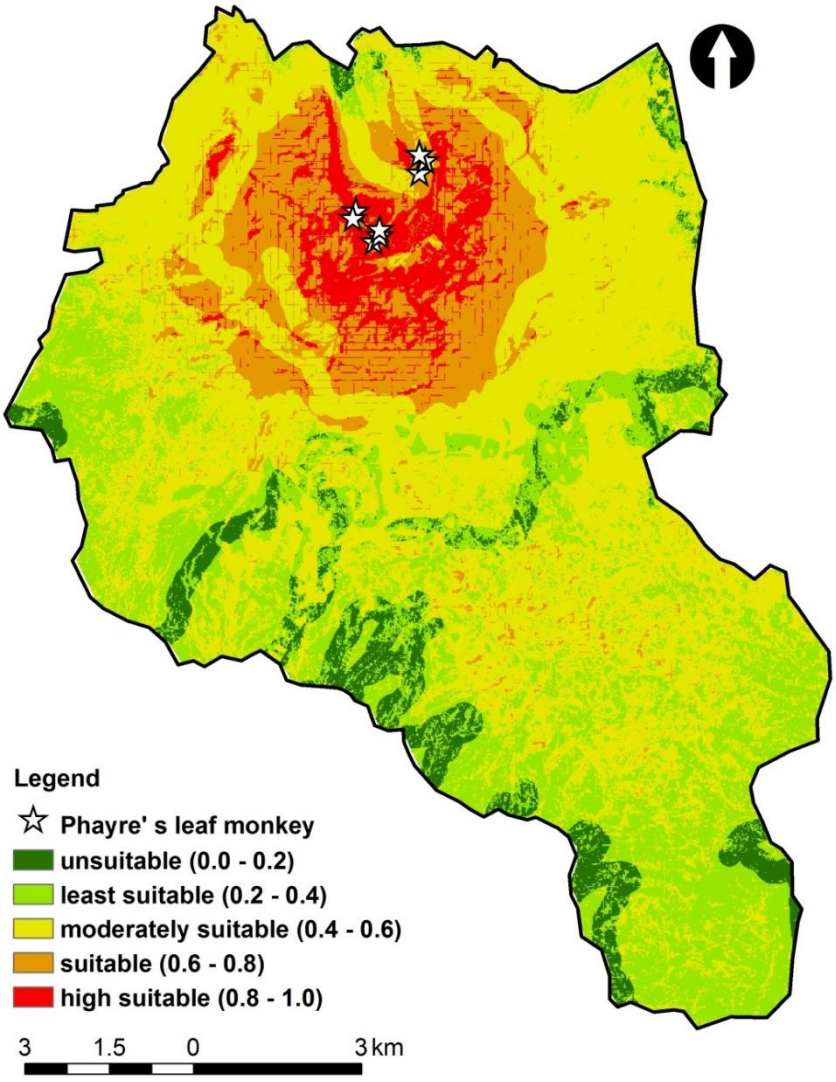


Figure 4.11 Habitat suitability map for Phayre's leaf monkey in Popa Mountain Park. Suitability index was divided into five categories from unsuitable to high suitable (0.0 – 1.0). Suitable habitats were found in the north and actual distribution of the monkeys was also restricted to the northern part.

According to the model, the habitats which were highly suitable for Phayre's leaf monkey laid in the northern part of the PMP. The predictive performance of the model could be implied in reality because the actual distribution of the monkeys were also concentrated in the northern part (**Figure 4.11**). Moreover, the 'suitable habitat' was also lying in the northern part. This is possibly due to dense vegetation cover which reflects high NDVI values in the northern part of PMP (**Figure 4.12a**). Dense forests play a vital role for life requisites of most of animals particularly herbivores. Zinner *et al.* (2004) had reported that the home range of the grivet monkey (*Cercopithecus aethiops*) revealed a significantly higher average NDVI value compared with the average of the survey area in Eritrea, Africa. Similarly, bodymass and calf survival of reindeer (*Rangifer tarandus*) was positively related with NDVI (Pettorelli *et al*. 2005b) and the expansion rate of roe deer (*Capreolus capreolus*) was significantly low in healthy vegetation area (Andersen *et al.* 2004). Moreover, semi-evergreen forests and dry mixed deciduous forests in which Phayre's leaf monkey often resides (Roonwal and Mohnot 1977) mostly occupied in the northern part of PMP (**Figure 4.12b**).

On the other hand, the unique topographical characteristics of PMP possibly favor the habitat suibility to the north. Mt. Popa is an extinct volcanic mountain and its slope rising gently at first and then steeply towards the north. The mountain was opened up to the north during the eruption and thus forming a deep valley with steep terrains to the eastern, southern and western rims (**Figure 4.12c**). Difficult access to reach this particular steep terrains and

high altitude can make little disturbances to natural habitats in which Phayre's leaf monkey prefers to reside (**Figure 4.12d**). Moreover, steep terrains possibly provide suitable habitats for Phayre's leaf monkey for sheltering as the monkeys used to cimb up the steep terrains in order to avoid heavy rain and wind (Thant, pers. obser.)

Mostly 'suitable habitats' were lying in the north and some fragmented ones found along the southern ranges where dry-mixed deciduous forest occupied. The 'moderately suitable habitat' was also mostly lying in the northern part of PMP. The major portion of the PMP was undergo the categories of 'least suitable habitat' and 'unsitable habitat' particularly in the south and south-west where than-dahat forests mostly occupied and these forest types may not provide food for Phayre's leaf monkey. Moreover, human settlements (about 17 villages) were concentrated around the water dam located southern foothill of PMP. Consequently southern part of the PMP possibly being seriously affected by human disturbances that were avoided by Phayre's leaf monkey.

Based on the results of habitat suitability model, the percentage of high suitable area was relatively low compared with the whole study area. However, this study also revealed that other potential areas particularly in the north. Therefore, the park authority is suggested to consider the habitat restoration at the other potential areas while giving the higher protection measures to the critical habitats of the monkeys.

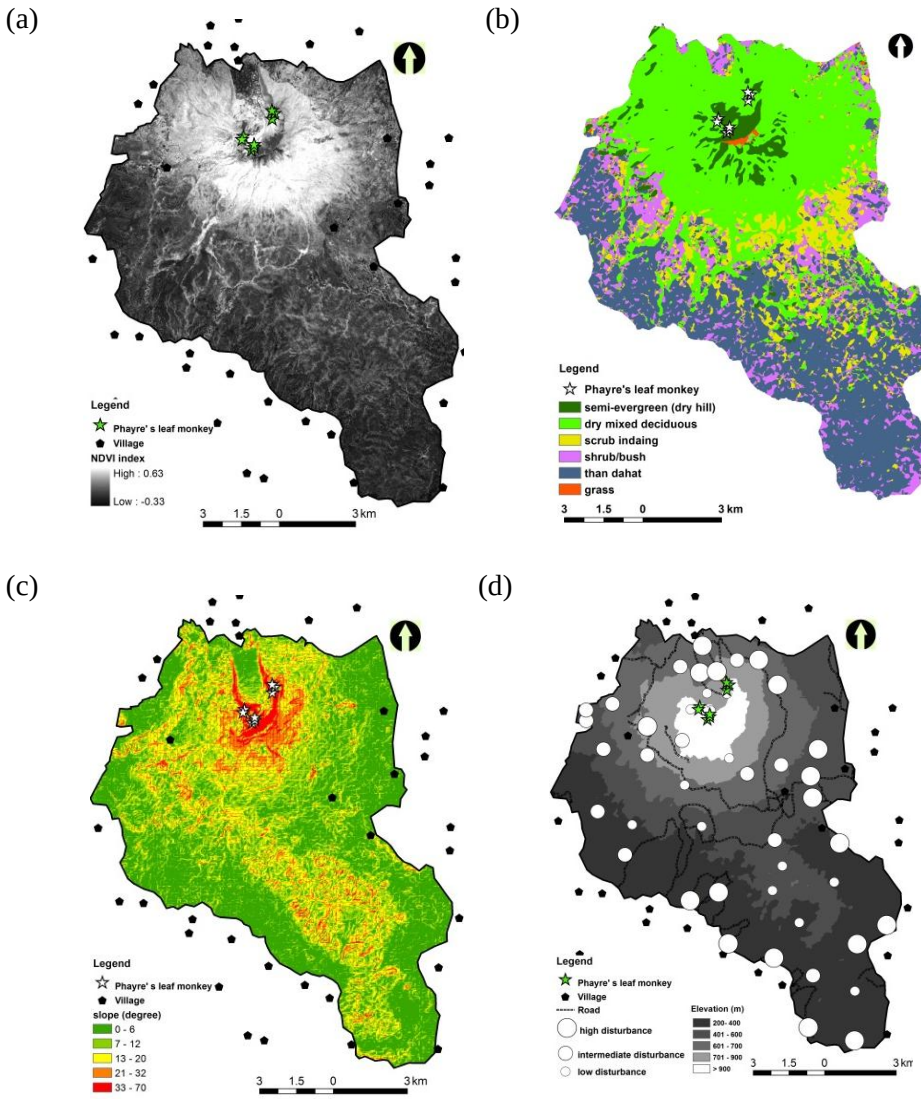


Figure 4.12 Actual distribution of Phayre's leaf monkeys related with (a) high forest density (high NDVI value), (b) forest types (semi-evergreen), (c) steep slope (> 20°) and (d) high elevation with less disturbances

4.4 Conclusion

In this study, the maximum likelihood classification (MLC) approach revealed high accuracy of vegetation cover classification for Popa Mountain Park. The result supports that using high resolution satellite image improves the efficiency and accuracy for mapping forest types although none of ancillary data was included in the classification. Suitable habitats for Phayre's leaf monkey were found in the northern part where food resources were more abundant rather than other site of PMP (Chapter III). In addition, the elevation is gradually uprising to the north which comprises steep terrains and open rock areas. These unique topographical features possibly favour the monkeys to live in. Therefore higher priority for protection measures should be given to northern part of PMP. Since the highly suitable habitats for Phayre's leaf monkey were relatively small (only 5.28% of the total area) compared with the total park area, habitat restoration program is also recommended to implement at the other areas representing suitable or moderately suitable habitats in Popa Mountain Park.

Chapter V. Conclusions

The global population of Phayre's leaf monkey (*Trachypithecus phayrei*) is declining due to the combination of habitat degradation and anthropogenic activities. Therefore, it has been given high attention for conservation as an endangered species categorized by IUCN. Since wildlife management aimed at species conservation needs adequate information of species identities and phylogenetic status among species, this study was investigated the phylogenetic status of the Phayre's leaf monkey using non-invasive DNA samples in two protected areas, Popa Mountain Park (PMP) and Alaungdaw Kathapa National Park (AKNP) in Myanmar. This study confirmed the identity of Phayre's leaf monkey in PMP both at species and subspecies level since it was misidentified as the closely related taxa, Dusky leaf monkey (*T. obscurus*) in the recent years. Moreover, the species existence of Phayre's leaf monkey was recorded as first time for AKNP. The results showed that the population from PMP is closely related to the subspecies *T. p. shanicus* from the southwest China while that from AKNP clusters together with the subspecies *T. p. phayrei* from the northeast India. This genetic variation between two populations could be the result of having the geographical barrier, Ayeyarwaddy River, between two study sites. This is in agreement with the general hypothesis that the habitat separation by the geographical barrier, particularly river, makes limitation for gene flows among taxa. Moreover, the results supported that the scenario of habitat continuity that

favors gene flow among taxa since the AKNP connects with the northeastern ranges of India where the distribution of *T. p. phayrei* was also reported.

On the other hand, the understanding of species adaptation to the source habitats is essential for the effective wildlife management. Until to date, the ecological studies for Phayre's leaf monkey were limited although there were a few studies conducted in India and Bangladesh. In order to fill up the gap, this study was conducted the ecological studies in terms of population size, species distribution, behavior and habitat use of Phayre's leaf monkey in PMP. About 100 individuals of Phayre's leaf monkey were distributed in PMP and they restricted to the forested areas comprised with high species density, diversity, basal area and mean tree height of food trees. The occurrence of Phayre's leaf monkey was positively related with the abundance of foods, a scenario which has not been reported elsewhere for this monkey. Additionally, the monkeys prefer to live in the steep terrains associated with the open rock areas with little or no human disturbances. The results of the habitat suitability model also predicted that the highest suitable habitats for Phayre's leaf monkey were laid in the northern part of the PMP where the semi-evergreen and mixed deciduous forests mostly occupied. The result was consistent with the other studies that had reported that Phayre's leaf monkey often resides in these forest types.

From the intensive study on behavior of Phayre's leaf monkey, the result was in agreement with the general hypotheses that food distribution and habitat structure influence the animal behaviors. In this study, the monkeys spent most of time (about 60 %) in the trees where they prefer to feed, rest and sometimes play.

However, ground uses for resting were also high for Phayre's leaf monkey since there were open rock spaces where the monkeys likely to take sunbathing especially in winter. Although the behaviors of Phayre's leaf monkey were expected to change according to seasons and times of day, this hypothesis was not in agreement and it may possibly due to the abundance of foods for year round in the areas where the monkeys inhabit.

Management and conservation implications

Management and maintenance of genetic diversity is an issue of concern for all biologists studying threatened species and of great relevance in animal populations. There is no doubt for that habitat fragmentation results in reduced population sizes and may in time result in significant genetic drift, inbreeding, and susceptibility to further stochastic demographic contraction. Given that the findings of genetic features and habitat isolation process, this study suggested that the Phayre's leaf monkey from PMP should be given high conservation efforts at least as a 'management unit (MU)'. However, further studies on morphological and demographic information are highly recommended to confirm whether it is a distinct unit for management and conservation. In this study, the population from AKNP was also confirmed as subspecies *T. p. phayrei* as a new record for the park. But further assessment on distribution and genetic features are suggested to cover the whole population of Phayre's leaf monkey in AKNP since the current study was limited to the population distributed in the eastern part of the park. In general,

the wildlife authority is highly recommended to develop molecular analysis for confirmation of species identities and to collect more DNA samples of Phayre's leaf monkey distributed throughout the country in order to realize the whole spectrum of genetic characteristics among subspecies of *T. phayrei*.

In terms of habitat uses, this study revealed that the Phayre's leaf monkey in PMP mostly occupied the forested areas comprising enough food resources. Phayre's leaf monkey prefers to eat evergreen species (e.g. Moraceae, Myrtaceae) and deciduous species (e.g. Ebenaceae, Combretaceae). Additionally, some climbers belong to family Viatceae and Piperaceae were also recorded as food resources for Phayre's leaf monkey. Therefore, the park authority is suggested to set up management plan particularly to conserve main food trees from harvesting in its habitat.

Furthermore, habitat assessment plays a vital role for wildlife management and also geospatial map of habitat suitability supports the species conservation implications. The current study provided information of geospatial parameters (e.g. slope, altitude, distance to roads, distance to village, water resources, and vegetation coverage) and also human disturbances such as cutting and farming which are likely to influence habitat selection of Phayre's leaf monkey in this study. Based on the findings of the habitat suitability index, the most suitable habitats for Phayre's leaf monkey were laid down in the northern part of the PMP. Therefore the study suggested the protection measures and habitat restoration should be given high priority at the most suitable habitats with careful

consideration of ecological and geographical requirements of the study species, Phayre's leaf monkey in PMP.

In general, further studies on the current distribution and the genetic characteristics of all subspecies of Phayre's leaf monkey in Myanmar are highly recommended since these aspects are fundamental for effective wildlife management particularly the conservation of endangered species.

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Appendices

Appendix 2.1 Polymerase Chain Reaction (PCR) recipes used for *Prm1* gene (a) and *Cyt- b* gene (b) of Phayre's leaf monkey in this study

(a)

PCR Mixture	Stock concentration/µl	Final reaction concentration/µl	Reaction volume µl/sample
$MgCl_2$	25 mM	2.5 mM	2.5
10x Buffer Mg^{+2} free	-	1 x	2.5
dNTPs	10 mM	200 mM (0.2 M)	0.5
BSA 10x Buffer	-		2.0
primer F	10 µM	1 µM	2.5
primer R	10 µM	1 µM	2.5
H_2O	-	-	11.4
Taq polymerase	5U	2U	0.1
DNA template	-	1	1.0
Reaction Volume			**25**

(b)

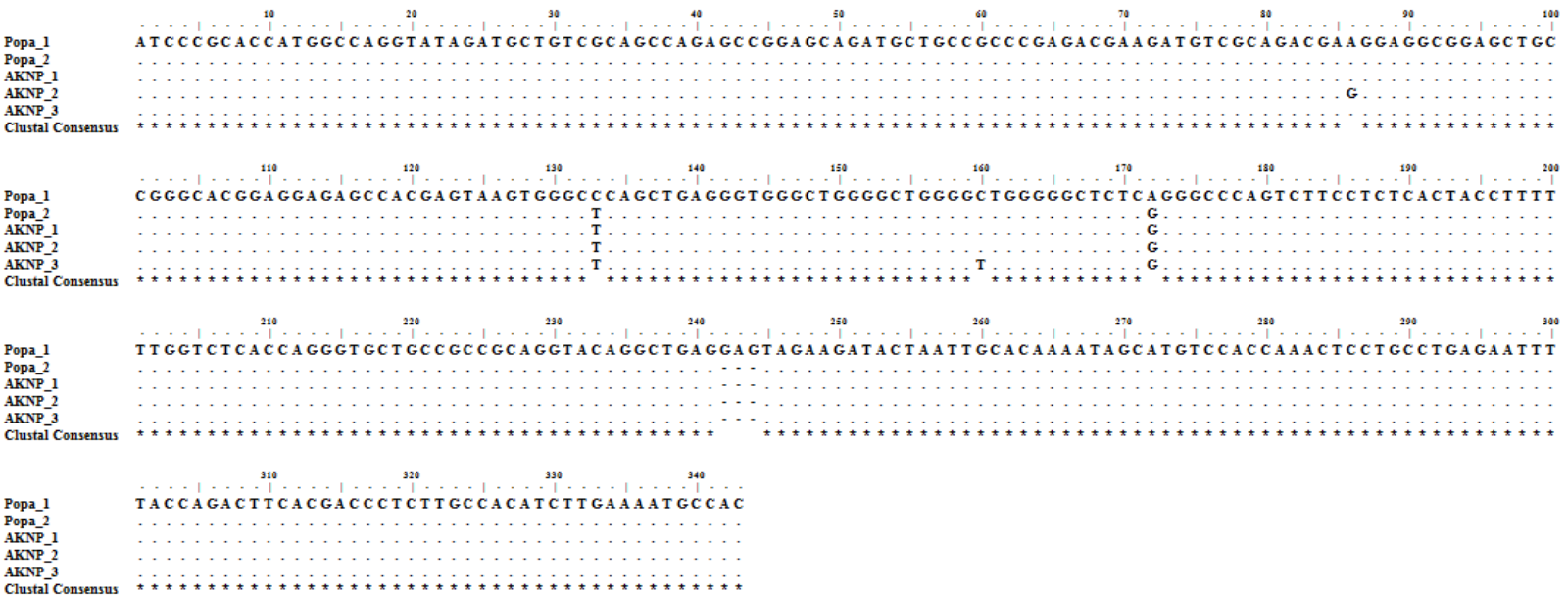
PCR Mixture	Stock concentration/µl	Final reaction concentration/µl	Reaction volume µl/sample
$MgCl_2$	25 mM	2.5 mM	2.5
10x Buffer Mg^{+2} free	-	1 x	2.5
dNTPs	10 mM	200 mM (0.2 M)	0.5
primer F	10 µM	1 µM	2.5
primer R	10 µM	1 µM	2.5
H_2O	-	-	13.4
Taq polymerase	5U	2U	0.1
DNA template	-	1	1.0
Reaction Volume			**25**

Appendix 2.2 The sample number, sample ID, type of sample, PCR and sequencing success, haplotype for *Prm1* and *Cyt-b* genes use in this study. O: Success; X : Fail ; N/A : no account; H: haplotype

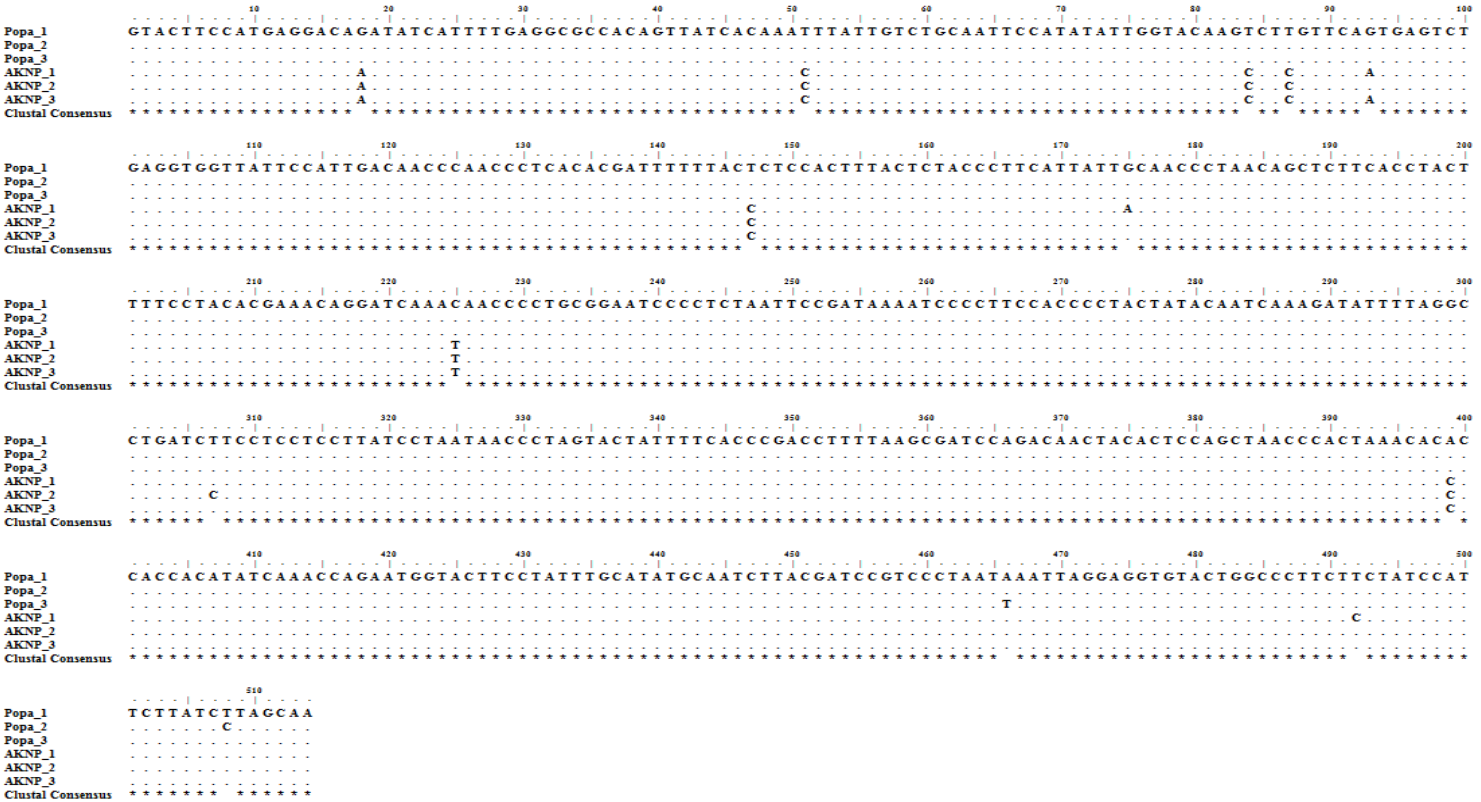
Sample no.	Sample ID	Sample type	PCR		Sequencing		Haplotype	
			Prm1	*Cyt-b*	*Prm1*	*Cyt-b*	*Prm1*	*Cyt-b*
1	Popa-B	Bone	O	O	O	O	H1	H1
2	Popa-01	Feces	O	O	O	O	H1	H1
3	Popa-02	Feces	O	O	O	O	H2	H1
4	Popa-03	Feces	X	X	N/A	N/A	N/A	N/A
5	Popa-04	Feces	X	X	N/A	N/A	N/A	N/A
6	Popa-05	Feces	X	X	N/A	N/A	N/A	N/A
7	Popa-06	Feces	X	X	N/A	N/A	N/A	N/A
8	Popa-07	Feces	O	O	O	O	H1	H2
9	Popa-08	Feces	X	X	N/A	N/A	N/A	N/A
10	Popa-09	Feces	O	O	X	X	N/A	N/A
11	Popa-10	Feces	X	X	N/A	N/A	N/A	N/A
12	Popa-11	Feces	X	X	N/A	N/A	N/A	N/A
13	Popa-12	Feces	X	X	X	X	H1	H1
14	Popa-13	Feces	O	O	O	O	N/A	N/A
15	Popa-14	Feces	O	O	O	O	H1	H2
16	Popa-15	Feces	X	X	N/A	N/A	N/A	N/A
17	Popa-16	Feces	O	O	O	O	H1	H2
18	Popa-17	Feces	X	X	N/A	N/A	N/A	N/A
19	Popa-18	Feces	O	O	O	O	H2	H3
20	Popa-19	Feces	X	X	N/A	N/A	N/A	N/A
21	Popa-20	Feces	X	X	N/A	N/A	N/A	N/A
22	Popa-21	Feces	X	X	N/A	N/A	N/A	N/A
23	Popa-22	Feces	X	X	N/A	N/A	N/A	N/A
24	Popa-23	Feces	X	X	N/A	N/A	N/A	N/A
25	Popa-24	Feces	X	X	N/A	N/A	N/A	N/A
26	Popa-25	Feces	O	O	N/A	N/A	N/A	N/A
27	Popa-26	Feces	O	O	N/A	N/A	N/A	N/A
28	Popa-27	Feces	X	X	N/A	N/A	N/A	N/A
29	Popa-28	Feces	O	O	N/A	N/A	N/A	N/A
30	Popa-29	Feces	X	X	N/A	N/A	N/A	N/A

31	Popa-30	Feces	X	X	N/A	N/A	N/A	N/A
32	Popa-31	Feces	X	X	N/A	N/A	N/A	N/A
33	Popa-32	Feces	O	O	N/A	N/A	N/A	N/A
34	AKNP-H	Hair	O	O	O	O	H1	H1
35	AKNP-01	Feces	O	O	O	O	H2	H2
36	AKNP-02	Feces	X	X	N/A	N/A	N/A	N/A
37	AKNP-03	Feces	X	X	N/A	N/A	N/A	N/A
38	AKNP-04	Feces	X	X	N/A	N/A	N/A	N/A
39	AKNP-05	Feces	O	O	O	O	H2	H2
40	AKNP-06	Feces	X	X	N/A	N/A	N/A	N/A
41	AKNP-07	Feces	X	X	N/A	N/A	N/A	N/A
42	AKNP-08	Feces	X	X	N/A	N/A	N/A	N/A
43	AKNP-09	Feces	O	O	O	O	H3	H3
44	AKNP-10	Feces	X	X	N/A	N/A	N/A	N/A
45	AKNP-11	Feces	X	X	N/A	N/A	N/A	N/A
46	AKNP-12	Feces	X	X	N/A	N/A	N/A	N/A
47	AKNP-13	Feces	X	X	N/A	N/A	N/A	N/A
48	AKNP-14	Feces	X	X	N/A	N/A	N/A	N/A
49	AKNP-15	Feces	X	X	N/A	N/A	N/A	N/A
50	AKNP-16	Feces	X	X	N/A	N/A	N/A	N/A
51	AKNP-17	Feces	X	X	N/A	N/A	N/A	N/A
52	AKNP-18	Feces	X	X	N/A	N/A	N/A	N/A
			33% (17/52)	33% (17/52)	23% (12/52)	23% (12/52)	2 (PMP) 3(AKNP)	3 (PMP) 3 (AKNP)

Appendix 2.3a Full-length (343 bp) of nuclear protamine P1 (*Prm1*) gene was assessed. Two haplotypes (Popa 1and 2) and three haplotypes (AKNP 1, 2, and 3) were assessed for populations of Popa Mountain and Alaungdaw Kathapa National Park, respectively.



Appendix 2.3b Partial length (514 bp) of mitochondrial cytochrome b (*Cyt-b*) gene was assessed. Three haplotypes (Popa 1, 2 and 3) and (AKNP 1, 2 and 3) were assessed for Popa Mountain Park and Alaungdaw Kathapa National Park, respectively.



Appendix 3.1 Data used for disturbance factors analysis, correlation between occurrence of Phayre's leaf monkey and habitat characteristics. **Plot**: sample plot (C01 – C26 for core area and B01 – B20 for buffer area); **ψ** : occupancy of monkey; **Alt.**: elevation (m); **H'**: Shannon tree species diversity; **DEN**: tree density (no./plot); **height**: mean tree height (m); **BA**: basal area (m^2/plot); **cutting**: stem/plot; **farming**: area percentage/plot; **vil-dist**: distance to village (m); **HDI**: human disturbance impact

No.	Plot	ψ	Alt.	H'	DEN	height	BA	cutting	farming	vil-dist	HDI
1	B01	0.025	307	0.56	5	5.2	0.32	0	100	1800	1.6
2	B02	0.025	467	0.35	13	5.2	0.33	13	0	2000	1.2
3	B03	0.025	393	0.75	26	5.8	0.82	0	50	1500	1.2
4	B04	0.025	615	1.3	16	7.2	0.46	0	100	2800	1.4
5	B05	0.025	289	0	0	0	0	0	100	1000	2.0
6	B06	0.025	530	1.44	24	4.4	0.48	0	0	1300	0.8
7	B07	0.025	616	2.25	16	5	0.25	15	0	1600	1.4
8	B08	0.025	330	0	0	0	0	0	100	1000	2.0
9	B09	0.025	382	1.8	18	4.6	0.29	10	0	1800	1.1
10	B10	0.159	738	1.2	15	7.1	0.62	0	100	1600	1.6
11	B11	0.025	336	0.28	26	5.1	0.33	5	0	2000	0.8
12	B12	0.025	749	1.75	7	9.1	0.29	0	100	1800	1.6
13	B13	0.025	363	1.06	10	6.2	0.23	0	100	1400	1.7
14	B14	0.025	303	0.85	9	6.4	0.21	0	90	1000	1.7
15	B15	0.025	594	1.01	27	5	0.63	0	100	1600	1.6
16	B16	0.025	620	1.24	6	6.1	0.18	0	100	1400	1.7
17	B17	0.025	454	0.5	5	6.4	0.13	0	100	1100	1.9
18	B18	0.025	344	0.85	28	5.9	0.7	0	50	1550	1.1
19	B19	0.025	620	1.61	5	4.6	0.05	0	100	1000	2.0
20	B20	0.025	512	1.67	13	6.1	0.53	0	100	1300	1.8
21	C01	0.025	614	1.56	29	5.3	0.66	0	0	2400	0.4
22	C02	0.025	613	2.8	63	5.6	0.95	5	0	1300	1.0
23	C03	0.025	611	2.2	47	6	0.78	0	0	2500	0.4

24	C04	0.025	614	1.76	17	5.1	0.34	0	0	2400	0.4
25	C05	0.025	612	0.79	48	5.7	0.8	0	0	3200	0.3
26	C06	0.025	976	2	61	6.7	1.38	0	0	2900	0.3
27	C07	0.159	613	2.29	33	11.8	1.95	20	0	2400	1.4
28	C08	0.025	467	0.77	25	7.5	0.61	3	0	2800	0.5
29	C09	0.025	611	1.84	16	5	0.42	0	80	2100	1.3
30	C10	0.025	611	0.88	22	5.8	0.54	0	0	1450	0.7
31	C11	0.025	602	2.71	46	6.3	1.02	7	0	1600	1.0
32	C12	0.025	522	2.13	40	5.9	0.93	5	0	3500	0.5
33	C13	0.768	1063	1.6	26	10.1	1.03	0	0	3400	0.3
34	C14	0.159	772	2.21	40	7.3	1.02	0	0	1500	0.7
35	C15	0.025	610	1.97	15	5.3	0.21	5	0	2200	0.7
36	C16	0.159	604	2.22	27	7.3	0.59	0	0	1000	1.0
37	C17	0.025	609	2.5	63	5.8	1.19	0	0	1900	0.5
38	C18	0.025	609	1.71	38	7.2	0.55	3	0	2000	0.7
39	C19	0.025	609	1.11	28	8.6	1.94	8	0	4200	0.6
40	C20	0.768	866	2.1	53	12.6	1.9	0	0	2600	0.4
41	C21	0.159	757	1.84	27	8.4	1.26	0	50	1900	1.0
42	C22	0.768	925	2.3	37	21.8	2.21	0	0	3000	0.3
43	C23	0.159	1099	0.51	55	12.1	2.43	0	0	3300	0.3
44	C24	0.025	738	2.25	30	5.8	0.8	5	0	1300	1.0
45	C25	0.025	681	2.22	55	6.3	1.08	5	0	3200	0.6
46	C26	0.159	1227	1.54	54	9.5	2.3	0	0	3200	0.3

Appendix 3.2 Tree density (DEN: no./ha), basal area (BA: m^2/ha) and importance value index (IVI) of tree species in three forest stands of Popa Mountain Park. LE = low elevation stand (301-600 m), ME = mid elevation stand (601-900 m), HE = high elevation stand (> 900 m)

Species	Family	LE			ME			HE		
		DEN	BA	IVI	DEN	BA	IVI	DEN	BA	IVI
Acacia catechu (L. f.) Willd.	Fabaceae	27	0.58	8.2	22	0.56	2.37	-	-	-
Acacia leucophloea Willd.	Fabaceae	11	0.33	4.5	-	-	-	-	-	-
Acacia spp.	Fabaceae	-	-	-	11	0.11	1.19	-	-	-
Aegle marmelos (L.) Corrêa	Rutaceae	11	0.11	2.9	11	0.33	1.51	-	-	-
Albizia chinensis (Osbeck) Merr.	Fabaceae	-	-	-	17	0.50	2.52	44	1.72	9.63
Amerimnon cultratum (Graham) Kuntze	Fabaceae	17	0.33	4.0	22	1.00	3.02	11	0.11	2.86
Anacardium occidentale L.	Anacardiaceae	100	1.44	13.5	19	0.33	2.86	-	-	-
Annona squamosa L.	Annonaceae	11	0.33	2.7	100	1.44	7.39	-	-	-
Artocarpus heterophyllus Lam.	Moraceae	17	0.72	5.2	-	-	-	-	-	-
Balakata baccata (Roxb.) Esser	Euphorbiaceae	-	-	-	-	-	-	78	4.56	7.90
Barringtonia arborea (Roxb.) F. Muell.	Lecythidaceae	-	-	-	11	0.61	2.42	-	-	-
Bauhinia acuminata Bruce	Fabaceae	-	-	-	11	0.11	1.19	11	0.33	3.21
Bauhinia racemosa Lam.	Fabaceae	22	0.33	4.4	44	0.89	3.92	-	-	-

Species	Family	LE			ME			HE		
		DEN	BA	IVI	DEN	BA	IVI	DEN	BA	IVI
Bombax ceiba L.	Malvaceae				11	0.89	2.33	-	-	-
Bridelia ovata Decne.	Euphorbiaceae	33	0.33	4.4	22	0.22	1.88	-	-	-
Buchanania lanzan Spreng.	Anacardiaceae	11	0.22	2.4	33	0.51	6.31	-	-	-
Carallia brachiata (Lour.) Merr.	Rhizophoraceae	-	-	-	-	-	-	150	3.89	20.31
Carum carvi L.	Apiaceae	-	-	-	-	-	-	17	0.56	5.87
Cassia fistula L.	Fabaceae	-	-	-	11	0.11	1.19	-	-	-
Cassia javanica L.	Fabaceae	-	-	-	11	0.22	1.35	-	-	-
Chukrasia tabularis A. Juss.	Meliaceae	-	-	-	11	0.11	1.19	-	-	-
Croton laevigatus Vahl	Euphorbiaceae	-	-	-	61	1.37	7.90	189	5.11	24.92
Dalbergia lanceolaria subsp. Paniculata (Roxb.) Thoth.	Fabaceae	28	1.03	8.7	44	6.33	11.92	-	-	-
Dalbergia oliveri Gamble	Fabaceae	-	-	-	26	0.46	4.89	-	-	-
Diasperus albizziodes (Kurz) Kuntze	Euphorbiaceae	-	-	-	11	0.11	1.19	-	-	-
Dillenia parviflora Griff.	Dilleniaceae	-	-	-	17	0.36	3.31	22	0.78	4.68
Diospyros burmanica Kurz	Ebenaceae	19	0.48	8.2	26	0.44	4.86	-	-	-
Diospyros mollis Griff.	Ebenaceae	22	1.22	6.2	28	0.72	3.38	11	0.78	3.92
Dipterocarpus tuberculatus Roxb.	Dipterocarpaceae	11	0.78	4.1	35	0.81	5.85	-	-	-
Engelhardtia spicata Blume	Juglandaceae	-	-	-	70	2.33	8.27	-	-	-

Species	Family	LE			ME			HE		
		DEN	BA	IVI	DEN	BA	IVI	DEN	BA	IVI
Eriobotrya bengalensis (Roxb.) Hook. f.	Rosaceae	-	-	-	-	-	-	11	0.56	3.57
Ficus auriculata Lour.	Moraceae	-	-	-	11	0.22	1.35	-	-	-
Ficus obtusifolia Kunth	Moraceae	-	-	-	-	-	-	17	0.94	6.49
Ficus pisifera var. obscura (Blume) Valeton	Moraceae	-	-	-	-	-	-	11	0.56	3.57
Flacourtia indica (Burm. f.) Merr.	Salicaceae	-	-	-	11	0.33	2.01	17	0.78	6.23
Gardenia obtusifolia Roxb.	Rubiacea	22	0.22	3.2	-	-	-	-	-	-
Gmelina arborea Roxb. ex Sm.	Lamiaceae	-	-	-	22	1.17	3.76	-	-	-
Grewia tiliifolia Vahl	Malvaceae	11	0.11	2.0	15	0.19	2.46	11	0.11	2.86
Haplophragma adenophyllum (Wall. ex G. Don)	Bignoniaceae	-	-	-	17	0.17	2.03			
Heteropanax fragrans (Roxb.) Seem.	Araliaceae	-	-	-	16	0.58	4.07	11	0.22	3.04
Hiptage candicans Hook. f.	Malpighiaceae	33	0.33	5.2	-	-	-	-	-	-
Homalium tomentosum Benth.	Flacourtiaceae	-	-	-	44	3.00	7.02	-	-	-
Jatus hamiltonianus (Wall.) Kuntze	Lamiaceae	111	2.88	25.8	137	6.33	17.33	-	-	-
Lagerstroemia speciosa (L.) Pers.	Lythraceae	-	-	-	15	0.11	2.36	-	-	-
Lagerstroemia villosa Wall. ex Kurz	Lythraceae	11	0.11	2.0	11	0.11	1.19	-	-	-
Lannea coromandelica (Houtt.) Merr.	Anacardiaceae	11	0.22	3.3	18	0.24	3.68	-	-	-
Litsea glutinosa (Lour.) C.B. Rob.	Lauraceae	-	-	-	63	2.78	8.57	11	0.94	10.31

Species	Family	LE			ME			HE		
		DEN	BA	IVI	DEN	BA	IVI	DEN	BA	IVI
Mallotus philippensis (Lam.) Müll. Arg.	Euphorbiaceae	-	-	-	59	1.85	7.03	50	1.75	6.11
Mallotus tetracoccus (Roxb.) Kurz	Euphorbiaceae	-	-	-	22	2.00	4.49	-	-	-
Mangifera indica L.	Anacardiaceae	52	2.30	13.5	31	1.08	5.03	-	-	-
Miliusa velutina (Dunal) Hook. f. & Thomson	Annonaceae	22	0.33	4.4	-	-	-	-	-	-
Millettia pulchra (Benth.) Kurz	Fabaceae	-	-	-	56	1.22	4.94	-	-	-
Morinda tinctoria Roxb.	Rubiaceae	22	0.33	8.0	11	0.11	2.18	-	-	-
Moringa oleifera Lam.	Moringaceae	11	0.67	3.7	-	-	-	-	-	-
Oleaceae spp.	Oleaceae	-	-	-	56	2.89	7.39	-	-	-
Osyris quadripartita Salzm. ex Decne.	Santalaceae	11	0.15	3.9	-	-	-	-	-	-
Pentacme siamensis (Miq.) Kurz	Dipterocarpaceae	72	1.72	12.3	63	1.36	8.94	-	-	-
Phoenix paludosa Roxb.	Arecaceae	11	1.67	6.8	-	-	-	-	-	-
Phyllanthus emblica L.	Phyllanthaceae	11	0.11	2.0	11	0.11	1.19	-	-	-
Pinus kesiya Royle ex Gordon	Pinaceae	-	-	-	-	-	-	100	10.67	13.90
Pittosporum napaulense (DC.) Rehder & E.H. Wilson	Pittosporaceae	67	1.11	9.2	78	1.25	9.02	17	0.67	25.75
Premna integrifolia	Verbenaceae	-	-	-	22	0.22	2.38	-	-	-
Premna pyramidata Wall. ex Schauer	Lamiaceae	11	0.78	4.1	17	0.20	4.06	-	-	-
Protium serratum (Wall. ex Colebr.) Engl.	Burseraceae	-	-	-	38	1.24	7.57	33	1.11	6.05

Species	Family	LE			ME			HE		
		DEN	BA	IVI	DEN	BA	IVI	DEN	BA	IVI
Psidium guajava L.	Myrtaceae	-	-	-	11	0.11	1.19	-	-	-
Pterospermum semisagittatum Schreb.	Malvaceae	22	0.33	3.5	-	-	-	-	-	-
Quercus serrata Thunb.	Fagaceae	-	-	-	-	-	-	272	12.11	14.50
Salacia insignis A.C. Sm.	Celastraceae	22	0.33	3.5	11	0.33	1.51	-	-	-
Schleichera oleosa (Lour.) Merr.	Sapindaceae	11	0.11	2.9	11	0.22	1.85	11	0.39	41.77
Shorea obtusa Wall.	Dipterocarpaceae	48	1.48	10.7	56	1.63	10.99	-	-	-
Spondias pinnata (L. f.) Kurz	Anacardiaceae	11	0.44	3.1	-	-	-	-	-	-
Spondias spp.	Anacardiaceae	-	-	-	11	0.44	1.68	-	-	-
Stereospermum neuranthum Kurz	Bignoniaceae	11	0.11	2.0	11	0.28	1.93	11	0.11	5.23
Strychnos nux-blanda A.W. Hill	Loganiaceae	22	0.33	3.5	44	0.56	3.43	-	-	-
Syzygium cumini var. cumini	Myrtaceae	-	-	-	22	0.89	2.86	31	1.33	13.87
Tamarindus indica L.	Fabaceae	17	0.33	4.0	22	0.61	2.95	-	-	-
Tectona grandis L. f.	Lamiaceae	129	2.10	22.1	48	0.89	7.04	-	-	-
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae	-	-	-	11	1.22	2.82	22	0.56	2.86
Terminalia chebula Retz.	Combretaceae	28	0.72	6.0	20	0.64	4.38	-	-	-
Terminalia crenulata Roth	Combretaceae	11	0.11	2.0	37	0.51	5.95	-	-	-
Terminalia oliveri Brandis	Combretaceae	27	0.62	10.1	17	0.67	2.76	-	-	-
Terminalia spp.	Combretaceae	-	-	-	17	0.50	2.52	22	1.17	4.33

Species	Family	LE			ME			HE		
		DEN	BA	IVI	DEN	BA	IVI	DEN	BA	IVI
Trema orientalis	Cannabaceae	-	-	-	-	-	-	11	0.11	7.23
Trevia nudiflora L.	Euphorbiaceae	-	-	-	-	-	-	78	7.11	18.57
Ulmus lancifolia Roxb.	Ulmaceae	11	0.11	2.0	56	6.11	12.12	22	1.33	5.57
Vitex canescens Kurz	Lamiaceae	-	-	-	22	0.22	1.88	-	-	-
Vitex limonifolia Wall.	Verbenaceae	31	0.58	7.6	13	0.20	3.41	-	-	-
Walsura trichostemon Roxb.	Meliaceae	11	0.22	2.4	22	0.44	2.21	-	-	-
Wendlandia glabrata DC.	Rubiaceae	11	0.22	2.4	22	0.33	3.53	56	0.94	9.16
Xylia xylocarpa Taub.	Fabaceae	17	0.17	3.5	11	0.22	1.85	-	-	-
Ziziphus jujuba Mill.	Rhamnaceae	44	2.89	12.9	-	-	-	-	-	-
Ziziphus rugosa Lam.	Rhamnaceae	22	0.22	3.2	39	0.78	3.99	-	-	-

Appendix 3.3 Tree species consumed by Phayre's leaf monkey. Information was obtained from Min (2006) and in this study. ** both study, *this study

**Mani-awga (*Carallia brachiata*): leave

*Yinnaung-new (*Cissus vitiginea*): leave, bud

*Kun-line (*Cordyline fruticosa*): leave

**Thin twe nyo (*Diospyros mollis*): leave, bud

** Tha bye (*Eugenia contracta*): leave, bud

**Nyaung-gyat (*Ficus obtusifolia)*: leave, bud, fruit

*Naywe (*Flacourtia indica*): fruit

**Sayo (*Piper attenuatum*): leave

**Thadi (*Protium serratum*): fruit

*Thit-e (*Quercus serrata*): leave

*Sein-nabaw (*Smilax perfoliata*) : leave

**Thit-seint (*Terminalia bellirica*): fruit

*YethaKhwar (*Trema orientalis)*: leave, fruit

*Sakadu (*Trevia nudiflora*) : leave, fruit

Abstract in Korean (국문 초록**)**

미얀마에 서식하는 Phayre's Leaf Monkey (*Trachypithecus phayrei*)의 분자계통학적 위치와 생태학적 특성

Phayre's leaf monkey (*Trachypithecus phayrei*)는 아시아의 열대 산림에 국한되어 서식하는 중형 영장류로서, 세계자연보전연맹 적색목록(IUCN Red List)의 '멸종위기종(endangered species)'에 해당하며 CITES 부속서 II (Appendix II)에도 기재되어 있다. 현재까지 세 아종(*T. p. phayrei*, *T. p. crepusculus*, *T. p. shanicus*)이 알려져 있으나, Phayre's leaf monkey의 분류학적 위치나 생태에 대해서는 아직까지 거의 알려진 바가 없다. 본 연구는 미얀마에 서식하는 Phayre's leaf monkey의 분류학적 위치를 확인하고 행동 및 서식지 이용을 파악함으로써, 해당종의 보전과 서식지 관리를 위한 기초 정보를 제공하는데 그 목적이 있다.

본 연구는 미얀마의 Popa Mountain Park (PMP)와 Alaungdaw Kathapa National Park (AKNP)에 서식하는 Phayre's leaf monkey의 계통분류학적 위치를 조사하기 위하여 비침습적 표본(털과 분변)과 비파괴적 표본(사체에서 확보한 뼈)를 이용한 첫 번째 분자계통학적 연구이다. 이를 위해 nuclear protamine P1 (*Prm1*)과

mitochondrial cytochrome *b* (*Cyt-b*) gene을 DNA 마커로 이용하였다. 그 결과 PMP 개체군은 중국 남서부의 *T. p. shanicus*와 계통학적으로 더 가까웠으나, AKNP 개체군은 인도 북동부의 *T. p. phayrei*와 동일 집단으로 분류되었다.

PMP에는 세 집단의 Phayre's leaf monkey가 서식하는 것이 확인되었으며, 1집단, 2집단, 3집단의 평균 개체수는 각각 41 ± 0.67, 41 ± 0.58, 21 ± 0.67개체였다. Phayre's leaf monkey는 교란이 거의 없는 반상록수림에 주로 분포하였다(occupancy value: ψ = 0.7679, SE = 0.182). Phayre's leaf monkey의 출현은 수목밀도(tree/plot), 흉고단면적(m^2/plot), 평균수고(m)의 임분 특성과 양의 상관관계를 보였다(Spearman's rank correlation; 수목밀도: Υ_s = 0.305, P = 0.039; 흉고단면적: Υ_s = 0.469, $P < 0.0001$; 평균수고: Υ_s = 0.583, $P < 0.0001$).

먹이자원의 가용성은 Phayre's leaf monkey가 이용하는 임분과 이용하지 않는 임분간에 큰 차이를 보였다. 본 연구에서 Phayre's leaf monkey는 뽕나무과(Moraceae)와 도금양과(Myrtaceae)를 포함한 14과의 식물을 먹이로 이용하였다. 다른 지역에서는 먹는 것이 확인된 바 없는 덩굴식물(*Cissus vitiginea*, *Piper attenuatum*, *Smilax perfoliata*)의 채이도 처음 확인되었다.

Phayre's leaf monkey의 주간행동 비율은 채이(feeding; 54%)가 가장 높았으며, 그 다음 휴식(resting; 32%), 이동 (travelling; 7%), 기타 행동(other activities; 7%) 순이었다. Phayre's leaf monkey의 이동행동은 지면과 교목층에서 무작위적으로 관찰되었지만(Kruskal-Wallis H test; $H = 3.527$, $df = 4$, $P = 0.474$), 채이, 휴식과 기타 행동(털손질과 놀이)은 선택적인 양상을 보였다 (Kruskal-Wallis H test; 채이: $H = 23.214$, $df = 4$, $P < 0.0001$; 휴식: $H = 23.291$, $df = 4$, $P < 0.0001$; 기타: $H = 11.107$, $df = 4$, $P = 0.025$).

Phayre's leaf monkey는 여름철(28.73%)과 우기(27.63%)보다 겨울철(31.65%)에 휴식에 소비하는 시간이 약간 더 많았다. 이른 아침(06:00-08:00)과 늦은 저녁(16:00-18:00)에 두 번의 섭식 정점이 관찰되었다. 그러나 모든 주간행동을 함께 분석한 자료에서는 기타 행동(예, 털손질과 놀이; Kruskal-Wallis H test: $H = 13.28$, $df = 5$, $P = 0.021$)을 제외하고는 유의한 차이를 보이지 않았다. 이 연구는 만약 서식지에 충분한 먹이자원이 연중 존재한다면, 계절과 일일시간대는 이 종의 행동에 영향을 주지 않을 것임을 제시하고 있다.

경관서식지적합성지수(HSI) 모델을 이용하여 PMP의 Phayre's leaf monkey에 대한 서식지 적합성을 구했다. PMP 북부지역에 위치한 최적합(highly suitable) 서식지는 전체의 5.28%를

차지하였으며, 적합(suitable)이 12.63%, 준적합(moderately suitable)이 23.54%, 부적합(least suitable)이 26.31%, 절대부적합(unsuitable) 서식지가 32.24%인 것으로 나타났다. 도출된 서식지 적합성 지도는 이해하기 쉬우며, Phayre's leaf monkey에게 적합한 서식지를 보전하기 위한 공원관리계획에 이용될 수 있을 것이다.

PMP의 개체군은 기타 *T. phayrei* 아종들과 다른 유전적 특징을 보여주고 있으므로, 본 연구에서 확인된 분류학적 정보는 멸종의 위협에 처한 종, 특히 PMP에 서식하는 Phayre's leaf monkey의 유전자원을 보전하기 위한 근거로 이용될 수 있다. 또한, Phayre's leaf monkey의 먹이자원과 적합한 서식지에 대한 연구결과는 주요 먹이자원을 보전하고 적합한 서식지를 보호하도록 공원관리 계획을 개선하는데 활용될 수 있을 것이다.

주 요 어: 분자계통, 비침습적 표본, 서식지 이용, 서식지 적합성, 주간행동, Phayre's leaf monkey (*Trachypithecus phayrei*)

학 번: 2009-31279

Abstract in Myanmar

စာတမ်းအကျဉ်းချုပ် (မြန်မာ)

မြန်မာနိုင်ငံ၌တွေ့ရှိရသော မျောက်မျက်ကွင်းဖြူ၏ မျိုးရိုးဗီဇနှင့် ဂေဟဗေဒစနစ်အားလေ့လာခြင်း

မျောက်မျက်ကွင်းဖြူမျိုးစိတ်များသည် အရွယ်အလတ်စားမျောက်မျိုးတွင်ပါဝင်ပြီး အာရှ အပူပိုင်း မုတ်သုန်သစ်တောများအတွင်း၌သာတွေ့ရှိရသည်။ IUCN ၏ ကာကွယ်မှု အဆင့်တွင် မျိုးသုဉ်းရန် အန္တရာယ်ရှိသည့်မျိုးစိတ်အဖြစ်လည်းကောင်း၊ CITES ကွန်ဗင်းရှင်း၏ ကန့်သတ်စာရင်းတွင် နောက်ဆက်တွဲ ၂ အဖြစ်လည်းကောင်း သတ်မှတ်ထားပါသည်။ ဤစာတမ်းသည် မျောက်မျက်ကွင်းဖြူမျိုးများနှင့် ၎င်းတို့၏ ကျက်စားဒေသများ ထိန်းသိမ်းကာကွယ် စောင့်ရှောက်ရေးကို အထောက်အကူပြုနိုင်ရန် ရည်ရွယ်၍ သိပ္ပံနည်းကျ မျိုးဗီဇခွဲခြားစမ်းသပ်ခြင်း၊ ကျက်စားနယ်မြေရွေးချယ် အသုံးပြုမှုကို လေ့လာခြင်းနှင့် အစာရေစာရရှိမှုပေါ်မူတည်၍ လူပ်ရှားသွားလာ နေထိုင်မှု ပြောင်းလည်းခြင်းများကို ဆန်းစစ်သုတေသနပြုလုပ်ခဲ့ခြင်း ဖြစ်ပါသည်။

ဤစာတမ်းသည် မြန်မာနိုင်ငံအတွင်းရှိ မျောက်မျက်ကွင်းဖြူမျိုးစိတ်များအနက် ပုပ္ပါးတောင်နှင့် အလောင်းတော်ကသပဒေသအတွင်း ကျက်စားသည့်မျိုးစိတ်များ၏ ကြွင်းကျန်ရစ်သည့် မစင်၊အမွှေးအမျှင်များနှင့် အရိုးစနမူနာများအသုံးပြု၍ မျိုးဗီဇကို ပထမဆုံး အကြိမ်အဖြစ် ဖော်ထုတ်လေ့လာနိုင်ခဲ့ခြင်းဖြစ်ပါသည်။ မျိုးဗီဇခွဲခြားစမ်းသပ်ရာ တွင် nuclear protamine P1(*Prm1*) နှင့် mitochondrial cytochrome b (*Cyt-b*) ဗီဇဆဲလ် ၂ မျိုးကို အသုံးပြုခဲ့ပါသည်။ ပုပ္ပါးမျောက်မျက်ကွင်းဖြူအုပ်စုသည် တရုတ်နိုင်ငံ အနောက်တောင်ဖက် ဒေသရှိ မျောက်မျက်ကွင်းဖြူမျိုးစိတ်ကွဲ *T. p. shanicus* နှင့် များစွာ နီးစပ်မှုရှိကြောင်းတွေ့ရှိရပြီး အလောင်းတော်ကသပရှိ မျောက်မျက်ကွင်းဖြူအုပ်စုသည်

အိန္ဒိယနိုင်ငံ အနောက်မြောက်ဖက်မှ မျိုးစိတ်ကွဲ *T. p. phayrei* နှင့် ဆင်တူကြောင်း တွေ့ရှိရသည်။

ပုပ္ပါးတောင်၌ မျောက်မျက်ကွင်းဖြူများကို ၄၁ ကောင်ခန့်ရှိ အုပ်စု ၂ စုနှင့် ၂၁ ကောင်ခန့်ရှိအုပ်စု ၁ စု၊ စုစုပေါင်း အုပ်စု ၃ စုတွေ့ရှိရသည်။ ကျက်စားနေထိုင်မှု ဆန်းစစ်တွေ့ရှိချက်အရ အမြိမ်းဆန်သောတောများရှိသည့် တောင်မြင့်ပိုင်းဇရိယာသည် ၎င်းတို့အတွက် အနောက်အယှက်နည်းပါးစွာ နေထိုင်နိုင်သည့်နယ်မြေအဖြစ် တွေ့ရှိရသည်။ အပင်ကြီးများ ထူထပ်မှုများပြားသည့် နေရာများတွင် အများဆုံးနေထိုင်ကျက်စားမှု ရှိကြောင်းကိုပါ လေ့လာတွေ့ရှိရသည်။

သတ်မှတ်ကျက်စားနယ်မြေ ရွေးချယ်သတ်မှတ်ရာတွင် အစာရေစာရရှိနိုင်မှု အခြေအနေ နှင့် ပေါက်ရောက်သည့် အပင်အမျိုးအစားသည် အဓိကအကြောင်းအရင်း တစ်ရပ်ဖြစ်ကြောင်း တွေ့ရှိရသည်။ မျောက်မျက်ကွင်းဖြူများ၏ စားပင်များအဖြစ် Moraceae နှင့် Myrtaceae အပါအဝင် မျိုးရင်းဝင်အပင် ၁၄ မျိုးကို မှတ်တမ်းတင်နိုင်ခဲ့သည့်အပြင် နွယ်ပင်မျိုးများ ဖြစ်သည့် ရင်းတောင်း (*Cissus vitiginea*), စရို (*Piper attenuatum*) နှင့် စိန်နဘော် (*Smilax perfoliata*) တို့ကိုလည်း စားသောက်တတ်ကြောင်း တွေ့ရပါသည်။ အဆိုပါနွယ်ပင်များကို မျောက်မျက်ကွင်းဖြူများ၏ စားသုံးပင်အဖြစ် ယခင်မှတ်တမ်းများ၌ ဖော်ပြထားခြင်း မရှိသေးကြောင်း တွေ့ရှိရပါသည်။

ပုပ္ပါးမျောက်မျက်ကွင်းဖြူမျိုးစိတ်၏ အလေ့အထတွင် အစာရှာဖွေစားသောက်မှု အတွက် ၅၄%၊ နားနေခြင်း ၃၂%၊ လှည့်လည်သွားလာခြင်း ၇% နှင့် ကျန်အခြား လှုပ်ရှားမှုအဖြစ် ၇% ခန့် အသုံးပြုကြောင်းတွေ့ရသည်။ အလေ့အထနှင့်အမူအကျင့်များ ဆန်းစစ်လေ့လာတွေ့ရှိမှုရလဒ်အရ ရံဖန်ရံခါ မြေပြင်ပေါ်နှင့်အပင်များပေါ်တွင် ကျပမ်းလှည့်လည်သွားလာတတ်သော်လည်း အစားအစာအတွက်ကိုမူ ပုံသေတစ်သမတ်တည်း သွားလာစားသောက်လေ့ရှိကြောင်း တွေ့ရှိရသည်။

ပုပ္ပါးမ်ောက်မျက်ကွင်းဖြူများ၏ ရာသီအလိုက်လှုပ်ရှားနေထိုင်မှုကို လေ့လာဆန်းစစ်ချက်အရ အနားယူမှုအချိန်မှာ ဆောင်းရာသီ၌ (၃၁.၆၅%)၊ နွေရာသီ၌ (၂၈.၇၃%)နှင့် မိုးရာသီ၌ (၂၇.၆၃%)စီ ရှိကြောင်း တွေ့ရှိရသည်။ နေ့စဉ်လှုပ်ရှားနေထိုင်မှုတွင် နံနက်ပိုင်း ၆ နာရီမှ ၈နာရီအထိ တစ်ကြိမ်၊ ညနေ ၄ နာရီမှ ၆ နာရီထိ တစ်ကြိမ် အစာစားသုံးမှု ပိုများကြောင်း တွေ့ရသည်။ မျောက်များကို လေ့လာဆန်းစစ်တွေ့ရှိချက်အရ တစ်နှစ်ပတ်လုံး အစာပေါများစွာ ရရှိမှုနှင့် လုံခြုံမှုတို့ရှိပါက ရာသီဥတုနှင့် အချိန်ကာလကိုလိုက်၍ အလေ့အထများ ထူးခြားစွာ ပြောင်းလဲမှုမရှိကြောင်း သုံးသပ်တွေ့ရှိရသည်။

ပုပ္ပါးမ်ောက်မျက်ကွင်းဖြူများ၏ ကျက်စားနယ်မြေလိုအပ်မှုဆိုင်ရာ အညွှန်းကိန်း (Habitat Suitability Index-HSI)ကိုတွက်ချက်မှုအရ ကျက်စားနယ်မြေသည် ပုပ္ပါးတောင်၏ မြောက်ဖက်အစွန်းပိုင်းတွင် အများဆုံးကျရောက်နေပြီး အကောင်းဆုံးအနေအထား ၅.၂၈%၊ ကောင်းမွန်သည့်အနေအထား ၁၂.၆၃%၊ အသင့်အတင့် ကောင်းမွန်မှု ၂၃.၅၄%၊ ညံ့ဖျင်းမှု ၂၆.၃၁% နှင့် မသင့်လျော်သည့်နယ်မြေ ၃၂.၂၄% အဖြစ် ဖော်ထုတ်ရရှိခဲ့ပါသည်။ ကျက်စားနယ်မြေ ဆန်းစစ်လေ့လာတွေ့ရှိချက်သည် မျောက်မျိုးစိတ်များထိန်းသိမ်းရေးနှင့် ဥယျာဉ်အုပ်ချုပ်လုပ်ကိုင်မှုစီမံချက် ရေးဆွဲရာတွင် သာမက မျိုးစိတ်ပြန့်နှံ့တည်ရှိမှုမြေပုံ ရေးဆွဲရေးအတွက် များစွာအထောက်အကူ ရရှိစေမည်ဖြစ်သည်။

ဤသုတေသနစာတမ်းတွင် ပုပ္ပါးနှင့် အလောင်းတော်ကဿဖရို မျောက်မျက်ကွင်းဖြူများ၏ မျိုးဗီဇကိုမျိုးစိတ်ကွဲအဆင့်ထိ ဖော်ထုတ်လေ့လာတွေ့ရှိရခြင်းကြောင့် အခြားဒေသရှိ ၎င်းမျောက်မျိုးစိတ်များအပါအဝင် ကျန်ရှားပါးမျိုးစိတ်များ၏ မျိုးရိုးဗီဇ ထိန်းသိမ်းရေးလုပ်ငန်း အတွက် မျိုးဗီဇခွဲခြားလေ့လာရေးလုပ်ငန်းများစတင်ရန် လိုအပ်ကြောင်းအကြံပြုအပ်ပါသည်။ မျောက်မျက်ကွင်းဖြူမျိုးစိတ်များ၏ ကျက်စားဒေသများအလိုက် ဂေဟဗေဒဆိုင်ရာ လေ့လာဖော်ထုတ်တွေ့ရှိရသည့် အချက်အလက်

များအရ ဥယျာဉ်အုပ်ချုပ်လုပ်ကိုင်မှုစီမံချက်များ ပြန်လည်ပြင်ဆင်ရေးဆွဲရာတွင် မျိုးစိတ်များထိန်းသိမ်းရေးနှင့် ကျက်စားနယ်မြေများကို ထိန်းသိမ်းပြုစုပေးခြင်း၊ ကာကွယ်ပေးခြင်းများကို ပိုမိုအလေးထားလုပ်ဆောင်သင့်ကြောင်း အကြံပြုထည့်သွင်း ရေးသားအပ်ပါသည်။

အဓိကစာသား - နေ့ဘက်သွားလာလှုပ်ရှားမှု၊ ကျက်စားနေမြေလိုအပ်မှု၊ စားကျက်အသုံးပြုမှု၊ မျိုးဗီဇနမူနာ၊ မျိုးဗီဇကွဲပြားပြောင်းလဲမှု၊ မျောက်မျက်ကွင်းဖြူ

ကျောင်းဝင်အမှတ် - ၂၀၀၉ - ၃၁၂၇၉