【Original Article】

A Behavioural Study and Gene Analysis of *Trachypithecus phayrei* (Blyth, 1847), Phayre's Leaf Monkey, an Old World Monkey in the Volcanic Crater of Popa Mountain Park, Myanmar

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(Received: 3/30/2011)
(Accepted: 4/15/2011)

Summary

A behavioural study of Phayre's leaf monkey, *Trachypithecus phayrei* (Blyth, 1847) conducted in Popa Mountain Park, Myanmar is described with photographic evidence. The condition of the study area and habitats of the monkeys are also presented. DNA extraction and amplification of both the mtDNA genes and LZM genes utilizing skin samples showed that *T. phayrei* leaf monkeys are more closely related with the species of India than Vietnam. Additionally, we assume that the leaf monkeys inhabiting Popa Mountain Park are descendents of leaf monkeys inhabiting nearby Chin Hill plateau rather than those distributed in Bago Yoma.

(Med Biol **155:** 332-345 2011)

Keywords: *Trachypithecus phayrei*, Popa Mountain Park, Myanmar, Feeding behaviour, Lysozome and mtDNA

Introduction

Next to humans and apes, monkeys are the highest taxonomic rank among mammals[1)] and the most social of all animals. As the closest living relatives to humans, they are important models for the study of human evolution. They are characterized by their exceptionally diverse social systems, ranging from solitary females and males with a polygamous and promiscuous mating system, plain-living monogamous species, groups with one male and females (polygamous) and with one female and several males (polyandrous), to groups with several adult males and several adult females[2)]. Similar to that of humans, the diversity in genetics and social phenomena in monkeys is closely related to the overall biodiversity of an area. This fact also has the potential to contribute to an understanding of human evolution. Plant diversity increases with enriched diversity in primate species as they play a part in the dispersal of seeds. Primates occupy a wide range of habitats and show a wide diversity of adaptations to their contrasting environment.s Monkeys include terrestrial species as well as arboreal ones, specialized insectivores as well as fruit and leaf-eaters [3)].

Old World monkeys, family Cercopithecidae, have been divided into two families: Cercopithecinae (including the genera *Miopithecus, Cercopithecus, Erythrocebus, Allenopithecus, Cercocebus, Macaca, Papio, Mandrillus, and Theropithecus*), and Colobinae (including the

genera *Presbytis, Seminopithecus, Thrachypithecus, Pygathrix, Rhinopithecus, Nasalis, Simias, Colobus, Piliocolobus*, and *Procolobus*[4]. Both are often referred to as leaf monkeys because of their predominantly specialized folivorous diet. In fact, many of the anatomical features distinctive to the Colobinae are related to their folivorous adaptation. Colobines have adapted to digest cellulose and denature toxins in leafy materials, though several species are not predominantly folivorous. This adaptation has influenced the design of their digestive system as well as their ecology and foraging behaviour. Colobines lack cheek pouches, that are used to keep food for a short time [5].

The Asian colobines commonly referred to as langurs, are comprised of seven genera (Semnopithecus, Trachypithecus, Presbytis, Pygathrix, Rhinopithecus. Nasalis and Simias) with 35 to 43 species and 90 to 93 taxa [6,7]. Of these genera, 5 species are found in Myanmar [9]. The genus Trachypithecus is the most widespread Asian langur with a distribution stretching from India to Vietnam and Sumatra, Java and Borneo. In the past in Vietnam, taxa of three major groups were recognized; the Cristatus group, (*T. cristatus*) a silvered langur closely related to other species of Java, Borneo, Sumatra, and some neighbouring islands around the Malay peninsula; the Obscurus group, a Grey langur most closely resembling Phayre's langur; hence, it was described as a subspecies of the Phayre's langur (*T. phayrei crepusculus*); and the Francoisi group, specified as the superspecies francoisi - with 6 to 7 different taxa of which four to five were recognized in Vietnam (*francoisi, policephalus, delacouri, hatinhensis, ebenus*). The data set excludes four species (*johnii, vetulus, geei* and *pileatus*) of the genus *Trachypithecus*, since these are more closely related to the genus *Semnopithecus* than they are to *Trachypithecus* and hence, should be recognized as species of *Semnopithecus*; these species are not found in Indochina. The results of the DNA analysis within the genus *Trachypithecus*, reveal that a first radiation into two main lineages occurred: 1) the Cristatus group, that is a silvered-Ebony langur group with three species (*germaini, cristatus and auratus*); 2) an Obscurus group that includes a Phayre's group and a Dusky langur group with two species (*phayrei, obscurus*) *francoist* (superspecies) including *crepsuculus*. Phayre's Leaf Monkey (*Trachypithecus phayrei*), also known as Phayre's langur, is a species of langur found in Southeast Asia. The species distribution includes Bangladesh, India, Myanmar, China, Thailand, Laos and Vietnam [8].

In Myanmar, a total of 14 species of primates can be found in different forests within the country. Among them, the monkey groups such as langurs and macaques are reported to be most abundant and found in different types of habitat [9].

The Phayre's leaf monkey, *Trachypithecus* phayrei is designated as a protected species mainly because of high hunting pressure, habitat deterioration by logging, and increasing agricultural land use. They inhabit one particular forest situated in the crater of Popa Mountain National Park in central Myanmar. This forest does not connect with any other corridor leading to other forests with well-defined margins. Thus, their distribution and seasonal movement in association with food availability is fragmented.

An isolated extinct volcano with a peak 1660m above sea level, Popa Mountain Park is situated in the central dry zone of Myanmar at 25° 56′ N and 95° 16′ E. Mountain ridges occur around the entire circumference of the inside of the crater that has a radius of 1667m. The northern wall of the crater is missing, due to the formation of a 667m deep valley. The park covers an area of 25,000 acres and serves as an important water catchment area in the middle of the dry zone. The original forest cover of the exterior of the mountain has long since disappeared and much of it is now covered

with plantations of Eucalyptus. Wildlife is scarce most likely due to its development for recreational camping and tracking.

The objectives of this paper are to present data on the distribution and behaviour of wild moneky groups obtained from previous studies, and new results on mtDNA and LZM gene analyses of the Phayre's leaf monkey (*Trachypithecus phayrei*).

Materials and Methods

Selection of the Study Site and Subject

Popa Mountain Park was selected as the study site as the population of leaf monkeys was estimated to be sufficient for research purposes (Fig. 1). Three troops of the Phayre's leaf monkeys were found to live separately in 3 different sites approximately 3km apart from each other and all located on the central level of the slope about 800m above sea level. Deep, densely forest valleys and slopes prevent the mixing of the habitats of the three monkey troops and also provide a safe shelter from the intrusion of hunters and enemies. The three elevated areas that the monkeys inhabit are: Nwa La Bo Hill, Hman Pya Hill and Say Hmon Hill. In our study, Nwa La Bo Hill was designated as site I, and defined as the main study site, while Hman Pya Hill was labeled site II, and Say Hmon Hill as site III. The groups of monkeys living in Nwa La BO, Hman Pya and Say Hmon mountain were designated as A, B and C, respectively. The present study focuses on group A as a subject due to the better visibility and easy access to the study site, as well as the largest grouping of monkeys (65-75 in total) (Table 1).

Study Period

The behavioural study was conducted during the period from July 2003 to July 2006, and Gene

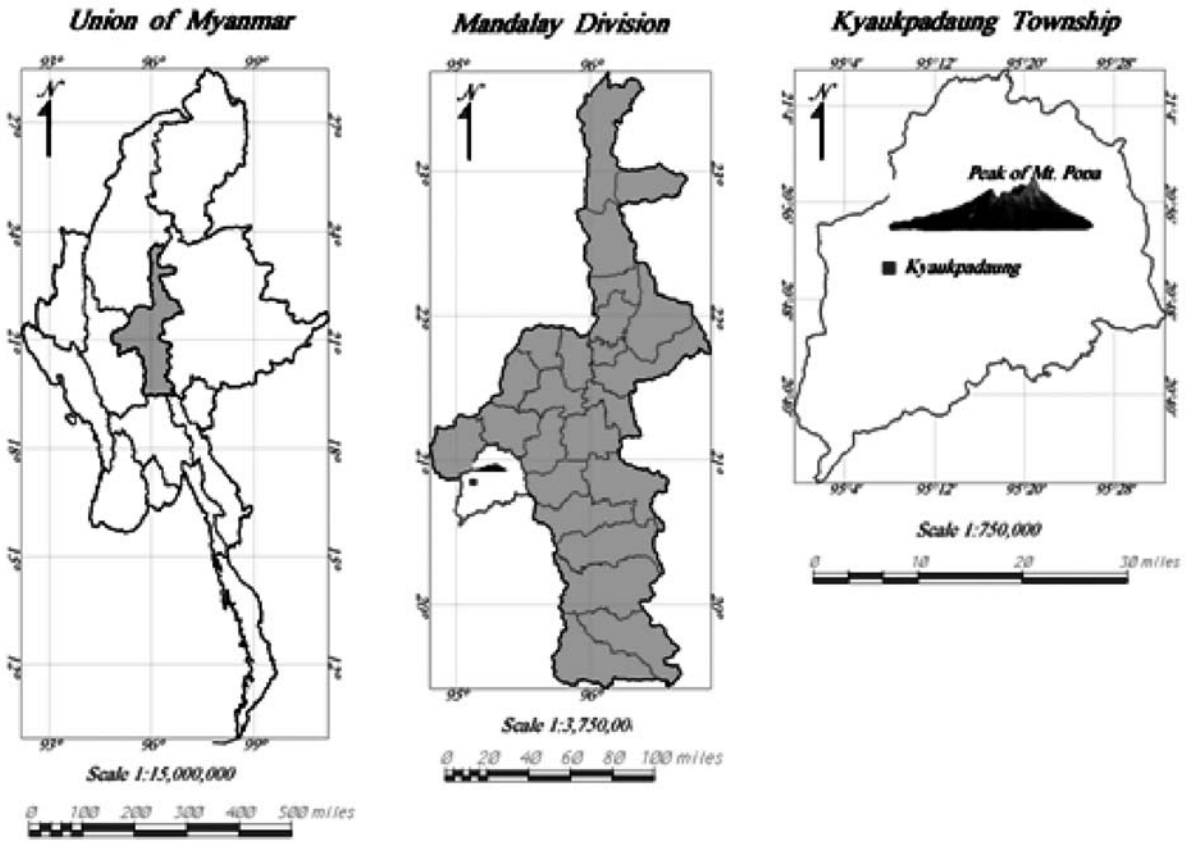

Fig. 1 Location of study area

A: The map of the Union Myanmar　B: Mandalay Division, Central Myanmar　C: Popa Mountain , Kyaukpadaung Township

Table 1. Group compositions of the leaf monkeys recorded in studied site (2004 - 2005).

Group Name	Study site	Percentage of animals in a troop						Group size	
		Adult/Sub Adult		Juvenile		Infant			
		2004	2005	2004	2005	2004	2005	2004	2005
A	I	66	68	31	20	3	12	65	75
B	II	51	69	42	18	7	13	84	89
C	III	70	69	26	25	4	6	27	32

analyses were conducted in collaboration with 2 research teams－the Forestry Department of Popa Mountain Park, Myanmar and Nihon University, College of Industrial Technology, Japan, from 2007 to 2010.

Camping and Sighting

A house made of logs near the Nwalabo Mountain was utilized as the base camp and daily observation was made around the site (Fig. 2). Locations of the studied places were noted using a GPS unit.

Sample collection

Skin tissue samples of T. phayrei were collected during the previous study in the crater of Popa Mountain Park, Myanmar, in 2004 with permission from the Park's Forestry Department.

Data Collection

Survey on distribution of the leaf monkey

Surveys were conducted by investigating audible sounds made by leaf monkeys. The presence of the leaf monkeys was confirmed by approaching in the direction of the sounds and visual checking. If leaf monkeys were observed, photographic and video records were taken as required.

Leaf monkey numbers

In the study area, points were drawn and the helpers (ranger, forester and staff from the park) were asked to count the number of monkeys as they were sighted. These results were then assessed and estimated. The composition of male, female and young within each group was recorded. This was done throughout the study period.

A　　B

Fig. 2. The main study site I in the crater of Popa Mountain Park, Mandalay Division, central Myanmar.

A: Nwalabo Mountain, the focus of the study area.　B: Showing the base camp of field study and the focus study area.

Behavioural study

With the help of binoculars (70x50) and video cameras (Canon inc.), data were collected for the following behaviours related to feeding habits: initial foraging time; preference of food sources; food search practices; behaviour regarding differentiation of food sources; feeding practices; and aggressive and competitive behaviour. Behavioural patterns and group activities (climbing, walking, running, playing) were recorded during foraging periods. Activities of the leader and followers were investigated. Social behaviour was also recorded during foraging periods. Social ranking as well as division of labour in the troop were recorded, this was also aided by the use binoculars and video cameras. Different patterns of resting behavior were also investigated and recorded. Sleeping sites were located based on the abundance of feces at/or around the habitats. These sites were confirmed by watching their activities before dawn and dusk.

DNA Extraction and Amplification

Small fragments of skin tissue (0.004g) were washed in 95% ethanol, and digested in 90μl of TBE buffer (89 mmol/l Tris, 88.9 mmol/l boric acid, and 2 mmol/l EDTA・2Na) and 10μl of 100 μg/ml proteinase-K for 3 hours in an incubator at 56ºC. DNA extraction was carried out using Magtration System 6GC (Precision System Science Inc., Chiba, Japan) according to the manufacturer's instructions. The forward and reverse primers used for the 1st and 2nd PCR were L14724 and H15915 [10]. PCR reactions were carried out with a total volume of 20 μl.

The protocol for the first thermal cycle using the primers (L14724 and H15915) was conducted by pre-heating at 94ºC for 5 min, 35 cycles of denaturation at 94ºC for 40s, annealing at 52ºC to 63ºC for 30 s, extension at 72ºC for 90s, and a further elongation step at 72ºC for 10min. The PCR parameters for KOD plus polymerase (Toyobo, Japan) were as follows: 1.0 μl of KOD plus (1.0 units/μl), 5 μl of 10× buffer for KOD plus, 5 μl of 2 mM dNTPs, and 2–10 μl of extracted DNA and templates. Half of 20 μl of the first PCR product was used as template for the second PCR. The second PCR was performed in the same way as the first PCR. The PCR products were electrophoresed in 2% agarose gels purified by Wizard® SV Gel and PCR Clean-Up System (Promega, USA).

The protocol for the first thermal cycle using the primers LZM (wm8 and wm9) was performed by pre-heating at 94ºC for 2 min, 38 cycles of denaturation at 98ºC for 10s, annealing at 50ºC to 56ºC for 30s, extension at 68ºC for 60s, and a further elongation step at 68ºC for 2 min. The PCR parameters for KOD plus polymerase (Toyobo, Japan) were done by mixing 1.0 μl of KOD plus (1.0 units/μl), and 5 μl of 10×Buffer for KOD plus. Extracted 5 μl of 2 mM dNTPs, and 2.5 μl were tested for DNA and templates. Half of 20 μl of the first PCR product was used as template for the second PCR. The second PCR was performed in 1.0 μl of I PCR product, 9.5 μl of Distilled water, 1.5 μl of primer (forward and reverse), and 8.0 μl of Master Mix. Half of 20 μl of the first PCR product was used as template for the second PCR. Cycling parameter was conducted by preheating at 96ºC for 1min, 30 cycles of denaturation at 96ºC for 20s, annealing at 58ºC for 20s and extension at 60ºC for 2min. The PCR products were electrophoresed in 2% agarose S gels purified by Wizard® SV Gel and PCR Clean-Up System (Promega, USA).

DNA Sequence Analysis and BLAST Search

In order to understand the species-specific sequences in DNA alignment, we focused on two major parts of the DNA locus, one is mtDNA and the other is *lysozyme* (*Lzm*), both located in the cytoplasma gene. These two genes have specific areas used to identify to species of monkeys. All

sequences are investigated using computerized BLAST Search systems.

The *cytochrome b* (*Cyt-b*) gene was sequenced to determine the mtDNA phylogeny of the langurs [10]. The DTCS Quick Start Kit (Beckman Coulter, USA) and the 2nd PCR product were used for sequencing. DNA sequences were manipulated and analyzed using the CEQTM 8800 SERIES Genetic Analysis System (Beckman Coulter). BLAST searches were carried out online (http:// blast.ncbi. nlm.nih.gov/blast.cgi).

Results

Vegetation types

Vegetation of the Popa Mountain Park consisted of three different forest types, dry forest, mixed deciduous forest and low Indaing forest. The foot of Mount Popa was covered with relatively dense forest and the species composition found in Popa Mountain Park was very luxuriant being comprised of herbs, shrubs, climbers and medicinal plants. In the foothills between 700m and 1000m elevation, the following plant species can be found: Kyun (*Tectona grandis* Linn.); Pyinkado (*Xylia xylocarpa* Roxb.); Taukkyant (*Terminalia crenulata* Heyne.); Phanga (*Terminalia chebula* Retz.); Ingyin (*Shorea siamensis* Kurz.); Thitya (*Shorea obtusa* Wall.); Yemane (*Gmelina arborea* Roxb.). Additionally, the only bamboo species of Hmyin-wa (*Dandrocalamus strctus* Nees) can be found in this location. The vegetation on the lowest slopes and foothills of Mount Popa are classified as dry mixed deciduous forest and low Indaing forest.

In the area between 1000m and 1300m, Te (*Diospyros burmanica* Kurz.), Thitya (*Shorea obtusa* Wall.), Ingyin (*Shorea siamensis* Kurz.) and In (*Dipterocarpus tuberculatus* Roxb.) were absent but Thitswele (*Engelhardtia spicata* Blume.), Zibyu (*Emblica officinalis* Gaerth.), Phanga (*Terminalia chebula* Retz.), Thabye (*Eugenia cuminii* L.), and Petsut (*Eriobotrya bengalensis* Hook.) were recorded as the dominant species. The exotic species of *Agaves* spp and *Pinus kesiya* Royle were planted at the same elevation. Tree species of dry hill forest, such as *Quereus* and *Castanopsis* species were also noted. Above 1300 meters in elevation a large part of the mountain becomes sparse and most of the trees stunted. Most of the area of this slope was covered with grasses. Some ferns of polychiaceae were also recorded.

Not only the vegetation types differed from one another along the slope, but the soil types also differed. The crater is about one mile wide, and descends from the top of the mountain to a depth of about 1300m. The vegetation was very luxuriant and included trees that attain heights of 23 to 27 meters. The undergrowth of the forest was very moist and dense with herbs similar to evergreen forests.

The lowest area inside the crater was about 1300m above sea level and Thitya (*Shorea obtusa* Wall.) and Ingyin (*Shorea siamensis* Kurz.) can be found in this area. The forest of the study area comprised of dry upper mixed deciduous forest and low Indaing forest. These two forest types were found mixed together in some places. Tree species composition in the entrances was completely different from that of the inside. Thitya and Indaing were absent and tree species of mixed deciduous forest type were recorded, such as Kyun (*Tectona gradis* Linn.), Pyinkado (*Xylia xylocarpa* Roxb.), Didu (*Bombax insigne* Wall.), Nabe (*Lannea coromamdelica* Houtt.), Phanga (*Terminalia chebula* Retz.) and Pet-wun (*Berrya mollis* Wall.). The inside of the crater was dominated by the tree species of evergreen forest type such as Kadut (*Ficus hispida* L.) and Pyinma (*Largastroemia macrocarpa* Kurz.). A natural water source was noted at 1000m at the study site. Animals inside the crater were quite diverse and various types of vegetations were noted forming a deep forest supported by the fertile soil of the crater.

The leaf monkey species of the study area

The leaf monkey species present in the study area identified according to Goodall (1996) and Francis (2001) was recorded to be diurnal and arboreal *Trachypithecus phayrei* Blyth 1847 (Phayre's langur). Distinctive morphological character of *T. phayrei* was grey to blackish brown body color. The hands are brown, the feet black; the upper arms, legs and tail are silvery grey. The eyes and lips respectively consisted of a white patch (Fig. 3). Neonates are straw colored. They inhabited the primary and secondary dense high forests. Their diet consists of 58.4% leaves, 24.4 % fruit and 9.7% petioles. They were generally found in high areas away from humans.

Behaviors

Feeding behavior

The monkeys are called leaf monkey because they mainly feed on young, tender leaves and sometimes young twigs and flower buds. Feeding on the leaves starts as soon as they got up early in the morning. Almost all the trees near their sleeping site were employed as food plants and they consumed the leaves as much as they could. These monkeys selectively fed on young, tender leaves and the old leaves with rough texture were usually removed and dropped onto the ground. By looking for dropped green leaves the presence of leaf monkeys could be predicted. Small green branches were also dropped after taking the tender leaves from them. The hanging tail from among the leaves indicated the presence of these monkeys while they were feeding on leaves among the branches. The leaf monkeys usually avoided ripening fruits as they prefer young fruits. Phayre's leaf monkeys feed exclusively on tender young leaves, fruit and buds that are available throughout the year. The fresh leaves appeared to provide the water required by the monkeys since the Phayre's leaf monkeys were never seen drinking in the wild. During the study period, feeding habits differed seasonally depending on the availability of various food sources. The phayre's leaf monkeys usually returned to the old trees when the new leaves sprouted. The trees of *Ficus spp*, produced a large amount of young leaves and fruits over a long period. The phayre's leaf monkey generally remained in the vicinity of food plants and returned frequently to the trees they had previously used. Both old and young leaves were often plucked from trees and bushes. Fruit, buds and young flowers were mostly eaten in the dry season when young leaves were scarce. They also showed preference in their choice of food. Fig plants of *Ficus* sp were the most preferred. Other plants such as *Treura nudiflora, Flacourtia cataphracta, Alseodaphne kee nanii, Protium serrata, Saussurea affinis, Sapium baccatum, Carallia brachiata, Piper attenuatun, Smilax* spp, *Eugenia* spp, *Ter minalia betterioa* Roxb and *Trema* spp were found to be included in their food sources and used alternatively in different seasons.

(A)　(B)　(C)

Fig. 3 . Phayre's leaf monkey in the crater of Popa Mountain Park, Myanmar,
(A) Male Sub adult , (B) Adult Female , and (C) Male Juvenile.

In this study, the leaf monkeys consumed 19 species of plants. Among them, various *Fig* spp, and *Piper attenuatun* were eaten throughout the year and served as the primary food source for the leaf monkeys. The *Saussurea affinis* was eaten in June and September. Fruits, except of some trees such as *Treura nudiflora* and *Protium serrata* were eaten. Fighting between individual monkeys for food was not observed. Seasonal movement and foraging areas were remarkably different, probably depending on the availability of foods sources. The foraging areas recorded in rainy season was smaller than that of the other two seasons.

Social hierarchy

Hierarchical behavior was recorded in the population of leaf monkeys especially during their movement from the sleeping site to feeding site. A group of monkeys is usually led by a strong adult male or female. Lactating mothers and juveniles following the leader are further protected by strong males and females following them. An aggressive, strong and healthy male leaf monkey stayed apart from the group acting as a guard. The alert guard monkey is always on the look out for approaching enemies. If any sign of enemies or if enemies are sighted, a loud warning call is produced in order to give time for the moving monkeys to seek hiding areas among the bushes and dense branches of the trees. When an enemy is directly encountered the guard monkey shows his aggressive manner and evades the enemy by swinging from one branch of a tree to another. A strong male monkey is also usually found guarding the members of the group during inactive periods. This monkey usually occupies higher a level in the tree looking out for enemies while other members are sleeping (Fig. 4).

Sleeping sites

Two sleeping sites, a sleeping site and a day sleeping site, were recorded. The night sleeping site was found to be more secured since they used large trees growing in the deeper valley and the canopies densely covered with leaves. The day sleeping site was not particularly selected and was found change depending on the place where they fed. The trees on which they fed were used as day

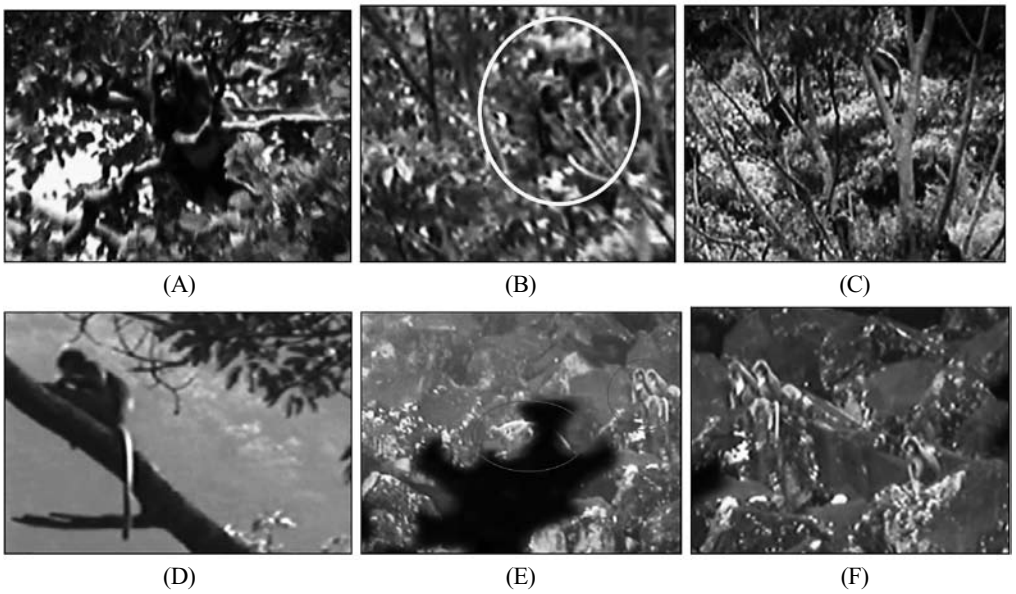

Fig. 4. Common behaviour of Phayre's leaf monkey.

(A) The leader making an alarm call. (B) Active period. (C) Foraging and locomotion. (D) Take duty and watching enemies when others are resting and sleeping. (E) Female with baby moving while two males watch and guard their group. (F) Males sitting and watching for others.

sleeping sites. Almost all the monkeys—with the exception of the one (usually a sub adult male) taking the sentinel duty—slept during the resting time after feeding at noon. Night sleeping sites could be easily recognized by the presence of a large number of droppings (faeces) and a bitter foul smell produced from the faeces and urine of the monkeys.

Interaction and relation among the members

A number of Rhesus monkeys were found to live within the group of leaf monkeys. Juveniles of the two species of monkeys were seen playing together. The young rhesus monkeys generally came to the leaf monkeys to join in playing. Both species of monkeys were found to sit on the same branch feeding on different parts of tree with the leaf monkeys on the young leaves and rhesus monkeys on the ripening fruits. Presence of lineated barbet (bird) in its vicinity was an indicator of the leaf monkey as they usually congregated around the same food plants. This bird species also helped the monkeys to avoid approaching enemies by sudden fluttering. The birds and monkeys were found to congregate at a food tree quite distant from the old one.

Gene analysis

Fig. 5 was shown result of the PCR amplification of tissue samples using lysozome wm 8 & wm 9. Based on our PCR procedure, DNA sampling in separate part of monkeys was carried ou.t.. The sequences in *Cty-b* and *LZM* were compared and studied in term of BLAST sequence study software.

The result showed our studied phayre's leaf monkey is closely related with the phayre's leaf monkey of India than Vietnam. *T. phayrei* of Myanmar has homology differences of *Cty-b* and *LZM* 5% with *T. phayrei* of India and 11% and 6% with T. phayrei of Vietnam (Table 2).

Discussion

Popa Mountain Park was chosen as the study site for leaf monkeys since the place was well inhibited by the monkeys and hagave easy access to study the leaf monkeys. The age of extinct volcano in Popa mountain park and its forest is 250 thousand years. It is younger than the nearby mountain ranges, like Chin hill to its north and Bago yoma to its South. The leaf monkeys inhibiting in Popa Mountain Park could be descendents of leaf monkeys inhibiting in Chin hill and Bago Yoma. Also it is worth studying these monkeys while they are listed by IUCN as a critically endangered species and are on the Red List. Also, no one has so far studied this monkey in Myanmar. According to Myint Myint Oo (1995) the population of the

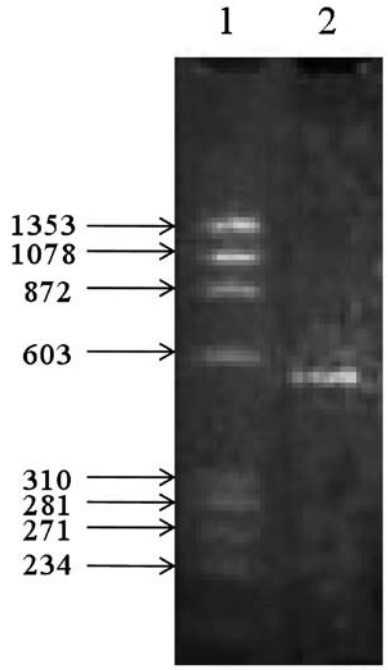

Fig. 5. Amplified product of *T. phayrei* of mtDNA *cyt b* gene.

Lane 1: Marker 4
Lane 2: Myanmar *Trachypithecus phayrei,* 569 bp

Table 2 Difference of DNA sequences between *T. phayrei* of India and Vietnam.

Gene	India	Vietnam
Cytochrome b	5%	11%
LZM	5%	6%

leaf monkeys at popa Mountain Park was found to be very small before that place was notified as a protected wild life park. The population of the leaf monkeys increased during the 3 years studied period due to law enforcement.

Although there were 3 groups of leaf monkey inhibiting in the forest of Popa mountain park, no sign of mixing among the groups was observed. Abundant food supply effectively supports the lives of monkeys and allowed them to build new colonies and disperse from the main group. The main group, having largest number of leaf monkeys, occupied the most secure and dense part of the forest. While the second and third group of leaf monkeys occupied a forest area with lesser security. As the evergreen forest of Popa mountain could provide enough food for the leaf monkeys, there were no signs of fighting for food between the groups of monkey observed.

Presence of social hierarchy is the character of advanced behavior in any group of vertebrate animals (1988). This social hierarchy is more distinct in a group of primates and this can effectively help the individuals of the group to get food, avoid enemies and to perform other duties for the colony. Similar social hierarchy was observed in the present study of leaf monkeys.

Night sleeping sites were found to be located in dense vegetation of large trees being difficult access by other terrestrial animals. This may be true for all the diurnal creatures that effective rest by sleeping at nigh time and need to be almost completely secure from attack by enemies. Day sleeping sites of these monkeys were found to be shifting in association with their choice of feeding site. Permanent day sleeping sites at particular locations were not recorded.

Presence of some rhesus monkeys among the leaf monkeys may be due to the partitioning in their requirements. Although both species feed on the same plant source, rhesus monkeys take the mature leaves and ripe fruit, while the leaf monkeys especially feed on young, tender leaves and fruit. Also, the peaceful behaviour of the leaf monkeys may probably encourage the rhesus monkeys to join them.

Rhesus monkeys could ingest mature leaves and fruit having a coarse texture because these monkeys possess cheek pouches for secondary rumination to aid more effective digestion. Polyphagous behavior of both monkey species enhances the possibility of their coexistence having separate food types.

Nineteen Species of plants were recorded as food plants, in which 5 species of Ficus seem to be staple food sources and are eaten throughout the year. When they utilize the young leaves and buds, they break some branches and they are dropped with the old leaves. In this study, leaf monkeys were found together with the Macaques (3-5 animals) in the same area probably sharing the food sources and without any competition.

Consumption of young leaves is the most important characteristic of phayrei leaf monkey. They are absent from the regions where food plants are not abundant and this appears to be significant factor for their limited their distribution.To understand the relationship between species, we carried out the gene analysis and sequence comparison in mtDNA and LZM region. As the data are shown in Fig. 6 and it is obious that the Popa mountain leaf monkey is genetically closely related to Indian species than that of Vietnam once. Based on that results, it is said that.

According to the gene analysis results by both mtDNA gene and *LZM* gene, studied *T. phayrei* leaf monkey is more closely related with the species of India than that of Vietnam. These data are shown in Table 3 and Table 4.

Previous to gene analysis study, the species T. Phayres is less and precise. However, gene analysis has confirmed the species comparing with those distributed in neighbouring countries.

The leaf monkeys inhabiting Popa Mountain

Fig. 6. Lysozome Sequences of studied *T. phayrei.*

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AF294866.1      ----------------------------------------------------GAAGAAAGAAGGTAAAA--
AF294867.1      ----------------------------------------------------GAAGAAAGAAGGTAAAA--
Myanmar         CTATGATGACAGCAGAATCAGAGGTGAGCTGACCTCACTTGCCACAGGGCGGAATAAAGAAGGTATAAAT
                                                                                    ***
                ********** **

AF294866.1      GATATA-AATACTG-GGACCAGCTCACCCTGGT-TAG-CGTA-GCAGTC--TGACCTAGCAGTC--AACA
AF294867.1      GATATA-AATACTG-GGACCAGCTCACCCTGGT-TAG-CGTA-GCAGTC--TGACCTAGCAGTC--AACA
Myanmar         GATATATAATACTGTGGACCAGCTCACCCTGGTATAGTCGTATGCAGTGCATGACCTAGCAGCTCAGACA
                ****** ******* ****************** *** **** *****     **********       ***

AF294866.1      TGAG-GGCTCTCATTATT-CTGGGGCTTGTCCTCCTTTCTGTCACGGT-CCAGGGCAAGATCTTTGAAAG
AF294867.1      TGAG-GGCTCTCATTATT-CTGGGGCTTGTCCTCCTTTCTGTCACGGT-CCAGGGCAAGATCTTTGAAAG
Myanmar         TGAGTGGCTCTCATTATGACTGGGGCGAGTCCTCCTTTCTGTCACGGTGCCAGGGCAAGATCTTTGAAAG
                **** ************   *******  ********************* ********************

AF294866.1      GTGTGAGTTGGCCAGAACTCTGAAAAAATTGGGACTGGATGGCTACAAGGGAGTCAGCCTAGCAAACTGT
AF294867.1      GTGTGAGTTGGCCAGAACTCTGAAAAAATTGGGACTGGATGGCTACAAGGGAGTCAGCCTAGCAAACTGT
Myanmar         GTGTGAGTTGGCCAGAACTCTGAAAAAATTGGGACTGGATGGCTACAAGGGAGTCAGCCTAGCAAACTGT
                ************************************************************************

AF294866.1      AAGTCTACTCTCCATACTTCCAGAGAATTAGCTACGTATGGAACAGACACTAGGAGAGAAGGAAGAAGAA
AF294867.1      AAGTCTACTCTCCATACTTCCAGAGAATTAGCTACGTATGGAACAGACACTAGGAGAGAAGGAAGAAGAA
Myanmar         AAGTCTACTCTCCATACTTCCAGAGAATTAGCTACGTATGGAACAGACACTAGGAGAGAAGGAAGAAGAA
                ************************************************************************

AF294866.1      GGGGCTTTGAGTGAATAGATGTTTTATTTCTTTGTGGTTTTGTATA-CTTACAATGGCTAAAAACATCAG
AF294867.1      GGGGCTTTGAGTGAATAGATGTTTTATTTCTTTGTGGTTTTGTATA-CTTACAATGGCTAAA --------
Myanmar         GGGGCTTTGAGTGAATAGATGTTTTATTTCTTTGTGGTTTTGTATATCTTACAATGGCTAAAAACATCAG
                ******************************************** **************

AF294866.1      TTTGGTTCTTTATAATCAGAGATACCCGATAAA- ----------------------------------
AF294867.1      ------------------------------------------------------------------------
Myanmar         TTTGGTTCTTTATAATCAGAGATACCCGATAAAGGAATAAGAGCATGGCAGGGGAAAATTCCATTCTAAG

AF294866.1      ----------------------------
AF294867.1      ----------------------------
Myanmar         TAAAACAGGAAGTACTAACTGCTAAAGAA
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AF294866.1 *Trachypithecus phayrei* 387 bp
AF294867.1 *Trachypithecus phayrei* 346 bp
Myanmar *Trachypithecus phayrei* 569 bp
Citing of CLUSTAL W (1.83) multiple sequence alignment

Table 3. *Cytochrome b* (*cyt-b*) gene analysis.

Sample type	Source	Cyt-b ID	Max Score	Max ident
Tissue (1)	India	AF29462	1186	95%
Tissue (1)	Vietnam	AF294622	965	89%

Table 4. LZM gene analysis.

Sample type	Source	Lzm	Max Score	Max ident
Tissue (1)	India	AF294866.1	623	95%
	Vietnam	AF294867.1	547	94%

Park could be descendents of the leaf monkeys inhabiting Chin hill. That is more likely than them being descendents of Bago Yoma.

Nature study is the basis for laboratory works. Behavioral Study and Gene Analysis of *Trachypithecus phayrei* (Blyth, 1847) Phayre's leaf monkey, Old World Monkey in the crater of Popa Mountain Park, Myanmar has thus highlighted the combination of field and laboratory studies. A long period of field surveys, involving many time consuming tasks, revealed the interesting behaviour of Phayre's leaf monkeys in the Popa Mountain area after. However, behavioural study alone is not sufficient. Therefore, genetic study needs to be conducted to confirm the species by comparing with the works conducted in neighbouring countries where leaf monkeys are distributed. Laboratory work is not as time consuming as field studies; however, one needs to gain the knack of the technique by practicing over and over. It is therefore concluded that both field and laboratory studies are of utmost important in scientific research.

In Table 4, the difference of sequence between two species is not so much outstanding, however, we understand in our experiments, the homology study in DNA sequences, in *Cty-b* is more significant than those of *LZM*. . We considerd that *Trachypithecus phayrei* of Myanmar is a species of more related to India species than that of Vietnam.

Acknowledgements

We would like to extend our profound gratitude to Professor Dr. Susumu Ishii, Vice-president of Nihon University for permitting us to conduct this work. We are obliged to Shigeru Oyama, Secretary General, and Association of Japan Myanmar Mutual Cooperation (AJMMC) for providing continuous support. We are obliged to the Association of Japan Myanmar Mutual Cooperation (AJMMC) for providing continuous support. We are indebted to Dr. Hiroshi Nemoto and our colleagues from the Department of Applied Molecular Chemistry, Nihon University, for aiding us when necessary. In Myanmar, Dr. Daw Tin Nwe, Professor (Retired), Zoology Department, Yangon University, Pro Rector Dr. Win Maung, Sittway University, Rakhine State, Professor Dr. Maung Maung Gyi, Head of Zoology Department, University of Yangon, Authorities from Forestry Department, Park wardens and staff members—without whose help, understanding, criticisms, and encouragement, this paper could not have been accomplished—all of their assistance is greatly appreciated. I would also like to express my gratitude to Nagao Natural Environment Foundation of Japan and Chairman, Executive Committee Members and staff of Forest Resources Environment Development, Yangon, Myanmar and Conservation Association from Yangon Training Coordinator of Wild life conservation Society (WCS), Yangon, Myanmar for kindly grating me the necessary scholarship for field studies.

References

1) Van Schaik C.P., Kappeler P.M. : Infanticide risk and the evolution of male-female association in primates. Proceedings of the Royal Society London B **264:** 1687-1694 1997.
2) Fleagle J.G. : Primate adaptation and evolution. 2nd edition, Academic Press, San Diego. 1999.
3) Oates J F, Davies A G and Delson E : The diversity of living colobines; in *Colobine monkeys: their ecology, behaviou and evolution* (eds) Davies A G and Oates J F (Cambridge:Cambridge University Press) 45–73 1994
4) Rowe N. : The pictorial guide to the living primates. Pogonians Press, East Hampton. 1996
5) Chapman CA and Onderdonk DA: Forests

without primates: primate/plant codependency. Amer J Primatol **45:** 127-141 1998

6) Eudey, A.A. : Asian Primate conservation - The species and the IUCN/SSC *primate specialist* network. Primate conservation **17:** 101-110 1996/1997

7) Groves, C.P. . Primate taxonomy. Smithsonian Institution Press, Washington D.C. 2001

8) Nadler, T., Momberg, F., Nguyen Xuan Dang and Lormee, N. 2003. *Vietnam Primate Conservation Status Review 2002. Part 2: Leaf Monkeys*. Fauna and Flora International and Frankfurt Zoological Society, Hanoi. pp. 145–164.Nadler, T., Momberg, F., Dang, N.X., and Lormee, N. 2003. Leaf Monkeys. Vietnam Primate Conservation Status Review, 2002, Part 2.

9) Tun Yin, U. : Wild Mammals of Myanmar. 1967

10) Karanth KP, Singh L, Collura RV: Molecular phylogeny and biogeography of langurs and leaf monkeys of South Asia. Mol Phylog Evol **46:** 683-694 2008

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Myanmar のポパマウンテンの火山クレーターに生息するオナガザル科リーフモンキー Trachypithecus phayrei (Blyth, 1847) の 行動学的研究と遺伝子解析

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要　旨

ミャンマーのポパマウンテンのクレーターに生息する *Trachypithecus phayrei*（BLYTH, 1847）の行動学的研究や研究場所とサルの生息地の状態に関して、写真等にて説明した。さらに、サルの皮膚サンプルから遺伝子を抽出し、ミトコンドリア遺伝子とリゾチーム遺伝子の 2 つをターゲットとして DNA 配列を解析した。その結果、本研究の *T. phayrei* は、ベトナムよりもインドに近い種であることが明らかとなりました。さらにポパマウンテンに生息するリーフモンキーは、バゴマウンテンから派生したサルではなく、チンヒル高原に生息するリーフモンキーから派生したと示唆されます。

キーワード：*Trachypithecus phayrei*, Popa Mountain Park, Myanmar,Feeding behaviour, Lysozome and mtDNA

連絡先：